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PHOTOPERIODIC AND FORMATIVE EFFECTS OF VARIOUS WAVELENGTH REGIONS IN *HYOSCYAMUS NIGER* AS INFLUENCED BY GIBBERELLIC ACID

by

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1. INTRODUCTION

In experiments with *Hyoscyamus* grown in narrowly defined spectral regions under long day conditions, STOLWIJK and ZEEVAART (1) reported stem elongation and flower formation in the blue and near infra-red regions, whereas there was little or no such response in the red and none at all in the green. In view of the findings of BRIAN and HEMMING (2) regarding the pronounced qualitative and quantitative effects of gibberellic acid on the growth of various plants, particularly in regard to stem elongation, it was felt that a repetition of the aforementioned experiments might be worthwile, with the addition of gibberellic acid in the various light treatments. While the work was under way, two reports (LANG 3, 4) appeared on the flowering of biennial *Hyoscyamus* in one year and under short day when gibberellic acid was supplied. These added to the interest of the present findings.

2. MATERIALS AND METHODS

The high light intensity cabinets described by WASSINK and STOLWIJK (5) were used in these experiments, with a few modifications. In the first experiment a blue (B+), green (G), red (R), and infra-red plus red (IR + R) cabinet were used. The first three spectral regions were obtained with banks of 40 watt fluorescent lamps of limited spectral emission, together with appropriate glass filters, while the IR + R was obtained with banks of 40 watt tungsten lamps and a red glass filter. In the latter the number of lamps was adjusted until the intensity of the photosynthetically functional (red) light was of the same order of magnitude as in the other cabinets. Light measurements in the cabinets were made with a calibrated thermopile and a KIPP portable galvanometer, whose sensitivity was regularly checked with calibration voltages from a voltage divider (STOLWIJK, 6). The spectral distribution of energy was estimated by means of serial measurements with various SCHOTT (Jena) filters (WASSINK and VAN DER SCHEER, 7). The blue glass filter used in the first experiment transmitted near infra-red and the lamp emission also contained a measurable amount of this light. The measured amount of near infra-red contamination in the blue cabinet

averages about 2% of the total light. Since the blue effect observed by STOLWIJK and ZEEVAART is in the same direction as the infra-red effect, it was deemed necessary to eliminate this contamination in the blue cabinet as far as possible.* Therefore, in the second experiment an additional blue light cabinet (B–) was arranged, using the same fluorescent lamps but with a set of blue plastic filters (Plexiglas, B 27 alt, RÖHM & HAAS, Darmstadt), recently available at our laboratory, instead of the blue glass. The transmission curve of the blue plastic filter, as obtained with a KIPP double monochromator and amplified photo-cell arrangement, indicates 0% transmission (certainly $< 0.05\%$) in the range from 600 to 840 mμ (photo-cell limit). When this filter set was installed and the energy in the 700 to 950 mμ region measured using the SCHOTT filters, no measurable amount of near infrared contamination could be detected. The cabinet with the blue glass filters was left intact so that for the second experiment there were two comparable blue cabinets, with (B+) and without (B–) near infra-red contamination. As will be shown below, these two cabinets gave the same results. It should be remarked that it was nesessary to add eight additional tubes (total 30) to the cabinet with the blue plastic filters in order to bring the blue intensity up to approximately the same level as in the cabinet with the blue glass filters.

The plant material used was the same annual strain of *Hyoscyamus niger* as used before (1). Seeds were germinated in the greenhouse and then grown under short day conditions until the beginning of the experiment. Plants were considered ready for treatment when there were 10 to 14 large rosette leaves. In the first experiment 12 plants, selected for uniformity, were used in each of the four cabinets. In the second experiment 16 plants were used in each of the five cabinets. The photoperiod temperature averaged 23°C while the night temperature averaged 19°C.

The gibberellic acid (GA) was kindly supplied by Prof. K. V. THIMANN, Harvard University, Cambridge, Mass. It was originally provided through the courtesy of Dr. P. W. BRIAN, Akers Research Labs., The Frythe, Welwyn, Herts., England. For each experiment a stock solution was made up by dissolving 10 mg of the cristalline material in 100 ml distilled water. These stock solutions were stored in the cold room in darkness. The solutions were applied to the plant daily with a dropping pipette. Three drops (total volume 0.1 ml) distributed as follows: one on the apex, the other two on randomly chosen, different leaves, either basal or stem (if a stem developed). In the second experiment the stock solution was diluted serially to obtain amounts of 10 μg (stock), 1 μg, and 0.1 μg GA in the 0.1 ml volume of the three drops applied.

