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THE INFLUENCE OF LIGHT QUALITY ON THE FLOWERING RESPONSE OF SALVIA OCCIDENTALIS

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INTRODUCTION

It has been found that an irradiation of low light intensity, supplementarily given to a short light period of high intensity (photoperiod or main light period), has a day lengthening effect on several plants. RASUMOV (1933) found that the red part of the spectrum has the greatest activity in this supplementary light reaction. Similar results were obtained by WITHROW *et al.* (1934, 1936 a and b), WASSINK *et al.* (1950, 1951) and by STOLWIJK (1952, 1954).

The same photoperiodic effect can be achieved when this period of supplementary light of low intensity is replaced by a "night break" i.e. an interruption of the dark period by a short irradiation of high light intensity.

The action spectrum of this night break reaction has been found to be similar for both long day and short day plants (PARKER *et al.*, 1946, 1950; BORTHWICK *et al.*, 1948).

A maximum of activity has been found in the red region of the spectrum with a second, smaller maximum in the blue part. Moreover it has been found by BORTHWICK *et al.* (1952) that the effect of a red light flash can be reversed by an infra-red irradiation.

Not very much is known about the influence of the main light period on photoperiodic effects.

HAMNER (1940) found that already one long dark period induces flowering in *Xanthium pennsylvanicum*, provided there is a light period of high intensity preceding the inducing long dark period. Whereas this light period can be substituted by a sugar treatment, BONNER *et al.* (1953) concluded that this light period is only necessary to produce photosynthates.

DE ZEEUW (1953) obtained flowering of *Perilla*, a short day plant, in 18 hrs light per day and even in continuous light, provided the plants were kept in light of a low intensity.

Only a few indications can be found on the influence of the light quality of the photoperiod on flowering.

ODÉN (1929) states that light of short wave lengths promotes heading of lettuce, whereas light of long wave lengths promotes flowering and fruiting.

KLESHNIN (1950) could confirm this conclusion for lettuce, as well as for some other long day plants and for a short day plant, *Amaranthus retroflexus*.

POPP (1926) found that *Petunia* and *Mirabilis* did not flower in daylight from which the shorter wave lengths were filtered out, whereas flowering occurred in white light of the same intensity.

WALLRABE (1943) working with *Kalanchoë Blossfeldiana*, a short day plant, came to the conclusion that a short day treatment in blue light is more effective than the same treatment in red light. In a short day in green light no flower initiation occurred at all.

STOLWIJK *et al.* (1955) growing *Hyoscyamus niger* in light of different spectral regions, concluded that this plant needs violet, blue or infra-red during the main light period to obtain flowering. A long day however is necessary.

In this paper experiments are described which show the same blue or infra-red necessity for inducing the long day effect on the short day plant *Salvia occidentalis*.

METHODS AND MATERIAL

In the following experiments "white" light and light of three different spectral regions have been used to grow plants: red, green and blue light.

The plant growth chambers in which the experiments were carried out under exclusion of daylight have been described by VAN DER VEEN (1950). The light was obtained by using different coloured fluorescent tubes (Philips) from which the undesired part of the spectrum was filtered out by suitable filters of "Plexiglas" (Röhm & Haas). The "TL"33 fluorescent lamp has been the light source for the experiments in "white" light. Red light was obtained by using a combination of the "TL" lamp no 103647 and the filter "Rot 1" (Fig. 1a.).

The "green" light, emitted by "TL" 17 lamps is purified by using the filter "Gelb 3" which cuts of all radiation with a wave length < 4700 Å (Fig. 1b).

Two different types of blue light were used. For both the light source was the same "TL" fluorescent tube, no. 103648, the filters however were different. One of them "Blau 27 alt" has only a transmission in the blue part of the spectrum, whereas the other one "Blau 27 neu", with a somewhat different transmission characteristic in the blue region, still has a transmission in the infra-red part. The pure blue light without infra-red contamination will be referred to as blue, the impure form (with infra-red) as blue + i.r. (Fig. 1c and d). In some experiments infra-red radiation was added by using incandescent light (100 Watt bulbs) from which the visible part of the spectrum ($\lambda < 7000$ Å) was filtered out by using a combination of the filters "Rot 1" and "Blau 27 neu". Light intensities were measured in $\mu W/cm^2$. The amount of added infra-red is given for the region 7000–8000 Å.

