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## The Effect of Daylength on the Hematin Content of the Leaves of Some Photoperiodically Sensitive Plants

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(Received December 7, 1961)

### Introduction

A ratio of about 60 molecules of total chlorophyll per l. of hematin has been found in mature leaves of several plants (Davenport 1958, Sironval 1958, DeKock *et al.* 1960).

DeKock *et al.* have shown for cultures of mustard plants that different iron, potassium, calcium, phosphorus and nitrogen levels, do not alter the constancy of this ratio. For some day-neutral species, the chlorophyll/hematin ratio appears also to be the same in long-days (16 hr) as in short-days (8 hr). It remains close to 60 in the two daylength conditions (Sironval 1958).

However hemp, a typical short-day plant, is an exception to the general rule. The transfer of hemp plants from long to short-days, induces a modification of the chlorophyll/hematin ratio. The ratio drops from its normal value of 60 in long-days to 30 in short-days. At the same time, flower induction occurs in short-days.

A preliminary report on hemp was previously published (Sironval 1958). We will present here some details of the behaviour of hemp and compare it with three other photoperiodic sensitive plants: two long-day plants (*Salvia splendens* Sello., var. Feu de la Saint Jean, and *Sinapis alba* L.), and a short-day plant (*Perilla nankinensis* Voss).

### Material and Methods

The variety of hemp (*Cannabis sativa* L.) used in the experiments is an Indian variety. When grown in long days, it does not flower until the 8th node in the males and not until the 10th node in the females. Short day treatment induces

immediate flower formation in very young plants. Twenty to thirty day old plants are induced to flower by about 8 short-days. Immediately after flower induction, the transfer back to long day produces a reversal to vegetative growth.

The photoperiodic behaviour of *Salvia splendens* Sello, var. Feu de la Saint Jean, has been studied by Crawford (1961). Flowering of this variety cannot occur when the daylength is under 7 hrs. In 8 hr-days, flower formation is strongly delayed. 80 day old plants grown in short-days are immediately induced to flower by some 16 hr-days.

*Perilla nankinensis* is the well known short-day plant. It does not flower at all in 16 hr-days, but it is induced by some 8 hr-days given at any time during its development in long-days.

The strain of *Sinapis alba* L. used in these experiments shows a typical long-day reaction. It does not flower at all in 8 hr-days, but in 16 hr-days flower induction takes place 14 days after germination (Bronchart 1957, Bernier 1961). The transfer of 20-30 day old plants from long- to short-day stops the formation of flowers. In short-days, the vegetative growth proceeds again.

All the plants, except hemp, have been grown in growth cabinets at 20°C under fluorescent lamps of the "Phytor" type providing about 5,000 lux to the upper leaves. Daylength was regulated by automatic switching on and off of the lights. Hemp was grown in an ordinary greenhouse in May-June-July. Short days were obtained by putting the plants in dark cabinets at the greenhouse temperature.

Total hematin was measured in the leaves by the method of Hill and Scarisbrick (1951). Total chlorophyll (a + b) was measured with the DU Beckman spectrophotometer in 80 % acetone using the formula of Mackinney (1941).

## Results

### 1. *Cannabis sativa* L.

Seeds are sown in 16 hr-days and in 8 hr-days. Figure 1 shows that in the cotyledons the total chlorophyll accumulates in greater amounts in long-days than in short. However the content in hematin does not differ significantly in the two series. It drops during the growth of the cotyledons and reaches a low (nearly constant) level after 6 days. Six days after sowing, the molecular ratio chlorophyll/hematin is 108 in short-days, against 167 in long-days.

In the leaves the ratio chlorophyll/hematin is lower than in the cotyledons. We shall restrict our description to adult fully expanded leaves.

Hemp plants 24 day old grown in 16 hr-days are transferred to 8 hr-days. Nineteen and twenty days later (after flower induction), the second leaf is analysed and compared with the second leaf of vegetative control plants in 16 hr-days. It is seen in Table 1 (data of June 17th and 18th; Experiment A) that the second leaf of the induced hemp has much less chlorophyll than the second leaf of the vegetative control. But the content in hematin is higher: there is 30 % more hematin in short-days than in long. The molecular chlorophyll/hematin ratio is about 30 for the induced hemp against 60 for the vegetative control.

