Responses of free running leaf movements to light, in particular to red and far red light during sunrise and sunset

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Summary. Daily leaf movements were compared with the intensities and proportions of red and far red light during sunrise and sunset. A linear relationship exists between the inverse of the $\frac{1}{3}$ power of the minimum leaf movement against the elapsed time for its occurrence since either sunrise or sunset. A minimum of the ratio of the intensities of red to far red light at 6 min after sunrise and 16 min before sunset are suggested to be related to minimal levels of far red phytochrome and of ATP activity.

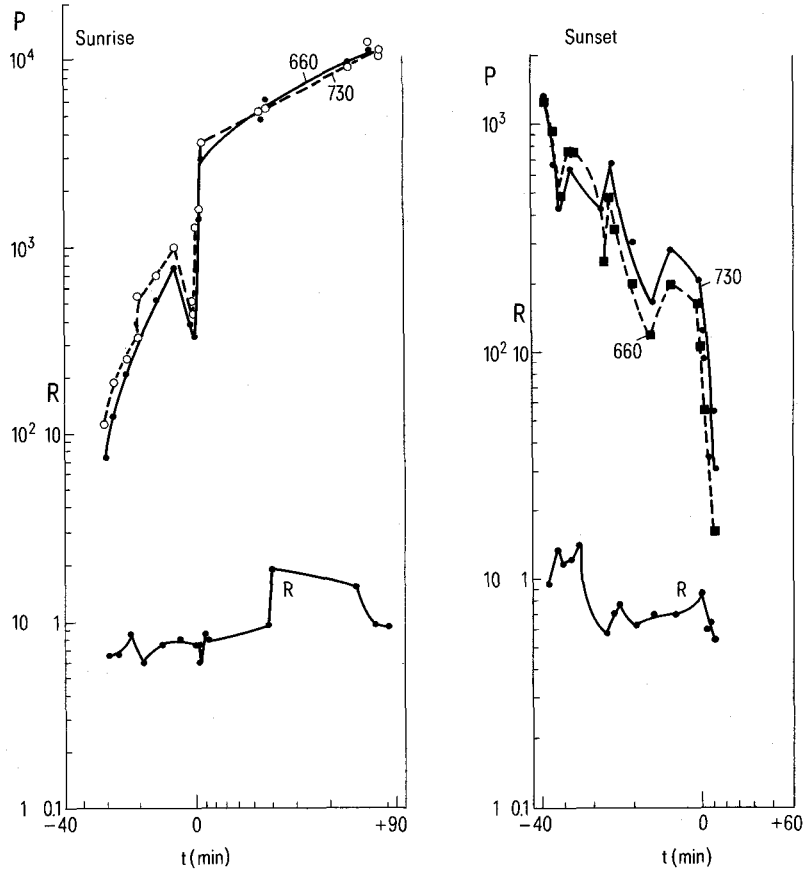
Previous observations of the free running rhythmic leaf movements of *Mimosa pudica* L. and *Sterculia planatifolia* had shown distinct fluctuations shortly after sunrise[1]. These have been less distinct prior to sunset. Roblin[2] and Fondeville[3] have reported a similar behavior. Therefore the question arose whether these fluctuations find a parallelism in the proportions of the intensities of natural red light to far red light, I_R/I_{FR}. Holmes and Smith[4-7] have measured these discontinuities, though in another context. They deduced concurrent transformations of phytochrome in several crop plants. In view of the phytochrome regulated rapid contractibility of tannin vacuoles of the pulvinar motor cells of *Mimosa pudica*[8] quantitative spectrometric measurements of natural daylight at sunrise and at sunset were made to demonstrate the parallelism between them and the free running movement of the primary pulvini of *Mimosa pudica* and of *Sterculia planatifolia.* The 1st plant was selected because of its sensitivity towards many external stimuli as for instance mechanical vibrations, electrical shock and microwaves. The 2nd plant was used as a comparator with its absence of such reactions.