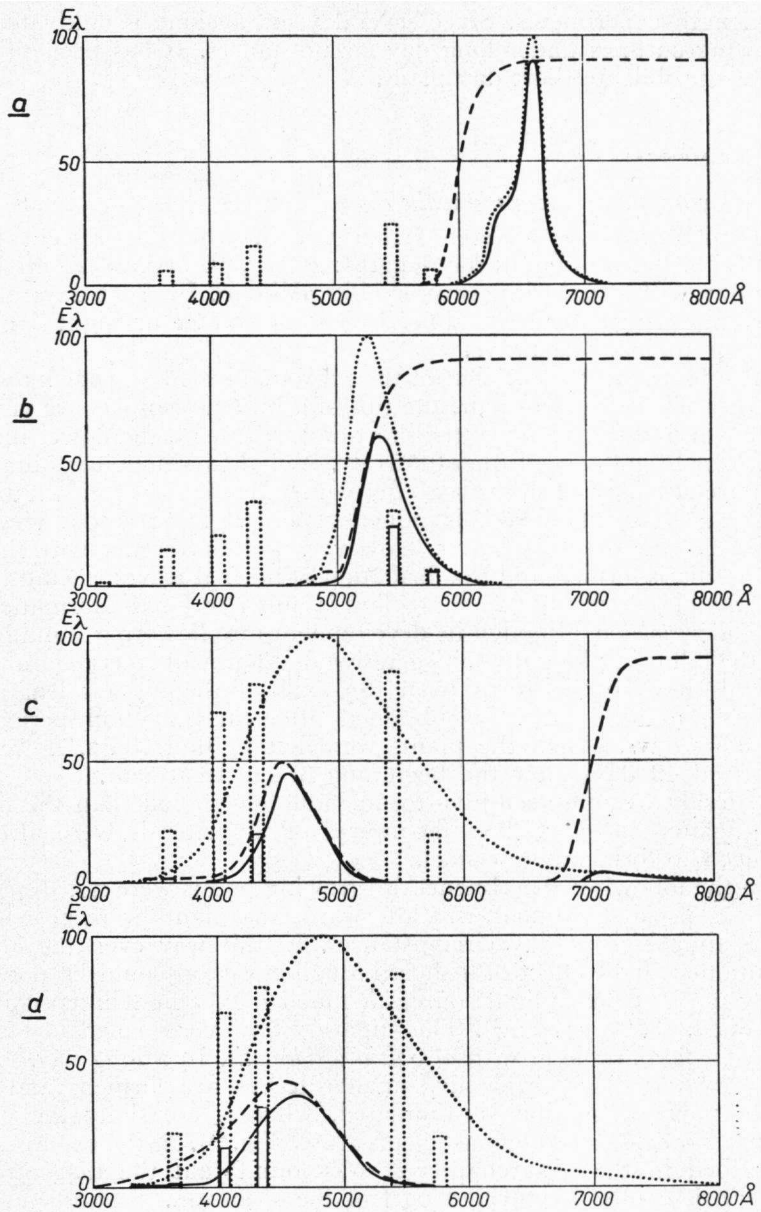

Fig. 1. The relative spectral energy distribution of the filtered light (solid line). a: red light; b: green light; c: blue light + infra-red; d: blue light. The emission curve of the fluorescent tubes is given by the dotted line, the transmission curve of the filters by the broken line.

As far as known *Salvia occidentalis* has never been used before in daylength experiments. Since several years a clone is cultivated by growing cuttings under long day circumstances. It has been proved to be an obligate short day plant.

Experimental data

1. *Day length experiments in white light*

These experiments were carried out in white fluorescent light ("TL" 33) in 3 different day lengths: 10 hrs, 16 hrs and 24 hrs light per day with a light intensity of 1100 $\mu W/cm^2$. In short days plants were flowering after some time. In 16 hrs and 24 hrs light per day plants remained vegetative.