3. EXPERIMENTAL RESULTS

Expt. 1. The seeds were sown on 22-3-56 and the plants were raised in the greenhouse on a 10 hr. day until 10-5-56. They were then placed under a bank of white fluorescent lights for two weeks, still on a 10 hr. day. The colored light (15 hr. day) and GA treatments began on 24-5-56. In each of the light cabinets 6 plants received no GA treatment while 6 were given 10 μg per day. The GA treatment for three of the latter was discontinued after two weeks, and for the other three after four weeks. Two representative plants from each group of 6 were photographed at weekly intervals. Plates 1A and 1B show the results after

* The desirability of this was emphasized by Dr. S. B. HENDRICKS (Beltsville) in a discussion with one of us (W) on the subject.

two and four weeks, respectively. Table I summarizes the data obtained 32 days after the beginning of the treatments.

The first response observed occurred in the IR + R cabinet within 36 hours. The leaves of all the IR + R plants assumed a more vertical position in this time. In a few days the GA treated plants in the other cabinets also showed this erection of the leaves while the untreated plants in R and G remained unchanged. Definite stems (> 1 cm) first became apparent in the B+, GA treated plants after 11 days. On the 12th day stems were also apparent on the IR + R, GA treated plants, and by the 14th day stems were apparent on all the B+ plants, on all the IR + R plants, and on the GA treated R and G plants. Flowers were first observed in the B+, GA treated plants after 29 days, in the IR + R, GA treated plants after 30 days, and in the R, GA treated plants after 60 days. No flowers were obtained in the G, GA treated plants after 68 days, when the experiment was terminated.

Expt. 2. The seeds were sown on 18-5-56 and after germination the plants were transferred to a bed outdoors where they were kept on a 10 hr. day until 2-8-56. At this time they were brought in and placed under the bank of white fluorescent lights (10 hr. day) for two days while the selecting, measuring, and tagging operations were carried out. The colored light (15 hr. day) and GA treatments began on 4-8-56. Eight additional plants were arranged in the greenhouse, 4 being kept in daylight under a 10 hr. and the other 4 kept under the natural daylength (long day) conditions. In each group of four, 2 plants were given 10 μg GA daily, the other two left untreated.

In each of the light cabinets 4 plants were given 3 drops of distilled water, 4 were given 10 μg GA/day, 4 others 1 μg GA/day, and the remaining 4 0.1 μg GA/day for the duration of the experiment. At ten day intervals one representative plant from each group of four was photographed. Plates 2A, B, and C show the results after 10, 20, and 30 days respectively. Plate 3 shows the results of the greenhouse experiment after 30 days. Table II summarizes the data obtained 26 days after the treatments were begun.

The sequence of events in this experiment was very much the same as in expt. 1, as may be see from Table III. This Table indicates the number of days required for the appearance of the first definite stems (> 1 cm) and flowers in each set. Most of the plants were cut up after the 31st day for examination for flower buds* and for dry weight determinations; treatment was continued on two R, GA (10 μg/day) plants and two similar G plants. The R plants flowered after 34 days, while the buds in the G plants then still showed no sign of flowering, they ultimately flowered after 67 days.

4. DISCUSSION

In the various light cabinets the untreated plants confirm the results of STOLWIJK and ZEEVAART (1), that is, flowering occurs in *Hyoscyamus niger* under long day conditions in the presence of blue and infra-red radiation, but not in red or green. In the second experiment the fact that the plants in the B+ and B– cabinets (i.e., with and without small amounts of near infra-red contamination) responded in an entirely equivalent manner makes it appear most probable that the blue response cannot be attributed to near infra-red contamination. The greater bud size in the blue cabinets also points in this direction, although the

* Collaboration of Mrs I. OLIVIER-LUYTEN on this point is gratefully acknowledged.

small bud size in the IR + R cabinet might be attributed to the lower amount of photosynthetically functional light.

It is apparent that gibberellic acid promotes stem elongation and flower bud formation in long day green and red and short day daylight, where no such response occurs without the GA treatment. It remains enigmatical whether the GA promotes the initiation of the flower buds or whether this ensues as a consequence of stem elongation or vice versa, since in all cases examined for this point the two processes seemed to occur simultaneously. Flower bud opening in the G, GA treated plants and in the short day, GA treated plants however is very slow. The GA response obtained with the short day plants confirms the results obtained by LANG (4) using a biennial variety of *Hyoscyamus*.