Materials and methods. Plants of *Mimosa pudica* and of *Sterculia planatifolia* were grown from seed in the greenhouse. Leaf movements were measured by means of a 3.5 MHz UHF Q-meter[1,9]. This instrument expresses a change of peak resonance UHF-current, when a leaf moves within the field of the UHF tuning capacitor of the UHF oscillator. The magnitude of pulvinar and petiolar rotation is expressed by deviations of Q, where a large displacement corresponds to a high value. Spectral light intensities were measured on open 6th floor balconies by an ISCO spectroradiometer equipped with a light guide and cosine receiver. This was mounted inside the narrow end of an opaque polyethylene powder funnel, which had a wide diameter of 100 mm and a narrow one of 18 mm with a total length of 107 mm. This arrangement eliminated direct stray side light and it also made possible the aiming of the receiver towards the sun. The amplified photocurrent was recorded by a strip chart recorder. The half-wave band was 30 nm. Light current readings were corrected by means of the manufacturer's calibration curve. The wave length accuracy of the scanner was checked out with the emission lines of a Hg-

Relation between leaf movement in Q-units and the ratios R between the light intensities at 660 nm and 730 nm at sunrise and sunset

	CS time of light measurement	CS time corrected to coincide with Q-measurement[a]	Min before and after sunrise or sunset	R[b]	Q/R		
					Q_1[c]	Q_2	Q_3
Sunrise t[d]	5:20				93	90	66
Q_{min}					40.3	43.9	48.7
	5:18	4:58	−2	0.79	51.0	55.7	61.6
	5:21	5:01	1	0.74	54.5	59.3	65.8
	5:24	5:04	4	0.74	54.5	59.3	65.8
	5:26	5:06	6	0.59	68.3	74.4	82.5
	5:31	5:11	11	0.86	46.9	51.0	56.6
	6:19	5:59	59	0.93	43.3	47.2	52.4
	6:23	6:03	63	1.09	37.0	40.3	44.7
	6:46	6:26	86	1.05	38.4	41.8	-
M ± SE[f]					49.2 ± 3.9	53.6 ± 4.4	61.3 ± 4.9
Sunset t[e]	19:12				15	360	300
Q_{min}					51	48.7	51.3
	18:36	18:39	−36	0.97	52.6	50.2	52.9
	18:40	18:43	−32	1.35	37.8	65.9	38.0
	18:43	18:46	−29	1.17	43.6	76.2	43.8
	18:48	18:51	−24	1.38	37.0	64.5	37.2
	18:56	18:59	−16	0.57	89.5	156.1	90.0
	18:59	19:02	−13	0.77	66.2	115.6	66.6
	19:03	19:06	−9	0.62	82.3	143.5	82.7
	19:06	19:09	−6	0.69	73.9	70.6	74.3
	19:09	19:12	−3	0.69	73.9	70.6	74.3
	19:12	19:15	0	0.79	64.6	61.6	64.9
	19:15	19:18	+3	0.84	60.7	58.0	61.1
M ± SE[f]					62.0 ± 5.6	84.8 ± 11.5	62.4 ± 5.6

[a] The difference in time in min between CS time of light measurement and CS time corrected to coincide with Q-measurements is the difference in time for sunrise or sunset for the spectral and Q-measurements. [b] $R = I_{660}/I_{730}$; [c] Q-measurements: Q_1 of *Stericulia planatifolia*, Q_2 and Q_3 for *Mimosa pudica;* [d] t is the time in min of the leaf measurements after sunrise at 5:00 CST, and [e] to the end of the decreasing tend of Q after sunset at 19:15 CST; [f] mean ± SE.



Intensities of natural daylight during sunrise and sunset (left and right) at 660 nm and 730 nm in $\mu W\ cm^{-2}\ nm^{-1}$ and their ratios R. Time t in min on a logarithmic scale with 0-time at sunrise or sunset. The logarithmic scale stresses the progression of the relation between the 2 light intensities immediately after sunrise (sr) and sunset (ss), which had been established by the local station of the National Weather Service.

lamp. The scanning range was 380–760 nm. The times of sunrise and of sunset of the measurements of the red/far red ratios were adjusted so as to coincide with the times of sunrise and sunset of the observations of leaf movements.

