# Light-Dependent Changes in the Leaflet Movement Rhythm of the Plant *Desmodium gyrans*

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Z. Naturforsch. **58c**, 81–86 (2003); received September 6/September 20, 2002

The movements of the lateral leaflets of the Indian telegraph plant *Desmodium gyrans* (L. F.) DC, have earlier been studied in detail with regards to the effects of chemicals, DC currents, and static magnetic fields. In the present paper we have discussed the oscillation of the lateral leaflets under the influence of white light of various light levels (0–75 $\mu mol \cdot m^{-2} \cdot s^{-1}$), produced by an array of LEDs (light emitting diodes). LEDs were used in contrast to fluorescense tubes as in earlier studies in order to minimize changes of wavelength when light intensity was decreased or increased. Furthermore, care was taken to ensure that the temperature in the experimental chamber was constant.

When the oscillations were first monitored in bright light, the oscillations were found to be very rapid and with decreasing light intensity the oscillations slowed down. For light levels lower than about 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ the period of the oscillation of the lateral leaflets was almost constant (or even decreased slightly towards complete darkness). We also show that the oscillations could completely stop under prolonged darkness (for longer than about 6 h) and that such halted oscillations could be restarted in most of the leaflets when he light was turned back on. Such stopping of the oscillation of the lateral leaflets in prolonged darkness suggests that these short period oscillations of the lateral leaflets could have a daily component and in natural environment these oscillations could serve the purpose of optimising the amount of light falling on the leaflets or/and facilitating transpiration of water through stomata. Such a finding could have an implication for the answer to the long standing question of adaptive significance of short period oscillation of the Indian telegraph plant *Desmodium gyrans* (L. F.) DC.

*Key words:* Ultradian Rhythms, Leaflets, *Desmodium*

## Introduction

The leaf system of the Indian telegraph plant *Desmodium gyrans* (or *Codariocalyx motorium*, Houtt, Ohashi) consists of a terminal leaflet and a maximum of two lateral leaflets, all on the same stalk. The terminal leaflet oscillates between upward and downward positions in a daily fashion and these movements are temperature compensated (Engelmann and Antkowiak, 1998). On the contrary, the movements of the lateral leaflets have a period of the order of few minutes and are temperature dependent. An increase of temperature decreased the period of the oscillations. The oscillations were also synchronised by temperature cycles (Lewis and Silyn-Roberts, 1987).

The oscillations in the lateral leaflets are generally believed to be generated by rhythmic swelling and shrinking of the motor cells located in the pulvinus of the leaflet base (Engelmann and Antkowiak, 1998). Electrical potentials across the motor cells oscillate and maintain a constant phase relationship with the leaflet positions (Antkowiak and Engelmann, 1995). The fluctuations in the electrical potentials in pulvini are due to the uptake and release of ions, especially $K^+$ and $Cl^-$ (Kumon and Tsurumi, 1984; Starrach and Mayer, 1989; Lowen and Satter, 1989). A considerable amount of $K^+$ is also shuttled from one part of the pulvinus to the other, acting as a cation reservoir (Freudling *et al.*, 1988; Mayer, 1990). The motor cells oscillate between electrically polarised and depolarised states. The state of depolarisation causes $K^+$ and $H^+$ efflux while hyperpolarisation causes $K^+$ and $H^+$ influx into the cells. The $K^+$ fluxes are believed to be responsible for the os-

0939–5075/2003/0100–0081 $ 06.00 

motic movement of water across the pulvinus, which in turn results in volume changes in the pulvinus and the observable leaflet movement.

The lateral leaflet oscillations are sensitive to electromagnetic field treatments. We have reported that the leaflet movement rhythm slowed down in the presence of a static magnetic field (Sharma *et al.*, 2000). Furthermore, the rhythms were affected by DC currents or 27-MHz radio frequencies and could also be temporarily stopped by stimuli, given at appropriate strength and at appropriate phase of the rhythm (Ellingsrud and Johnsson, 1993; Johnsson *et al.*, 1993; Fostad *et al.*, 1997). Recently we have also demonstrated that the phase of these oscillations could be reset in both directions (advances and delays), due to DC current pulses (Sharma *et al.*, 2001).