In light conditions under which *Hyoscyamus* does not require GA for stem elongation and bud formation (namely, the B, IR + R, and natural long day) it is clear that the GA still has an effect, both in increased stem elongation and earlier bud formation and flowering. This additive effect of the GA and light is least apparent under the IR + R conditions. In this case the time sequence of events is practically identical for the GA treated and untreated plants. This type of response presents an interesting parallel to the cases of differential growth response to GA reported by BRIAN and HEMMING (2) and PHINNEY (8) in various tall and dwarf varieties of peas, beans, and maize. Obviously, certain spectral qualities of light, and certain conditions of daylength influence the plants in a way similar to application of GA. This might be interpreted as the influence of light treatments on the level of a natural gibberellin-like factor in the plants, or as a parallel effect of GA and some light treatments on the enzymic regulations required to produce the observed effects, viz., promotion of stem elongation and flowering. Experiments reported by LONA and BOCCHI (9) and LOCKHART (10) may also point in the direction suggested.

From the data of these experiments it is clear that the response to GA depends on the length of treatment and the total amount applied. In the first experiment the plants in the green cabinet in particular reverted to an almost negligible rate of elongation shortly after cessation of the GA treatment. This result again parallels the results of PHINNEY (8), who found that the mutant maize seedlings, after an initial response to a single GA application, reverted to dwarf habit of growth.* The second experiment indicates that the response is a function of the concentration of GA applied. Under the red and green conditions the threshold for this response seems to lie close to 0.1 μg GA/day/plant.

In both experiments there is an obvious effect of the different spectral regions on the petiole length of the basal leaves. The additional effect of the GA treatments is not so clear, since an additive effect is indicated in the first experiment but to a much lesser extent in the second. The petiole length of the basal leaves seems to be directly correlated with the stem length; in all cases stem elongation is accompanied (or preceded) by elongation of these petioles.

Neither the light nor the GA treatments appeared to have a very marked effect on the shape of the basal leaves. An exception to this is the contrast between the basal leaves of the long day (daylight) GA treated plants and the other greenhouse plants. One might argue here that the stem elongation response is so

* In other experiments, DE LINT in our laboratory, under some conditions did not find a large difference between single and repeated application of GA to *Hyoscyamus*. It is now investigated further in how far this is related to the dose of GA applied.

rapid and immediate in these plants that the process of basal leaf expansion is left far behind or cannot compete successfully.

Besides the differences in the response of the petiole lengths to GA in the two experiments, a difference is apparent in the rate of elongation of the non-GA treated plants in the blue cabinets. In the second experiment these plants proceeded toward flowering much more rapidly than did the equivalent plants in the first experiment. These differences are probably attributable to the pretreatment differences, particularly the fact that the plants in the second experiment were approximately two weeks older than those in the first.

5. SUMMARY

When grown in narrowly defined spectral regions under long day conditions, *Hyoscyamus niger* (annual strain) flowers in blue and infra-red + red radiation, whereas neither stem elongation nor flower bud formation occur in green and red light. With the addition of gibberellic acid to these treatments, the rate of the flowering process is increased somewhat in the blue and infra-red + red, and stem elongation and flower bud formation occur in the green and red. Under short-day conditions in daylight plants which would normally remain vegetative responded in a similar manner. Stem elongation and flower bud formation were always found closely associated. Results are collected in Tables I to III and shown on Plates 1 to 3.

6. LITERATURE

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TABLE 1. Formative and flowering effects of gibberellic acid on *Hyoscyamus*, annual strain, grown in light of restricted spectral regions, in relation to duration of treatment. Experiment 1, 32 days after beginning of treatment

Spectral region, and intensity in ergs/cm²sec	GA treatment	Condition of apex	Stem length (cm)	Number of leaves (>1 cm)		Dimensions of mature basal leaf (cm)		
				basal	stem	petiole	blade length	blade width
Blue (+) 7800	*None	Flower buds	14	14	20	6	10	5
	2 weeks, 10 μg/day	Flowers or Flower buds	53	11	23	8	11	6
	4 weeks, 10 μg/day	Flowers or Flower buds	60	13	22	9	11	6
Green 11,200	None	Vegetative	0	18	–	3	8	5
	2 weeks, 10 μg/day	Flower buds	14	13	19	7	10	5
	4 weeks, 10 μg/day	Flower buds	20	12	19	8	12	6
Red 5700	None	Vegetative	0	20	–	4	9	4
	2 weeks, 10 μg/day	Flower buds	20	15	20	9	12	5
	4 weeks, 10 μg/day	Flower buds	31	14	18	10	12	6
Infra-Red 49,500 + Red 3400	None	Flower buds	21	18	12	11	10	5
	2 weeks, 10 μg/day	Flowers or Flower buds	41	13	21	13	11	5
	4 weeks, 10 μg/day	Flower buds	20	14	12	13	10	5