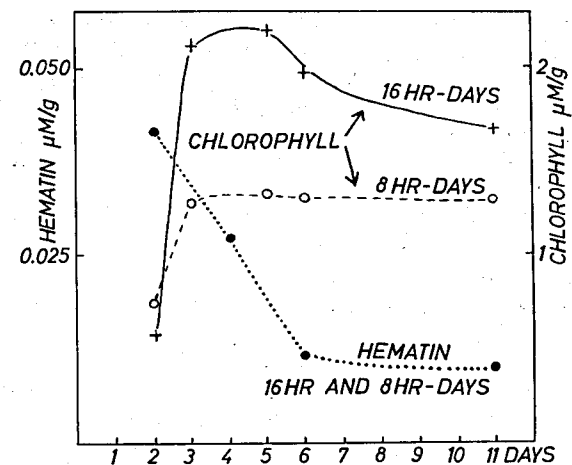


Figure 1. Evolution of the chlorophyll and hematin contents of hemp cotyledons grown in short and long days.

Table 1. Effect of daylength on total chlorophyll and hematin contents in the 2nd and 4th leaves of *Cannabis sativa*. Values expressed in µmol/g. fresh weight.

Experiment A (43 day old plants; second leaf)	June 17th			June 18th		
	Hematin	Chlorophyll	Ch/H	Hematin	Chlorophyll	Ch/H
Induced plants in 8 hr-days (19 and 20 short-days) ..	0.089	2.65	30	0.085	2.68	38
Vegetative control plants in 16 hr-days .....	0.069	4.08	59	0.064	4.10	64
Experiment B (50 day old plants; fourth leaf)	June 24th					
	Hematin	Chlorophyll	Ch/H			
Induced plants in 8 hr-days (26 short-days) .....	0.086	2.91	34			
Vegetative control plants in 16 hr-days .....	0.065	3.76	58			
Plants transferred back to 16 hr-days after induction in 8 hr-days (14 short-days followed by 12 long-days)	0.077	4.58	60			
Experiment C (56 day old plants; fourth leaf)	June 30th					
	Hematin	Chlorophyll	Ch/H			
Vegetative control plants in 16 hr-days .....	0.065	3.82	59			
Plants transferred to 8 hr- days on June 25th .....	0.085	3.28	37			

The effect is completely reversible. After 14 short-days induced plants are transferred back to long-days. Flower induction ceases and a reversal to vegetative growth occurs. At the same time, the chlorophyll content of the leaves increases to very high values and the hematin content falls. The chlorophyll/hematin ratio goes back to its normal value in long days: 60 molecules of chlorophyll per 1 of hematin (see the data of June 24th for the 4th leaf on Table 1; Experiment B).

The modification of the chlorophyll/hematin ratio needs only a few days. On June 25th some vegetative plants grown in long-days, are transferred to short-days. The content of the 4th leaf is measured 5 days later on June 30th. The data of table 1 (June 30th; Experiment C) show that 5 short-days have been sufficient to modify the content in chlorophyll and hematin of the 4th leaf. In that time the ratio chlorophyll/hematin has decreased to the short-day value of 37.

In conclusion, short-day induction of hemp flowering corresponds to a rapid increase of the total hematin content of the leaves and to a simultaneous decrease of the total chlorophyll. The reversal of induction by long-days corresponds to the reverse process.

## 2. *Salvia splendens* Sello., var. *Feu de la Saint-Jean*

Plants are grown from seed in 8 hr-days. At the age of 80 days, some of them are transferred to 16 hr-days. About 10 days later, flower induction takes place in long days. At that time, leaves of corresponding ages are analysed in short and in long days.

The chlorophyll and hematin contents are given in Table 2. The chlorophyll content of young and adult leaves decreases after the transfer to long days. Simultaneously the hematin content increases, while the chlorophyll/hematin ratio decreases.