The first macroscopic sign of the reproductive stage appears when instead of a young leaf pair, the tips of a bushel of bracts are visible.

By varying the length of the short day treatment, the flower induction can be influenced quantitatively. A slight induction is obtained by a treatment of 14 short days after which the plants are grown under long day circumstances. This induction period gives rise to a development of some bractlike leaves. However, no inflorescence with flower primordia is formed and the formation of normal leaves is continued. An induction period of 21 short days is sufficient for the formation of the inflorescence, although its development into flowering is inhibited by the following long days. Vegetative development starts again after laterals have been grown from the axils of the highest leaf pair. The same development is obtained after an induction period of 28 short days. When the plants were left in short days, flowering occurred 49 days after the beginning of the treatment.

Laterals are more sensitive to an induction period than the main stem. After 14 short days the apices of the laterals were already induced to form inflorescences.

In the following experiments the growing points were not dissected after a special treatment, but afterwards the plants were grown for some time in long day circumstances. In this way even the above mentioned slight effect of a short induction period could be noticed.

By using stronger light sources a much higher light intensity was obtained. (3500 $\mu W/cm^2$). This intensity however seemed to be too high for favourable growth of *Salvia occidentalis*. In a long day, stems are growing diageotropically, smaller leaves are formed, and the anthocyanin formation is stimulated, whereas especially the older leaves show a marked epinastic curvature (Fig. 2). In the experiments described in the next chapter it was found that the diageotropic growth is caused by the red part of the spectrum. To find out the most favourable light intensity, plants were grown in four different light intensities which were obtained in one plant growth chamber by using gauze filters. The intensities used are 2500, 1250, 850 and 250 $\mu W/cm^2$. The higher the light intensity, the more pronounced are the symptoms described above. It is remarkable that in the lowest light intensity plants entered into the reproductive phase although a long day of 16 hrs light per day was given.

The tips of the bracts appeared 30 days after the beginning of the

Fig. 2. The influence of the light intensity of the main light period on the photoperiodic effect of a long day treatment on *Salvia occidentalis*. From left to right: plants grown in 16 hrs of light per day with a light intensity of 2500, 850 and 250 $\mu W/cm^2$. 39 days after the beginning of the treatment. The plant grown under 250 $\mu W/cm^2$ shows inflorescences.

treatment and after 55 days these plants were flowering. The plants grown in the other intensities remained vegetative throughout this time (Fig. 2).

2. *Influence of light quality of the main light period*

In order to investigate the influence of light of different spectral regions on length growth, plants were grown in red, green, blue and blue + infra-red light.

It has been found that the elongation of internodes of different plants, grown in blue light is stimulated when the infra-red part of the spectrum, emitted by the fluorescent tubes, is not filtered out (Fig. 3).

This stimulation is entirely due to the infra-red contamination and not to differences in the blue region of the spectrum (fig. 1c and d). The same effect was obtained when plants were grown in blue light (fig. 1d) to which infra-red was added.

Next to this effect on elongation the apical dominance is influenced by infra-red irradiation. The development of the laterals which are normally formed is more or less inhibited when grown in blue + infra-red (Fig. 3).

The most remarkable effect however is the flowering response of *Salvia*, grown in a long day (16 hrs light per day) in light of different spectral regions.

Decapitated plants were grown in short day (10 hrs) and long day (16 hrs) during 32 days. The light intensities in quanta were equal. The observations were made on the laterals growing from the axils of the highest leaf pair after decapitation. After the treatment plants were kept in a glasshouse under normal long days. The last observations were made 77 days after the beginning of the treatment. The results are given in Table I. No difference was obtained in response to a short day treatment in red and in blue light. The number of leaves formed up to the inflorescences was the same (5 leaf pairs). Plants treated with long days in blue and blue + infra-red remained vegetative for the whole time.

Although in green light a long day was given, the flowering response of this lot of plants was the same as the plants exposed to short days in blue and red light. The bracts became macroscopically visible after the same time and the same number of leaves up to the inflorescence was formed.