* Data for "None" category are averages of 6 plants, for other two categories averages of 3 plants

TABLE 2. Formative and flowering effects of various concentrations of gibberellic acid on *Hyoscyamus*, annual strain, in light of restricted spectral regions, and in long and short days. Experiment 2, 26 days after beginning of treatment

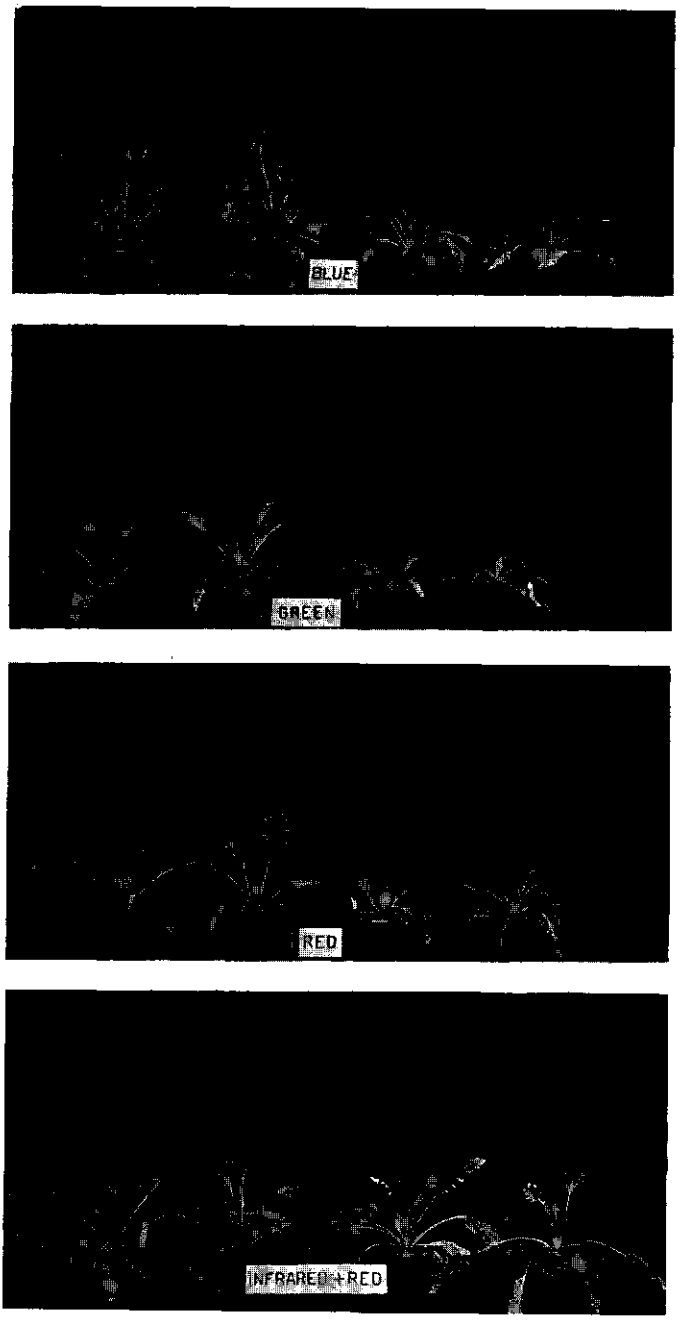
Spectral region, and intensity in ergs cm/sec.	Daily GA treatment (μg)	Condition of apex	Bud size (mm)	Stem length (cm)	Number of leaves (> 1 cm)		Dimensions of mature basal leaf (cm)		
					basal	stem	petiole	blade length	blade width
Blue (–)	0.0	Flower buds	3–5*	20.8	14	20	6.9	9.5	5.3
5600	0.1	Flower buds	3–5	19.4	12	18	6.3	9.4	5.3
	1.0	Flower buds	2–5	23.5	12	18	6.1	8.5	5.1
	10.0	Flower buds	4–7	37.8	13	25	7.3	9.6	5.8
Blue (+)	0.0	Flower buds	3–5	26.5	13	22	6.9	9.5	5.5
7800	0.1	Flower buds	3–5	25.8	12	21	6.6	9.1	5.6
	1.0	Flower buds	4–6	32.8	11	22	6.9	9.0	5.9
	10.0	Flower buds	5–10	42.0	11	23	7.0	9.0	5.5
Green	0.0	Vegetative	–	0	24	–	4.9	10.5	5.5
13,000	0.1	Vegetative	–	0	23	–	5.5	10.6	5.9
	1.0	Flower buds	1	4.4	17	11	4.8	9.8	5.0
	10.0	Flower buds	1	15.9	15	15	6.5	9.3	5.0
Red	0.0	Vegetative	–	0	25	–	4.0	9.5	5.4
15,500	0.1	Flower buds	< 1	< 1	24	–	4.1	7.9	5.0
	1.0	Flower buds	< 1	4.0	19	11	4.9	9.9	5.5
	10.0	Flower buds	1–2	21.3	16	16	4.9	8.8	5.1
Infra-Red	0.0	Flower buds	1–2	24.3	13	19	9.3	8.3	4.5
45,300	0.1	Flower buds	1–2	18.5	13	17	8.0	8.3	4.2
+ Red	1.0	Flower buds	1–3	25.1	12	17	7.6	7.6	4.9
4,300	10.0	Flower buds	2–4	36.0	12	20	7.9	8.8	5.0
Daylight	0.0	Flower buds	7–11	43.0	16	27	14.5	18.5	11.0
(Long Day)	10.0	Flowers	10–18	64.8	12	33	9.0	12.3	7.0
Daylight	0.0	Vegetative	–	0	25	–	6.0	17.3	12.0
(9 hours)	10.0	Flower buds	1	34.0	20	23	13.0	17.0	10.8