The process is similar to that observed in hemp. In both cases the inductive daylength (short-days for hemp; long-days for *Salvia*) increases the hematin content of the leaves and decreases the chlorophyll content. However there is an evident discrepancy between the absolute values of the

Table 2. Effect of the transfer of 80 day old *Salvia splendens* plants from 8 hr- to 16 hr-days (10 long-days) on the total chlorophyll and hematin contents of the leaves. Mean of 4 repetitions; values expressed in  $\mu\text{mol/g}$ . fresh weight.

Series	Expanding leaf			Fully expanded leaf		
	Hematin	Chlorophyll	Ch/H	Hematin	Chlorophyll	Ch/H
Control in 8 hr-days (= non-induced plants) .....	0.026	3.32	127	0.026	4.31	166
Plants transferred from 8 hr- to 16 hr-days (10 long-days = induced plants) ...	0.032	2.89	90	0.034	3.34	98
Difference: (induced)-(non-induced) .....	+ 0.006**	- 0.43*	-	+ 0.008**	- 0.97**	-

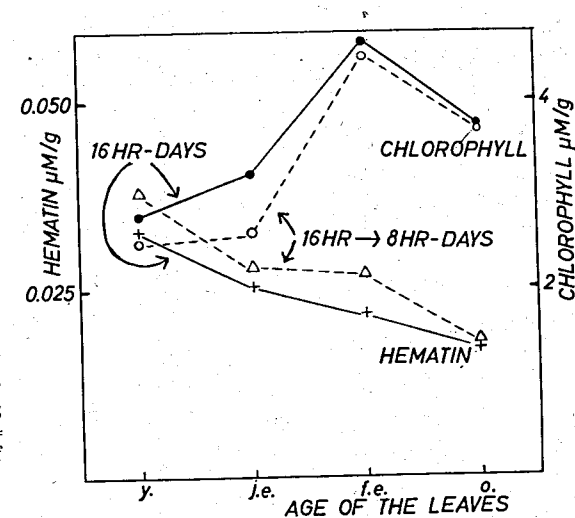


Figure 2. Effect of the transfer from 16 hr- to 8 hr-days on the chlorophyll and hematin contents of the leaves of *Perilla* plants (y=young leaf no. 1; j.e. = just expanded leaf no. 3; f.e. = fully expanded leaf no. 5; o=old leaf no. 8; see the text).

chlorophyll/hematin ratio found in hemp and those found in *Salvia*. This could be due to differences in the light given: natural day for hemp, artificial fluorescent light (about 5,000 lux) for *Salvia*.

## 3. *Perilla nankinensis* Voss.

Vegetative plants are grown in 16 hr-day for 100 days. After that time, some of them are transferred to 8 hr-days. The content of the leaves is measured 8 days later. Counting from the top the following leaves are analysed: leaf no. 1 (young expanded leaf), no. 3 (just expanded leaf), no. 5 (fully expanded leaf) and no. 8 (old leaf).

Figure 2 shows that the short-day treatment has no effect on the old leaf, no. 8. The leaves no. 1, 3, and 5 show in the inductive 8 hr-day, a decrease of the chlorophyll content and an increase of the hematin content. The decrease in chlorophyll represents 5 to 12 % of the initial content in long-days; the increase in hematin, 10 to 12 % of the initial content. In leaf no. 1, the ratio chlorophyll/hematin goes from 85 in long-days to 66 in short-days; in leaf no. 3, it goes from 124 to 94; in leaf no. 5, from 202 to 165.

It is clear that the process observed in *Perilla* shows fundamentally the same pattern as that already found in hemp and in *Salvia*.

## 4. *Sinapis alba* L.

Plants are grown from seed in 16 hr-days for a period of 10 days. Just before flower induction, some are transferred from long-days to short. Forty days later, the plants in 16 hr-days are in full bloom, but the plants transferred to 8 hr-days are fully vegetative. At that time a corresponding adult leaf is analysed in the two series.

Table 3. Effect of the transfer from 16 hr- to 8 hr-days on the total contents of hematin and chlorophyll in the leaves of *Sinapis alba*. Values expressed in  $\mu\text{mol/g}$ . fresh weight.