The periodicity of these oscillations was reported to remain unchanged when recorded at various intensities of white light in a moderate range or in darkness (Engelmann and Antkowiak, 1998). High intensities of light of the order of 50 $W/m^2$ could, however, modulate the rhythm. It was therefore speculated that an alteration between high and low intensity of light could bring about synchronisation of the oscillations (Engelmann and Antkowiak, 1998). Notwithstanding the importance of light for these oscillations, the role of light has never been systematically studied. An extensive investigation of the effects of light of various intensities and wavelengths could give a better understanding about the functional significance of these short period oscillations. In this paper we have discussed the effect of white light of various intensities on the lateral leaflet rhythm of the Indian telegraph plant *Desmodium gyrans.*

## Materials and Methods

The plants, *Desmodium gyrans* (L. F.) DC – also denoted *Codariocalyx motorius*, Houtt, Ohashi – were cultivated under light/dark cycles (12 h light and 12 h darkness) at about 27 °C. The humidity level was about 65%. Further details on the cultivation are given in Johnsson *et al.* (1993). Leaflets displaying a regular oscillation were cut from the mother plants and were kept in water in an acrylic glass holder. The terminal leaflet was cut off and vaseline applied to the cut surface to prevent excessive water loss. The leaflets were then placed inside an acrylic glass box to minimize temperature fluctuations (and kept at 27 ± 0.5 °C).

*Use of LEDs and experimental protocol*

Leaflets were illuminated from above by an array of LEDs (Nishigia NSPW 500BS bf) mounted in a 15 times 6 array on a printed board. The spectral composition of the LEDs was checked and was not changing when the current through the diodes was varied (in the irradiance range used). Different light levels were easily adjusted by changing the current thought the circuit. Sequences of light steps were used, *e.g.*, 0.5 , 7.0, 20, 36, 55 and 76 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Leaf movements were recorded by a video camera (FUJITSU TCZ-250E), positioned in front of the box containing leaflets. Eight infrared LEDs (Everlight IR333C/H2 ($\lambda$ = 940 nm, view angle 25 degrees) were mounted around the camera lens and directed towards the leaflets. The position of the LEDs were slightly off the optical axis, light thus directed forwards at a slight angle with respect to the axis. A highly reflecting sheet (3M Scotchlite 3290) reflected the infrared light from the LEDs, thus creating a silhouette picture of the moving lateral leaflets. By using this method it was avoided to construct a continuous background of infrared irradiation behind the leaflets. The additional LED light was checked not to affect the movements.

The video images were digitised in a digitising unit (VIDEO ST 1000) and the digitised images were then processed in an ATARI 1040 ST computer using software ("OXALIS") developed by J. Schuster and Engelmann at the University of Tübingen in Germany. The digitised image is presented in black and white and allows the user to define rectangular fields on the screen where the average horizontal and vertical position of the object within the field can be recorded. The leaflets were positioned in order to facilitate the recording of both the vertical and horizontal movements. The OXALIS program version 5.1 was used and the setting fields were: FIELDS definition – single, object colour-white, mode-quick, time span-10 h, recording time-5 s, x-coordinate (X), y-coordinate (Y), thus sampling horizontal (x-coordinate) and vertical (y-coordinate) leaflet movement once every five seconds for ten hours.

*Analysis of the time series*

For analysis of the time series, the recordings were split into one series per leaflet per intensity. From these, the last 200 samples were used to generate the data series that we used as a basis for further analysis. We chose to use the last 200 samples to ensure that the oscillations had reached a stable period before analysing the period of the signal further. We then calculated the autocorrelation function for these sub-series, and found the first peak, representing the dominant period in the oscillations. Finally, we computed the mean and standard error of the period as a function of light intensity.

*Statistical analysis*

The data obtained on the periodicity of leaflet oscillations were subjected to one way analysis of variance (ANOVA) and the periodicities at different intensities were compared using Tukey's test for multiple comparison (STASTICA, 1996). The period values obtained at various intensities were also analysed using non-linear regression to estimate the intensity after which the relationship between periodicity of leaflet oscillation becomes a linear function of light intensity.

## Results

A stepwise change in light intensity applied to the leaves caused a change in the period of the leaf movements. Sequences from a leaf movement recording are shown in Fig. 1. The position of the leaflet is recorded as a function of time, with start of the experiments as time zero. Since the leaflet oscillations are often very stable for a long period of time, it was possible to record the movements while we increased the light level in steps up from 0 to about 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and reduced the level in the same stepwise manner back to zero. The period of the oscillations changed as a function of light intensity.