*Data for all cases are averages of 4 plants per treatment, except in the two "Daylight" categories where they are the means of 2 plants per treatment.

TABLE 3. Number of days from beginning of treatments to appearance of stems and flowers in *Hyoscyamus*, annual strain, grown in light of restricted spectral regions, and in long and short days in daylight. Experiment 2

Spectral region	Daily GA treatment μg	Number of days to stem appearance	Number of days to flowering
Blue (−)	0.0	15	–
	0.1	15	–
	1.0	15	28
	10.0	13	27
Blue (+)	0.0	15	–
	0.1	15	29
	1.0	15	28
	10.0	13	27
Green	0.0	–	–
	0.1	–	–
	1.0	23	–
	10.0	15	(67)
Red	0.0	–	–
	0.1	25	–
	1.0	23	–
	10.0	15	(34)
Infra-Red + Red	0.0	14	–
	0.1	15	–
	1.0	15	–
	10.0	13	31
Daylight (Long Day)	0.0	14	30
	10.0	11	26
Daylight (9 hours)	0.0	–	–
	10.0	15	–

Note: Experiment terminated on 31st day except for R 10 μg, and G 10 μg plants



PLATES 1A, 1B. Formative and flowering effects of gibberellic acid (GA) on *Hyoscyamus niger*, annual strain, grown in light of restricted spectral regions, in relation to duration of treatment (Experiment 1).

PLATE 1A: After 2 weeks; 2 plants on left: 10 μg GA/day for 2 weeks; 2 plants on right: no GA. Photographed 9-6-'56