	Hematin	Chlorophyll	Ch/H
Experiment A (leaf no. 8; 50 day old plants)			
Flowering plants in 16 hr-days .....	0.038	2.55	67
Vegetative plants transferred from 16 hr- to 8 hr-days (10 long-days followed by 40 short-days) .....	0.016	1.92	120
Experiment B <sub>1</sub> (leaf no. 4; 25 day old plants)			
Induced plants grown in 16 hr-days .....	0.034	2.62	78
Non-induced plants grown in 8 hr-days .....	0.016	1.76	110
Experiment B <sub>2</sub> (leaf no. 4; 32 day old plants)			
Induced plants grown in 16 hr-days .....	0.043	3.58	83
Plants transferred from 16 hr- to 8 hr-days (25 long-days followed by 7 short-days; the induction is stopped in short-days) .....	0.015	2.59	173

As shown in Table 3, experiment A, the hematin content in 8 hr-days is only 42 % of the content in 16 hr-days. The chlorophyll content reaches in short-days 75 % of its value in long-days. As a result of this, the chlorophyll/hematin ratio is 67 in long-days, (flowering plants) against 120 in short-days (vegetative plants).

The short-day content can be induced rapidly as is shown by the following experiment (B<sub>1</sub> and B<sub>2</sub> in Table 3).

Two series are grown in 8 hr and in 16 hr-days. When they are 25 days old (at that time flower induction occurs in long-days), corresponding leaves are analysed in the two series. In short-day, the hematin content is found to be 47 % of its value in long-days, and the chlorophyll content is 67 % of its value in long-days (B<sub>1</sub>, Table 3). The chlorophyll/hematin ratio is 78 in long against 110 in short-days. At the time of the B<sub>1</sub> analyses, some plants grown in long-days are transferred to short day. Seven days later, a new analysis is carried out on the leaf already studied in B<sub>1</sub> (B<sub>2</sub>, Table 3). During these seven days, the hematin content drops from 0.034  $\mu\text{mol/g}$ . fresh weight in long-days (B<sub>1</sub>) to 0.015  $\mu\text{mol/g}$ . fresh weight in short days (B<sub>2</sub>). The chlorophyll content remains constant; it starts from 2.62  $\mu\text{mol/g}$ . fresh weight in long-day (B<sub>1</sub>) to the almost identical value of 2.59  $\mu\text{mol/g}$ . fresh weight (B<sub>2</sub>). Compared with the control remaining in long-days (B<sub>2</sub>), the content in hematin after seven short-days is about 35 % of the control and the content in chlorophyll about 72 %. Seven short days are sufficient to move the chlorophyll/hematin ratio from 78 in long to 173 in short-days. Simultaneously, the induction of flower formation is stopped in short-days.

A process opposite to that described above occurs when *Sinapis* plants grown in short-days are transferred to long-days (from the non-inductive to the inductive day-length). Plants are grown in 8 hr-days during 48 days. At that time, the content of the leaf no. 4 is measured. The leaf shows both

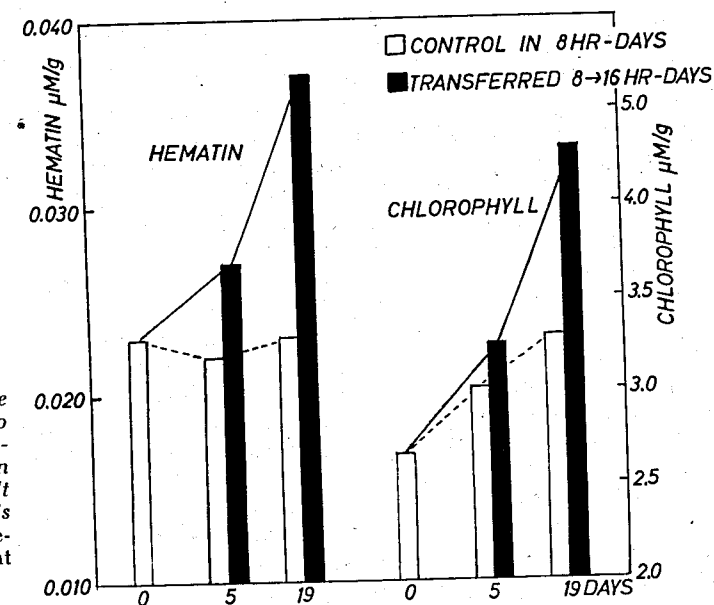


Figure 3. Effect of the transfer from 8 hr- to 16 hr-days on the chlorophyll and hematin contents of the adult leaf no. 4 of *Sinapis alba* plants (see the details of the experiment in the text).