Figure 2 shows the period of three individual leaflets when the light intensity was increased in a stepwise fashion. The period of the oscillations of the lateral leaflets, at a given intensity of light, did not depend on whether the intensity was reached by increasing or by decreasing the intensity of light. The altered periodicity under any light intensity was achieved fairly rapidly. The period of lateral leaflet oscillation of *D. gyrans* as a function of the light intensity is presented in Fig. 3. The period stays constant (or even increases) up to a light intensity of about 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The data were collected from several experimental series, n varies between 3 and 17 for the experimental points in the figure. Multiple comparison using Tukey's test showed that the periodicity of leaflet oscillation in an illumination of about 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was significantly different from the periodicity in all the other illuminations ($p < 0.05$). The periodic-

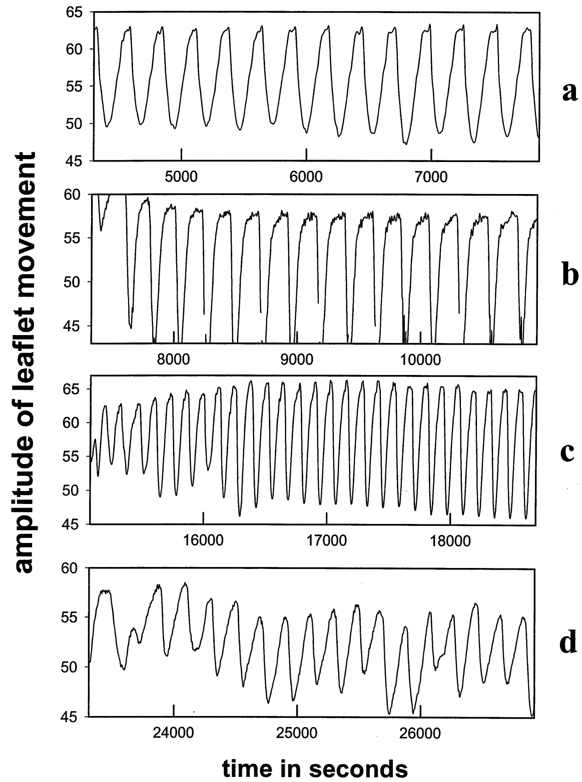


Fig. 1. An example of the oscillation of a lateral leaflet of *Desmodium gyrans*, recorded continuously at several illumination levels. The up-and-down leaflet movement was recorded as a function of time (seconds after start of recording), the position of the leaf is given in arbitrary (pixel) units. The light irradiance was first increased from 0 to 76 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and then decreased to 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The period of the oscillations, as calculated by the time distances between maxima of leaf position for the different time sequences, was as follows: (a) in constant darkness, about 265 s, (b) at a light flux of 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, about 228 s, (c) at a light flux of 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$, about 130 s and, finally, (d), at a light flux of 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, about 295 s.

ity of lateral leaflet oscillation of *D. gyrans* decreased with increasing light intensity after a light intensity of about 20 μmol·$m^{-2}$·$s^{-1}$ ($r$ = – 0.60, $p < 0.0001$; n = 44 experiments). The effect of light intensity on the lateral leaflet oscillations was completely reversible.

The oscillations of (n = 6) leaflets stopped in prolonged darkness (*i.e.* longer than about 6 h) and the oscillations could be initiated after light was turned on. An example of such a halt of the oscillations is shown in Fig. 4.

## Discussion

The light intensity changed the period of the lateral leaflet movements in *Desmodium gyrans*. The period in absolute darkness (as recorded with infrared LEDs) was about 3 min and remained un-