The plants showed about a similar reaction when treated with a long day in red light. Flower formation was obtained but it was somewhat retarded as compared to the other flowering groupes. In red light 8 pairs of leaves were developed before flower initiation occurred.

Already 3 days after the beginning of the treatment in red light leaves are showing a marked epinastic curvature. This curvature was less visible in the newly formed leaves. The size of the last ones is smaller than the size of the comparable leaves from the other light treatments. The diageotropical growth already mentioned above is only found in plants grown in red light. In other experiments light intensities were used as high as possible and by means of gauze filters 4 different light intensities could be obtained. The plants were exposed during 4 weeks to 16 hrs of red, blue and blue + infra-red light per day of different intensities (table 2). After this treatment

TABLE I

The response of *Salvia occidentalis* to a short day and a long day treatment in different spectral regions (equal amounts of quanta). Duration of the treatment 32 days. Day temperature 22° C. Night temperature 17° C.

Spectral region	intensity ($\mu W/cm^2$)	daylength hrs per day	condition of the apex	number of leaf pairs up to the inflorescence
blue	600	10	generative	5
red	370	10	generative	5
blue	600	16	vegetative	> 10
blue + i.r.	600	16	vegetative	> 11
green	500	16	generative	5
red	370	16	generative	8

plants were kept under long day circumstances in white light during another 6 weeks.

As can be seen in Table II even in the lowest blue light intensity used, 90 $\mu W/cm^2$, no flowering occurs. In red light however the lowest light intensity used, 360 $\mu W/cm^2$, gave rise to flower initiation.

Table II

The flowering response of *Salvia occidentalis* to a long day treatment in different intensities of blue, blue + i.r. and red light. Duration of the treatment 28 days. Day temperature 22° C. Night temperature 17° C.

Spectral region	Condition of the growing point — light intensity in $\mu W/cm^2$											
16 hrs. day	75	90	250	300	360	375	420	750	880	1200	1800	3600
blue		veg.		veg.			veg.		veg.			
blue + i.r.	veg.		veg.			veg.		veg.				
red					gen.					veg.	veg.	veg.

In the higher intensities of red light, the plants remained vegetative. Representative plants grown in light of an intensity of 900 $\mu W/cm^2$ are shown in Fig. 3.

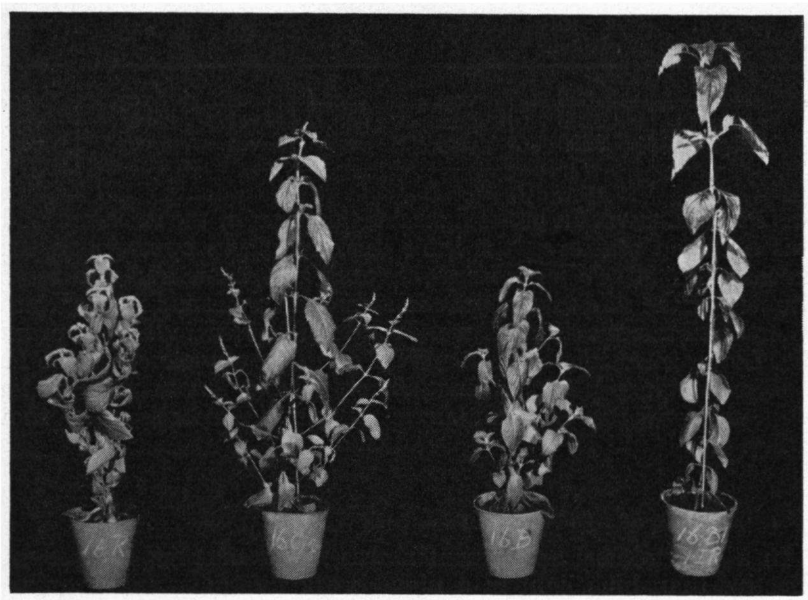

Fig. 3. The response of *Salvia occidentalis* to a long day treatment in light of different spectral regions. From left to right: plants grown in red, green, blue and blue + infra-red light, 16 hrs per day, 900 $\mu W/cm^2$, 56 days after the beginning of the treatment.