a low hematin and a low chlorophyll content, the molecular chlorophyll/hematin ratio being 117. On the 49th day, one part of the plants is transferred from short-days to long. Five and nineteen days later, the content in hematin and chlorophyll is measured again. Figure 3 shows the results. In long-days the hematin content increases markedly; it remains constant in the control leaf in short-days. The chlorophyll content increases very strongly in long-days; it increases also in the control leaf in short-days, but the increase is less than in long-days. In the control in short-days, the chlorophyll/hematin ratio goes up from 117 to 144, 19 days later. In the series transferred to long-days, the ratio remains constant. However, it does not fall owing to the very strong increase of the chlorophyll content after the transfer from short- to long-days. This very strong increase reflects what we have called a "transfer-effect" (Sironval *et al.* 1961) (cf. also Table 1, B). The normal chlorophyll content of the leaves of *Sinapis* in long-days is much lower than the figures found after the transfer from short- to long-days. In fact, 40 days after the transfer (at that time the plants transferred to long-day have built their flower primordia) the adult leaves in long-days have 3 to 3.5  $\mu\text{mol}$  total chlorophyll/g. fresh weight, the hematin content being 0.035 to 0.040  $\mu\text{mol}$  hematin/g. fresh weight. This corresponds to the chlorophyll/hematin ratio of about 80 normally found in *Sinapis* plants grown in long-days from the seed.

Therefore, if we neglect the "transfer-effect", *Sinapis* behaves fundamentally like hemp, *Salvia*, and *Perilla*. In the inductive long-day conditions, it shows a high level of hematin correlated to a chlorophyll/hematin ratio of about 80. In the non-inductive short-day conditions, it shows a low level of hematin correlated to a higher chlorophyll/hematin ratio.

### Discussion

In the figures presented, the value of the chlorophyll/hematin ratio, differs in many cases from the 60 molecules of chlorophyll per l. of hematin previously found in adult leaves of some plants. In our working conditions, — especially under artificial fluorescent lamps at 20°C constant —, we could take ratios between 60 to 90 to be "normal" for adult leaves. Thereby the figures nearest the "normal" are found in hemp in long-days; in Sinapis, in long-days; in Salvia, in long-days; in Perilla, in short-days. The chlorophyll/hematin ratio is under the "normal" in hemp in short-days. On the contrary, Salvia in short-days, Perilla in long-days and Sinapis in short-days show high values of the ratio. In each cases the ratio found in one daylength differs from that found in the other. This could be the general behaviour of the photoperiodically sensitive plants (contrary to that of the day-neutral plants, whose chlorophyll/hematin ratio is the same in short- and in long-day).

The variations of the chlorophyll/hematin ratio are due to distinct processes:

a) — In hemp, Salvia and Perilla, the hematin and chlorophyll contents of the leaves do not move parallelly when the plants are transferred from one daylength to another. Hematin increases when chlorophyll decreases, or hematin decreases when chlorophyll increases. This behaviour is the contrary of that generally found in plants (specially in day-neutral plants) where chlorophyll and hematin increase or decrease in the same time and in the same proportion when passing from one daylength to another.

b) — In Sinapis, the hematin content and the chlorophyll content increase or decrease at the same time, but not in the same proportion. (We have unpublished indications that this situation occurs in some long-day plants.)

It appears therefore that for photoperiodically sensitive plants, the relations between hematin and chlorophyll are not simple and depend upon the species under study.