PLATE 1B

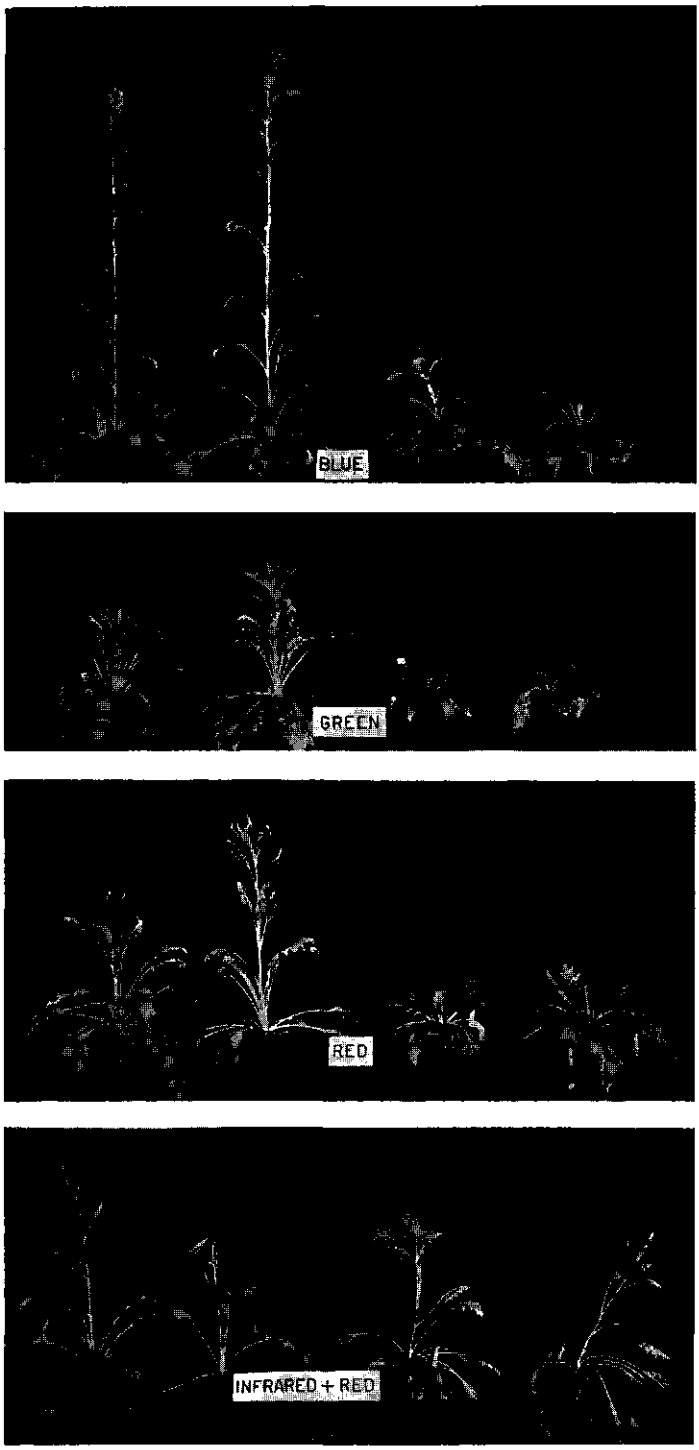
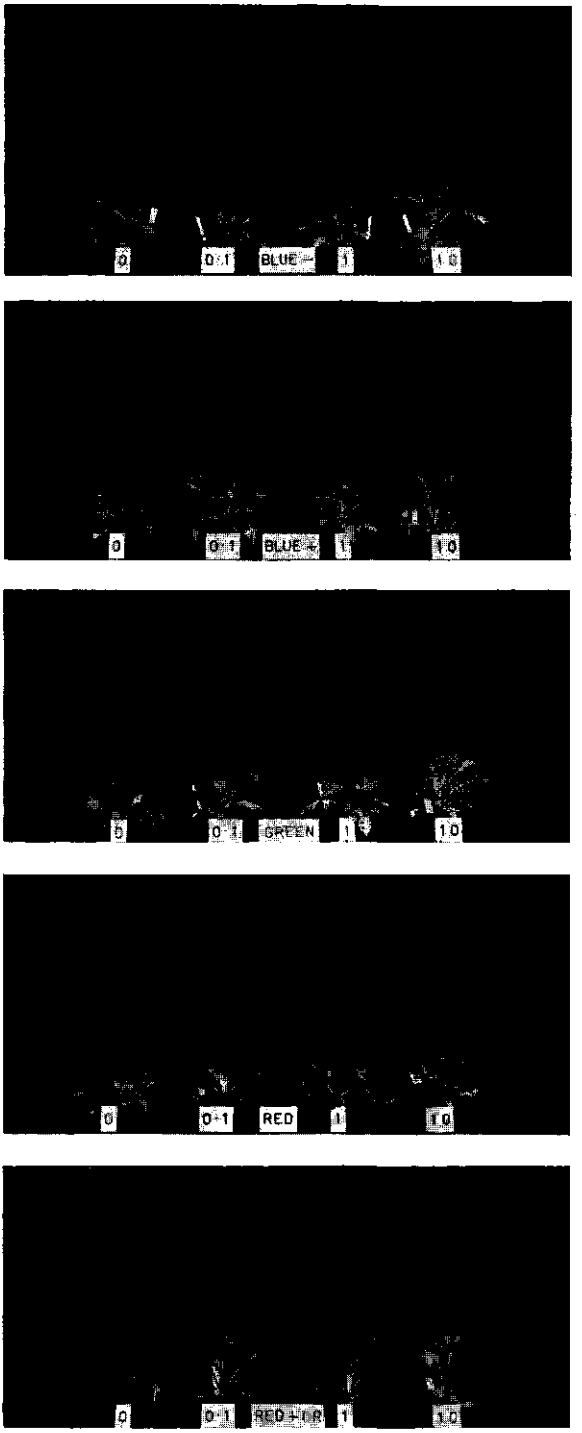