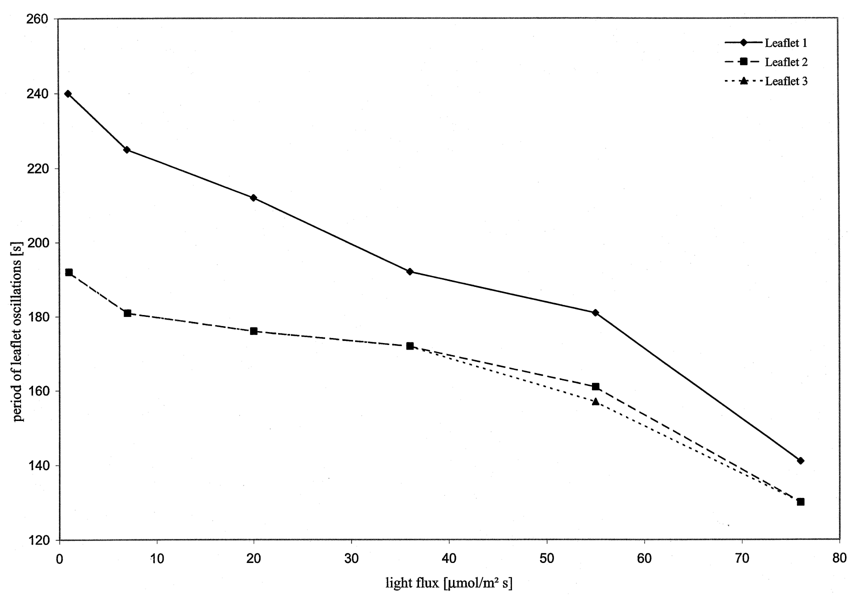


Fig. 2. Examples of the period of leaflet movement rhythm of *Desmodium gyrans* as a function of light intensity. The results from recordings of the rhythm in three different, individual leaflets. Increasing the light intensity stepwise (see text) also decreased the period of the leaflet oscillation in a stepwise manner.

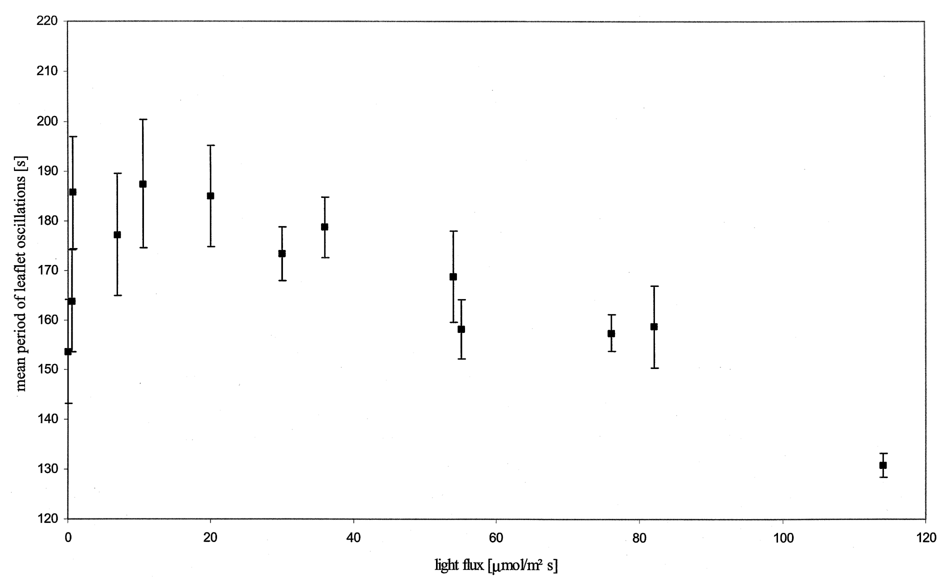


Fig. 3. The period of the oscillations (in seconds) of the leaflet movement rhythm of *Desmodium gyrans* as a function of light flux (μmol·$m^{-2}$·$s^{-1}$). The period of the oscillation remained unchanged with increase in light intensity up to about 20 μmol·$m^{-2}$·$s^{-1}$, however, a further increase in light flux decreased the period of the leaflet oscillation. The error bars represent standard error around means.