STOLWIJK *et al.* (1955) found that infra-red added to red light has a great effect on the flowering response of *Hyoscyamus niger*. In the following experiment infra-red was simultaneously added to red, green and blue light during 16 hrs per day. 3 or 4 different intensities of infra-red were used by treating the plants at different distances of the infra-red source.

In Table III the results are given of plants which were exposed during 4 weeks to this light treatment. At the end of the experiment the plants were kept another 5 weeks in long days in white light.

The intensity of the red and blue light was too high for flower initiation. Therefore an infra-red addition could not have any effect. Whereas this experiment was carried out simultaneously with the preceding one in which this intensity dependence was investigated, the long day effect of the red light intensity used in this experiment

TABLE III

The influence of the addition of infra-red on the day length effect of a long day treatment in blue, green and red light on flower initiation of *Salvia occidentalis*. Duration of the treatment 28 days. Day temperature 22° C. Night temperature 17° C. Day length: 16 hrs of light per day.

Condition of the growing point						
16 hrs per day		Intensity of infra-red, added simultaneously ($\mu W/cm^2$)				
Spectral region	intensity $\mu W/cm^2$	0	25	30	40	60
blue	880	veg.	veg.	veg.	veg.	veg.
green	1200	gen.	gen.	veg.	veg.	veg.
red	3600	veg.	veg.	veg.	veg.	veg.

was still unknown. In green light a clear effect of the infra-red added was obtained and even the limit of activity was approached. Irradiated with 30 $\mu W/cm^2$ infra-red the main stem and the laterals situated at the side towards the infra-red source remained vegetative, whereas all laterals situated at the shadowside initiated flower primordia. Lower intensities of infra-red did not have any effect (Table III).

Representative plants of another experiment grown in green light with and without an addition of infra-red irradiation are shown in Fig. 4.

3. *Influence of light quality on the night break effect*

In a preleminary experiment it was found that red light is more effective than blue light in a night break treatment. The results of another experiment are presented in Table IV. The plants were exposed to a short day, 10 hrs of light per day, in white fluorescent light (1500 $\mu W/cm^2$). In the middle of the dark period groups of plants were exposed to blue or red light. The total amount of light was varied by using different exposure times, which did not exceed 10 minutes. It was also found that the effect of a dark interruption by red light can be reversed by an infra-red irradiation (Table IV).

Fig. 4. The influence of an addition of infra-red on the day length effect of *Salvia occidentalis*, grown in 16 hrs green light per day. From left to right: plants grown in green light (900 μW/cm²) without infra-red (generative) and with 25 (generative), 40 (vegetative) or 60 μW/cm² infra-red (vegetative). 60 days after the beginning of the treatment.

Discussion

Until now it has been shown that only *Kalanchoe* (Wallrabe, 1943) and *Hyoscyamus niger* (Stolwijk *et al.*, 1955) have a special spectral requirement concerning the influence of the main light period on flowering.

The results presented in this paper make it obvious that *Salvia occidentalis*, a short day plant, has a similar spectral requirement as the long day plant *Hyoscyamus*.

In earlier experiments, Van der Veen could confirm Odén's statement, when growing lettuce in unfiltered neon and blue fluorescent light ("TL" 10). The difference was a quantitative one, the red light plants were flowering about two months earlier than the blue light plants.

Trifolium pratense showed the same reaction. This plant started bolting almost immediately when brought into a long day in neon light. Flowering occurred after 59 days. In blue light of the same intensity (1000 μW/cm²) plants were poorly flowering after 98 days.

From these experiments and from all the earlier work referred to in the introduction it is not possible to conclude whether there is

TABLE IV

Day lengthening effect of an interruption of the long dark period with red and blue light. Main light period 10 hrs white light per day (1500 $\mu W/cm^2$). Duration of the treatment 28 days. Day temperature 22° C. Night temperature 17° C. Observation 49 days after the beginning of the treatment. Number of plants 3.