PLATE 1B: After 4 weeks; from left to right: 10 μg GA/day for 2 weeks, 10 μg GA/day for four weeks; 2 plants without GA. Photographed 23-6-'56



PLATES 2A, 2B, 2C. Formative and flowering effects of various concentrations of gibberellic acid (GA) on *Hyoscyamus niger*, annual strain, in light of restricted spectral regions (Experiment 2). Concentrations of GA applied: 0, 0.1, 1, and 10 μg/day/plant

PLATE 2A: After 10 days, photographed 14-8-'56

PLATE 2B

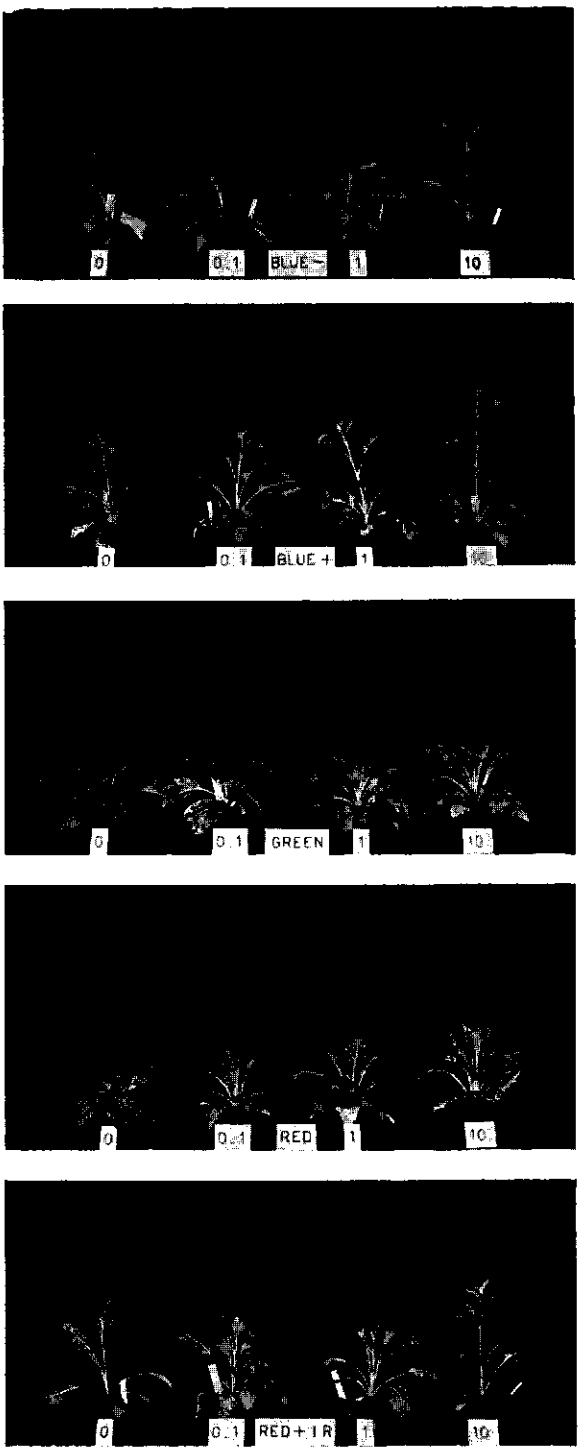