Results and discussion. The photocurrents at 660 nm and 730 nm at sunrise and at sunset on 18 to 21 August of completely clear days and the ratios $R = I_{660}/I_{730}$ are given in the figure. A sharp reduction of I_{660} and of I_{730} is evident at the moment of sunrise with a concurrent reduction of R. At 60 min after sunrise this ratio rises sharply and falls off 30 min later. An inverse situation exists during sunset, when the light intensity decreases momentarily about 8 min before sunset. The ratio R rises about 12 min before sunset, falls off at 10 min and rises again to a peak at the moment of sunset. These fluctuations can be related to the intensity of the Fraunhofer absorption bands for O_2 and $H_2O_{(g)}$ in the atmosphere at changing zenith angles[1].

One test for the directive effect of the light on these leaf movements at sunrise and at sunset would be an attempt to correlate the Q-value to events occurring subsequent to these instances of time. The initial minimal Q-values after sunrise (sr) for both species are in the range from 40 to 49 units. The periods of time t to reach these minima of Q can be related empirically to them by $tQ^{-1/3} = at$, where 'a' is the slope of the plot of $tQ^{-1/3}$ against t and equals 2.67 in this case.

$$\text{Hence } t_x = t_1 \frac{Q_1^{1/3} - a}{Q_x^{1/3} - a},$$

where Q_1 = the initially measured Q-minimum and Q_x = any other initial minimum at time t_x. The time for the 1st minimal Q-values at sunset (ss) is defined as the period extending to the termination of the nightly trend of the reduction of Q, which occurs shortly before sr. The minimal Q-values at sunset for both species are in the range from 48.7 to 51.3 units. The periods of time to reach these minima follow the same mathematical relation. Its slope 'a' is however 0.38.

These 2 regression lines suggest a programing effect by the light conditions at sr and at ss. It manifests itself within a time span, the duration of which is inversely related to the $\frac{1}{3}$ power of the intensity of the light effect. This effect is measurable by the magnitude of the displacement 'Q' of the leaves. The rate at which the effect goes into action is expressed by the slopes of the regression, which slope is 7.0 times greater for the stimulus at sr than for it at ss.

It is now necessary to search for the stages during the periods of sr and ss, when the light effect is initiated. This initiation of the process of programing is thought to be a dependent variable of the ratio R. When the minimal Q-values are related to R by the quotient Q/R (table 1), then specific maxima and minima appear. A minimum of R at 6 min after sr results in a maximum value of Q/R. A corresponding inverse situation exists beyond 1 h after sr. A minimum of R 16 min before ss results in a maximum of Q/R and a lesser peak of the quotient appears at 9 min before ss. The inverse minima of Q/R correspond to high values of R at 24 min before ss and just during and 3 min after it. Specific components of these pronounced fluctuations of R, also shown by others[4–10], can be thought of as being the initiators of the subsequent behavior of petioles and leaf blades. Based on the premise that drooping of leaves during a daily cycle is concurrent with a loss of available ATP by ATP-ase action and a reduction of far-red phytochrome (P_{FR}), then, for instance, a low value of R suggests a reduction of P_{FR}. The greatest reduction of P_{FR} is

thus indicated to occur 6 min after sr and 16 min before ss. Its highest level seems to occur 1-1.5 h after sr and 29 min before ss. The coincidences of absorption bands of the Fraunhofer lines and of phytochrome are noted in this context: The P_{FR} absorption at 660 nm compared with the Fraunhofer water vapor line at 650 nm and the C-line at 656 nm and also the absorption of P_R at 730 nm against the Fraunhofer water vapor absorption band also at 730 nm. Whether these coincidences are accidental or evolutionary in nature is an unresolved question.