Quantity of the light, interrupting the dark period. $\mu W/cm^2 \times sec.$		Number of leaf pairs formed from the beginning of the treatment						% of laterals in vegetative condition
		generative plants			vegetative plants			
blue	27000	5	5	5				0
	54000	5	5	5				0
	108000	6	5	5				0
	216000	5	6	6				0
	432000	5	5	6				0
red	24000	7	6	7				0
	48000	6	9		> 9			50
	96000	5			> 10	> 10		50
	192000	7			> 9	> 8		60
red	384000 followed by							
infra-red	33600	5	5	5				0
control		5	5	4				0

an influence on flower initiation or only on the development of the flower primordia which might have been initiated in red light as well as in blue light. Moreover one cannot conclude which part of the spectrum has a special effect, because the light used was contaminated by light of other spectral regions. As was shown in the experiments described here a small contamination of infra-red, which is transmitted by the blue filter, may have a very strong elongating effect on plants, growing in blue light.

Also an addition of small amounts of infra-red light to 16 hrs of green light causes a long day effect on *Salvia*, which effect was not obtained in a long day in green light without this infra-red addition.

Whether WALLRABE (1943) working with *Kalanchoe* has used "pure" light or not is questionable as no characteristics of the filters in the infra-red region of the spectrum were given. At least the red light must have contained a contamination of infra-red radiation.

STOLWIJK *et al.* (1955) concludes from Wallrabe's experiments that *Kalanchoë Blossfeldiana* has the same spectral requirement as *Hyoscyamus niger*, concerning the spectral activity of the photoperiod on flowering. Blue light was more promotive for flower initiation than red light, whereas green light did not induce flowering at all. However this conclusion is not correct.

In Wallrabe's *Kalanchoe* experiments a short day treatment was given, i.e. a daylength favourable for flower induction in this short day plant. In this case it was shown that a short day in red light is less effective in flower initiation than a short day in blue light;

whereas a short day in green light did not result in a short day effect. But the photoperiodic effect of a long day treatment in red, green and blue light on flowering has not been studied.

In the *Salvia* experiments however, a long day treatment was given, i.e. a daylength unfavourable for flower induction in this short day plant. For this plant it was shown that a long day in red is less effective in inhibiting flower initiation than a long day in blue light. Moreover a long day in green light did not have any long day effect at all, fòr flower formation was not inhibited.

This holds true for *Hyoscyamus niger* as well but, as this plant is a long day plant, flowering is initiated or is inhibited, when in *Salvia* flower initiation is inhibited, or is induced respectively.

Whether the "long day effect" of a long day treatment in red light of high intensity is due to the red light itself or to the unavoidable contamination of infra-red (in our experiments $\pm$ 2 % of the red radiation) is not sure.

However, it is not impossible that the action spectrum of the main light period will show some activity in the red part of the spectrum as well.

In the short day experiments an equal effect was obtained in red and in blue light.

The results of the night break experiments — red light being more effective than blue light — are in agreement with the action spectrum which has been thouroughly investigated by BORTHWICK and coworkers.

Examining a few other plant species (*Plantago major*, *Petunia hybr. Violacea*, *Arabidopsis thaliana*) we found clear indications that these plants too have the same spectral sensitivity for the main light period as *Salvia* and *Hyoscyamus*. It seems probable that this spectral requirement is a rather common phenomenon.

CONCLUSION

The results with *Salvia occidentalis* described above are in agreement with the response of *Hyoscyamus niger* described by STOLWIJK *et al.* (1955).

For *Hyoscyamus* and *Salvia* it is obvious that the photoperiodic effect is determined by at least two different photoreactions which have a completely different spectral sensitivity.

One of them, the reaction of the so called main light period, has the greatest activity in blue and infra-red.

In the other reaction, the so called night break, the red part of the spectrum is photoperiodically much more effective than light of the blue region. A red- infra-red antagonism exists in this night break reaction.

The statement of BONNER *et al.* (1953) that the main light period is only necessary for the production of photosynthates does not hold true for the plant species, mentioned above.

SUMMARY

1. *Salvia occidentalis* is an obligate short day plant.
2. However, flower initiation may occur after a long day treatment, depending on light intensity and the colour of the main light period.
3. In *Salvia occidentalis* as in *Hyoscyamus niger* the effect of a long day treatment is influenced by at least 2 different photoreactions.

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