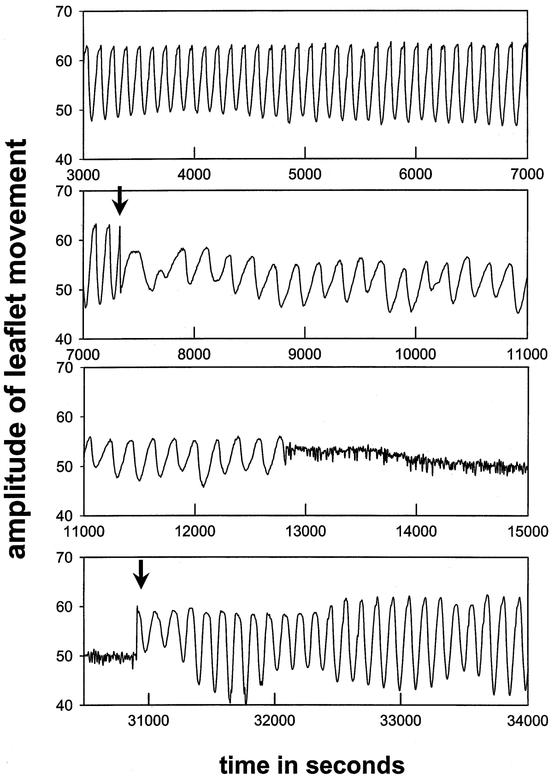


Fig. 4. Example of lateral leaflet oscillation of *Desmodium gyrans*. The leaflet oscillations were first recorded in constant illumination of 76 $\mu$mol · m$^{-2}$ · s$^{-1}$. At a time indicated by an arrow (between 7,000 and 8,000 seconds) the illumination was' turned off. The oscillations slowed down considerably, and finally disappeared altogether at about 13,000 seconds. The leaflet oscillations reappeared immediately after the illumination of 76 $\mu$mol · m$^{-2}$ · s$^{-1}$ were reapplied (at a time indicated by an arrow between 31,000 seconds to 32,000 seconds). The leaflet oscillation exhibited a periodicity of 130 s in an illumination of 76 $\mu$mol · m$^{-2}$ · s$^{-1}$ and 200 s in constant darkness before the oscillation disappeared.

changed with increasing light intensity up to about 20 $\mu$mol · m$^{-2}$ · s$^{-1}$. A further increase in light intensity casued a drop in period down to about 130 s (Fig. 2, 3). We used white light LEDs that do not emit in the infrared spectral region. We also kept the temperature constant during the experiment, however, it should be noted that the photon reactions within the leaflets can cause an increase in temperature. In our experiments we do not suspect that the period changes could be caused by an incerase/decrease in temperature. Cihlar (1965) reported on the temperature dependence of the period of the *Desmodium* leaflet rhythm. A change in period from about 200 s to 130 s, corresponding to a temperature rise of about 10 °C, is unlikely to occur under our experimental temperature controlled conditions. The nature of period changes, especially at low intensities (below about 10–15 $\mu$mol · m$^{-2}$ · s$^{-1}$) suggests that the period changes are not due to changes in temperature (Fig. 3).

It is believed that the movement of the lateral leaflets of *D. gyrans* originates in the volume changes of the motor cells of the pulvinus. These volume changes occur due to ionic movement between different parts of the motor cells. The depolarization of the membranes of the motor cells of *D. gyrans* causes $K^+$ and $H^+$ efflux and their hyperpolarization causes $K^+$ and $H^+$ influx into the cells. The movement of water across the pulvinus occurs due to $K^+$ fluxes, which in turn results in volume changes in the pulvinus and the observable leaflet movement. Exposure to a number of stimuli can alter the period of the lateral leaflet movements in Desmodium (Engelmann and Antkowiak, 1988). The slowing down of the leaflet movement rhythm in the presence of static magnetic fields (Sharma *et al.*, 2000) as well as electromagnetic fields can possibly be mediated through the manipulation of the ionic movements in the motor cells in the pulvini (cf. Garcìa Reina, and Arza Pascual, 2001). The effect of light on the ultradian leaflet movement rhythms of *D. gyrans* as demonstrated in the experiments described in the present paper, suggests that the ionic movements across the motor cells of the pulvini can also be manipulated by the exposure to light. This also opens up a possiblity to investigate light induced phase resetting and entrainment studies on ultradian rhythm. We have recently reported that DC currents can phase shift these short period oscillation in a phase dependent manner (Sharma *et al.*, 2001).

The results of our experiments also suggests a possible role of light/dark cycle in the leaflet oscillation in these species of plants which is mostly found in tropical region. There have been many speculations about the functional significance of these short period oscillations. The fact that the leaflets oscillate with different frequencies in different illuminations and that the oscillations halt in prolonged darkness suggest that the biological clock that regulates the ultradian rhythm of leaflet movements oscillates with different frequencies in the natural environment, where the intensity of light first increases to reach the maximum at mid day and then decreases towards the evening. The results also suggest a 24 h component in the ultradian oscillation which should be investigated in plants maintained in constant illumiation for several days. It appears that the ultradian oscillations of the lateral leaflets have a functional significance for the plant which grows under tropical climate in terms of optimising the light falling on the leaflet surface and/or helping in the transpiration of water through stomata.

*Acknowledgements*

We acknowledge the Norwegian Research Council for supporting the travel of VKS to NTNU, Trondheim, Norway.

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