

# Effects of Static Magnetic Field on the Ultradian Lateral Leaflet Movement Rhythm in *Desmodium gyrans*

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The rhythmic leaflet movements of the plant *Desmodium gyrans* (L.f.) DC slow down in the presence of a static magnetic field. The leaflet positions were digitally retrieved from sequential CCD camera images of the moving leaflets. The experiments were performed under constant light (ca. 500 lux) and temperature (about 20 °C) conditions. The period of the leaflet was then around 5 min. Leaflets moving up and down in a magnetic field of approximately 50 mT flux density increased the period by about 10% due to a slower motion in the “up” position. Since during this position a rapid change of the extracellular potentials of the pulvinus occurs, it is proposed that the effects are mediated via the electric processes in the pulvinus tissue.

## Introduction

Although ultradian rhythms of the lateral leaflets in the plant *Desmodium gyrans* have been studied intensively, its functional significance still remains unclear. The movements are due to swelling and shrinking of motor cells in pulvini at the base of these leaflets. Electrophysiological and chemical perturbation studies indicate that such swellings and shrinkings are caused by ion and water movements across the cell membranes of the motor cells (Antkowiak *et al.*, 1991). It was also speculated that Ca<sup>2+</sup> ions and phosphatidyl inositol may be involved in these chains of reactions (Chen *et al.*, 1997). The motor cells were found to continue their movements even in isolated pulvini (Mitsuno, 1986).

The amplitude, phase and period of the lateral leaf movement rhythm in *Desmodium gyrans* could be changed by a number of stimuli (reviewed in Engelmann and Antkowiak, 1998). Among all the factors affecting the leaf movement rhythm in *Desmodium gyrans*, the effects of electrical currents (Hufeland, 1790; Bose, 1913; Guhathakurta and Dutta, 1962) and 27 MHz radio frequencies (Johnsson *et al.*, 1993) could perhaps be directly related to the ion movements across

the motor cells of the pulvinus. When direct currents (DC) of strength 10 to 100 µA was applied for 10 sec to the tip of lateral leaflets, the phase of the rhythm was delayed. Pulses of DC current were also found to stop the leaf movement rhythms in these plants if applied at an appropriate phase. However, it was not possible to entrain the rhythm using such pulses (Johnsson *et al.*, 1993).

Ellingsrud and Johnsson (1993) perturbed the leaf movement rhythm of *Desmodium gyrans* using pulses of 27 MHz radio frequencies. It was observed that such pulses could change the amplitude, phase, and period length of the rhythm. Similar to the DC electric currents, radio frequencies of dose 8 W/cm<sup>2</sup> were also found to stop the leaf movement.

The results of experiments where electrical stimuli affected the ultradian leaf movement rhythm further support a model which explains the leaf movement rhythm in terms of proton pumps, membrane potential changes and ion transports. It would therefore be of interest to investigate whether static magnetic fields can influence the leaf movement rhythm.

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## Materials and Methods

The plants, *Desmodium gyrans* (L.f) DC (new name *Codariocalyx motorius*, Houtt, Ohashi) were cultivated under 12:12 hour light/dark cycles at about 28 °C. They were about 3 months old and had a height of approximately 60 cm when lateral leaflets were used. The humidity was about 65%. For details see Johnsson *et al.* (1993).

Leaflets displaying a regular oscillation were cut from the mother plants and the terminal leaflet removed. They were kept in distilled water in an acrylic glass holder inside an acrylic glass box to minimize temperature fluctuations ( $29 \pm 0.5$  °C). Three light tubes (Osram 40 W/15–1, 1500 lux) on top of the box illuminated the leaflets. A small white styrofoam ball was attached to the tip of each leaflet, serving as an optical marker. A black cloth at the rear of the box increased the contrast to the white marker.

Leaf movement was recorded by a video CCD camera (Fujitsu TCZ-250E), positioned in front of the box containing the leaflets. The video signal was digitized by a digitizer (VIDEO ST 1000, Ingenieurbüro Fricke, Berlin) and then processed in an ATARI 1040 ST computer using special software (Schuster and Engelmann, 1990). The horizontal and vertical position of the marker was determined by the program once every five seconds for ten hours and the data stored on disk.

Neodymium magnets (Elfa, Oslo, Norway) with dimension  $3 \times 3 \times 1$  mm were used in pairs and fastened to a holder at a distance of 10 mm between them. This allowed a *Desmodium* leaflet pulvinus to be centered between the magnets and to move at a distance of 5 mm from each one.

The magnetic flux density was measured by using a Hall element flux density meter (Unilab, Oxford). In the middle between the magnets the flux density was recorded to be 50 mT. In the plane between the magnets the field gradients were fairly small and in the area that was swept by the pulvinus the magnetic flux density was never less than about 30 mT. The magnetic flux density was higher near the magnets (as measured by the Hall probe) and the highest value could be measured at a distance of approximately 0.3 mm from the magnet (about 200 mT).

Care was taken that the pulvinus did not move sidewise during an experiment.

The normal earth magnetic field vector was likewise estimated in the Trondheim laboratory (flux-gate magnetometer, Bartington, Oxford, England) and amounted to 40  $\mu$ T, pointing slightly obliquely with respect to the plumbline.

Period of the rhythmic movement was estimated using a digital filter on the data and calculating the time between successive leaf position maxima. The periods of the leaf movements in the presence and absence of magnetic field were compared using Students' t-test.

## Results

When a static magnetic field of strength 50 mT was applied to the pulvinus of the leaf preparations of *Desmodium gyrans*, the period of the ultradian leaf movement lengthened in most of the experiments. However, in about 15% there was no significant change. The magnitude of change in period varied from leaflet to leaflet. The response to the magnetic field in most of the cases occurred in the first cycle.