However our results are consistent on the following point: we find in each experiment (except for hemp cotyledons) more leaf-hematin in the inductive daylength than in the non-inductive daylength, whatever the photoperiodic properties of the studied species. This situation results in a lower molecular chlorophyll/hematin ratio under induction. It appears from our data that the observed hematin increase is highest for Sinapis (the hematin content of Sinapis leaves is doubled under induction!) and lowest in Perilla (the hematin content of Perilla leaves increases about 10 % under induction!). We conclude that for the studied species *the biochemical pathways of synthesis of tetrapyrrolic-pigments rearrange under induction in such a way that the hematin content of the leaves definitely increases.*

Many authors report a variation of the chlorophyll content of the leaves in relation to the applied daylength (Kar 1937, Withrow and Withrow 1949, Cheuvar 1954, Chailahjan *et al.* 1957, Sironval 1957 a and b, Enloe 1959, Von Witch 1959, Friend 1961). If this effect is of a photoperiodic nature, is still controversial (see Sironval 1957 b, Friend 1961, Mitrakos 1961). Virgin (1961 a) suggests that the daily changes in chlorophyll formation in the young growing leaf "may have some bearings on the photoperiodic response

of different plants". Sironval (1957 a, p. 212–213) has discussed a similar hypothesis. The action spectrum for the elimination of the lag phase in chlorophyll formation is shown by Virgin (1961 b) to be essentially the same as those previously reported for other photomorphogenetic phenomena. Some reversibility by far-red light is found in several cases (Wolff *et al.* 1957, Enloe 1959, Virgin 1961 b). This could support the assumption that some mechanism on the way of chlorophyll formation, could be coupled to the red- and far-red light absorbing pigment system.

The effect of daylength on chlorophyll accumulation in the leaves is most evident in the young growing leaf. It is less marked, but still existing in the adult leaf (just expanded). It is almost negligible in the old leaf. In fact, the turnover of chlorophyll is many times greater in the young leaf than in the old one. (See Sironval *et al.* 1961.) The present paper shows that *parallel to the action of daylength on chlorophyll accumulation, there is another action on hematin accumulation.* This speaks for a generalised effect of daylength on the metabolism of tetrapyrrolic compounds. An effect of this kind has been suspected by Bünning (1959) who writes: "Of course, chlorophyll itself is not the lightabsorbing pigment in photoperiodism. But what about those substances which must increase or decrease in quantity along with the changes in chlorophyll content?".

### Summary

The experiments show that in some photoperiodically sensitive plants the induction of flower formation by proper daylength is related to an increase of the hematin content of the leaves. The effect is well marked in hemp (SDP) and in Sinapis alba (LDP). The modification of the hematin content is correlated to a modification of the chlorophyll content. This speaks for a generalised action of daylength on the metabolism of tetrapyrrolic compounds.

The author is greatly indebted to the "Institut pour l'Encouragement de la Recherche Scientifique appliquée à l'Industrie et à l'Agriculture; IRSIA", Belgium, for financial aid.

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## Bicarbonate Utilization by Marine Phytoplankton in Photosynthesis

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### Introduction

Although molecular CO<sub>2</sub> has long been considered a primary source of carbon for photosynthesis, recent studies of CO<sub>2</sub> system in sea water have strongly indicated that another form of CO<sub>2</sub> is the preferred source of carbon for photosynthesis in certain marine algae.

Arens (1933, 1936) observed that bicarbonate ions are taken up by the lower surfaces of leaves of higher plants and that carbonates are set free in the upper surface. Steeman Nielsen (1952) substantiated these results and presented data showing the rate of CO<sub>2</sub> assimilation from bicarbonate ion as the carbon source. Furthermore, evidence by Österlind (1951) indicates that bicarbonate ion absorption is an active process in *Scenedesmus quadricauda* under certain conditions.

Recently, Smith *et al.* (1960) reported that cultures of *Platymonas sp.*, *Nitzschia closterium*, and *Porphyridium cruentum* utilize the carbamino complex of alanine or sea water concentrates in preference to inorganic forms of components of the CO<sub>2</sub> system.

In a typical surface sea water sample having a pH value of 8.2, a chlorinity of 19 ‰ and a temperature of 20°C there are approximately 2 millimole/liter of the CO<sub>2</sub> system components (Harvey 1955). Bicarbonate ion is the most predominant, with a per cent fraction of approximately 86.4; whereas the molar fractions of molecular CO<sub>2</sub>, undissociated carbonic acid, and carbonate ion are 0.5 and 13.1, respectively (Saruhashi 1955). Faurholt (1924), Brinkman *et al.* (1933), and Himmelblau and Babb (1958) have reported the ratio of carbonic acid to molecular CO<sub>2</sub> in aqueous solution to be about