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Time-course of changes in lipofuscin-like pigments in rat liver homogenate and mitochondria after whole body gamma irradiation

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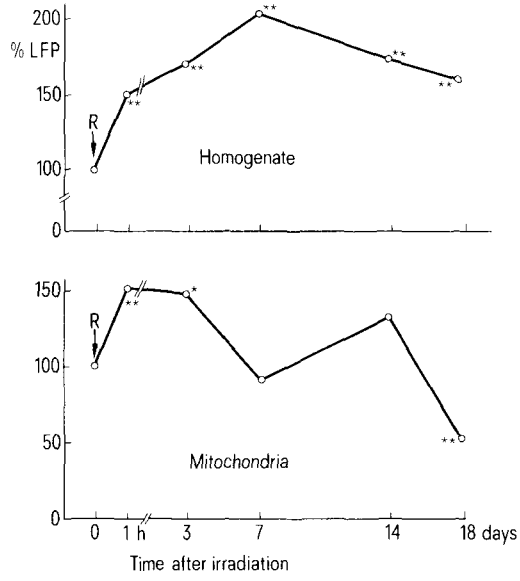
Summary. An increased level of lipofuscin-like pigments in rat liver homogenate was observed 18 days after whole body gamma irradiation, while in mitochondria they decreased below the control value.

Ionizing radiation leads to the formation of lipid peroxides[1]. The product of their decomposition, malondialdehyde[2], reacts readily to form very stable lipofuscin-like pigments[3]. These are formed both after ionizing irradiation and during aging as the so called age pigment[4]. The chromophore is probably a Schiff base with a characteristic fluorescence[5]. We studied in the present work the changes in the content of lipofuscin-like pigments (LFP) in rat liver homogenate and mitochondria in the course of 18 days after whole body gamma irradiation.

Materials and methods. Male white rats, Velaz breeding, were divided into 6 groups of 6 animals, weight 150-180 g. One group served as a control, the other groups were gamma irradiated by ^{60}Co (3.83 Gy with a dose rate 4.5 mGy/sec). The irradiation was performed in a turning cage to assure field homogeneity. The animals were decapitated without narcosis after 1 h, and on the 3rd, 7th, 14th and 18th days after irradiation. A 10% liver homogenate was made in sucrose (0.25 M sucrose, 0.001 M EDTA, 0.02 M Tris · HCl pH 7.4). After separation of nuclei, mitochondria were sedimented at 7000 g and washed twice. All operations were carried out at 0-4 °C. A standard solution of quinine sulfate was used for the estimation of the LFP fluorescence (0.05 mg quinine sulfate per ml of 0.1 N H_2SO_4 represented 550 relative units).

2 ml of homogenate or of mitochondrial suspension were added to 8 ml of a chloroform-methanol mixture (2:1, v/v) and extracted under argone 1 h on a motor-driven shaker. The fluorescence was measured at 435 nm after excitation at 365 nm on the Hitachi-Perkin Elmer MPF 2A spectrofluorimeter. The values were expressed as relative fluorescence units per mg of protein, determined according to Miller[6]. For statistical analysis Student's t-test was used.

Results. The content of LFP in homogenate or mitochondria during the time course of 18 days after irradiation is summarized in the table. The ratio of LFP content in the homogenate to that in mitochondria increased steadily up to the 7th post-irradiation day. On the 14th day it decreased, and increased again on day 18. While the LFP content in both homogenate and mitochondria increased equally up to the 1st post-irradiation h, it differed afterwards (figure). The LFP content of the homogenate rose on the 7th post-irradiation day up to 205% and then steadily decreased. Nevertheless, the LFP value was still 60% above the controls on day 18.



The time course of changes in LFP content in rat liver homogenate and mitochondria after irradiation. The control value is taken as 100%. The statistical significance of the difference between the particular and the control value is given by 1 asterisk ($p < 0.05$) and 2 asterisks ($p < 0.01$).