PLATE 2B: After 20 days, photographed 24-8-'56

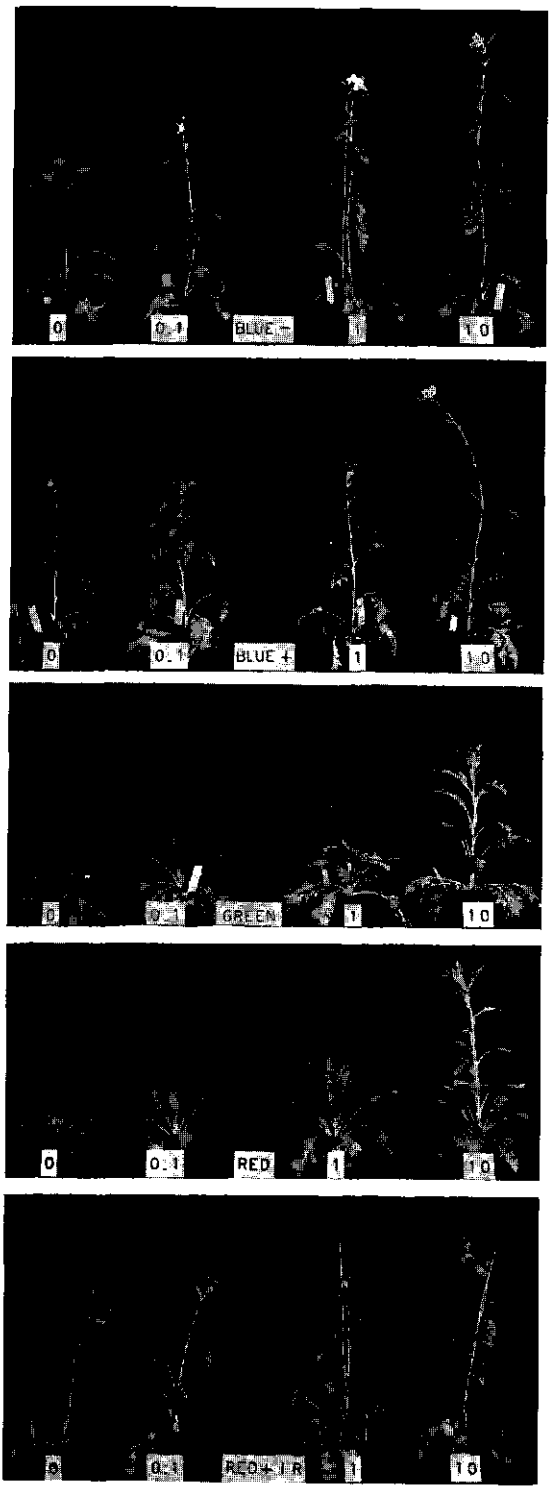


PLATE 2C: After 30 days, photographed 3-9-'56

PLATE 3

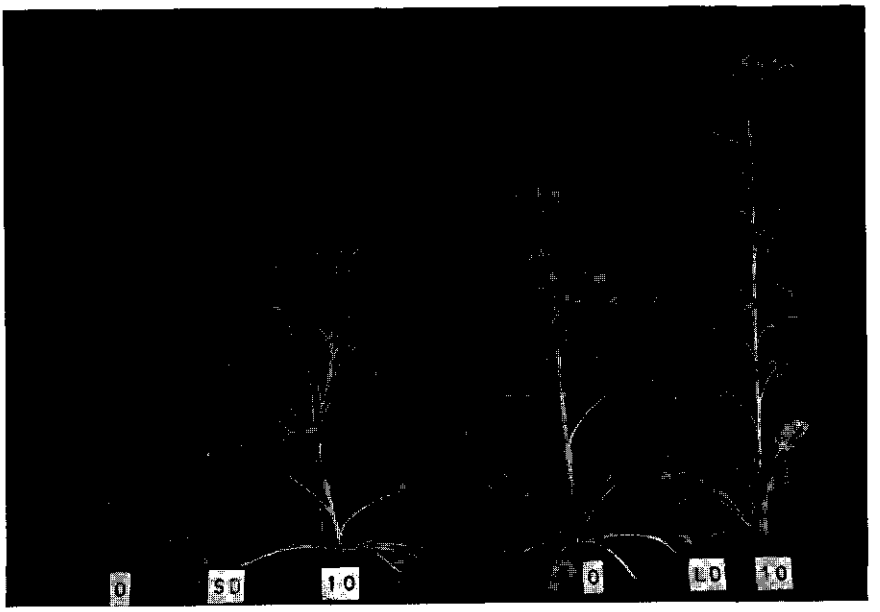


PLATE 3. Formative and flowering effects of gibberellic acid (GA) on *Hyoscyamus niger*, annual strain, in natural daylight in the greenhouse, in long (natural) days and in short days (9 hrs) (Experiment 2). Concentrations of GA applied: 0 and 10 μg/day/plant. Photographed 3-9-'56, after 30 days of treatment