Fig. 1 illustrates three examples. Thick vertical bars indicate times when magnetic fields were introduced and the thin bars when the field exposure was stopped. Estimating the period from such recordings ( $n = 42$ ) showed the lengthening of the period in the magnetic field and is illustrated in Fig. 2. The period lengthening of the leaflet rhythm in a magnetic field is rapid and completed within at most a few cycles.

Furthermore, in some cases there was a reduction in the period lengthening during longer exposures to the magnetic field, as exemplified at the very end of the recording b of Fig. 2.

Table I. Amplitude and period lengths (minutes) with standard deviation from experiments with and without magnetic field applied as illustrated in figures 1 and 2 (a–c).

Experiment	No magnet		With magnet	
	Amplitude (pixels)	Period (min)	Amplitude (pixels)	Period (min)
a	82.6 $\pm$ 15.1	6.1 $\pm$ 0.1	63.8 $\pm$ 21.6	7.1 $\pm$ 0.4
	62.1 $\pm$ 1.6	5.6 $\pm$ 0.1	68.2 $\pm$ 8.0	6.0 $\pm$ 0.01
	64.5 $\pm$ 0.7	5.9 $\pm$ 0.5	69.7 $\pm$ 1.4	6.3 $\pm$ 0.4
b			61.7 $\pm$ 2.2	6.1 $\pm$ 0.4
	70.6 $\pm$ 6.4	6.5 $\pm$ 0.1	75.1 $\pm$ 0.7	7.2 $\pm$ 0.4
	78.3 $\pm$ 0.9	6.7 $\pm$ 0.3	81.3 $\pm$ 0.3	7.1 $\pm$ 0.2

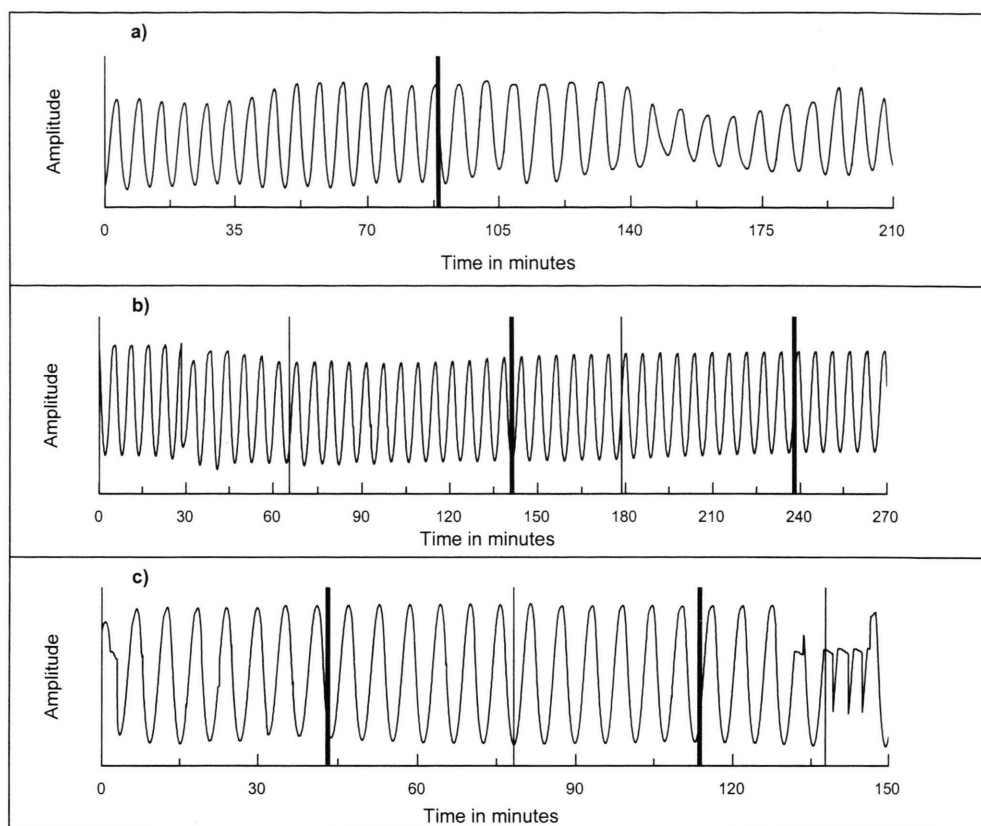


Fig. 1. Effects of static magnetic fields on leaflet movements. The vertical leaflet movements were recorded as a function of time. Thick vertical bars indicate when a magnetic field of about 50 mT was applied across the leaflet pulvinus; thin vertical bars indicate when field was removed.

1a) Introduction of magnetic field increased the period length of the movements. Long term treatment.

1b) At the start of the recording a magnetic field was present; it was then removed, applied etc for short time intervals. The period of the leaflet rhythm changed accordingly.

1c) In this recording the field was absent at the beginning of the experiment, was then applied, removed etc. but the alterations were done with shorter intervals of time than in 1b).

We have not yet studied the detailed transient response of the leaflets to the magnetic fields. Visual inspection shows that the increase in period is mainly due to a lengthening of the “upper phase” of the leaflet movement. In some recordings this is also revealed by a more “rounded” curve form in the upper position in the magnetic field than without the field.

## Discussion

Kuznetsov and Hasenstein (1996) showed that (diamagnetic) starch grains move in a high gradient magnetic field and the position of intracellular amyloplasts could, therefore, be affected in a mag-

netic field gradient, causing tropistic movements. To move a (supposedly existing) diamagnetic particle in a pulvinus cell, the difference in diamagnetic properties between the cytoplasm and the particle must reach a certain level.

In the study cited, it was estimated that a force of  $10^{-3}$  times the gravitational force could be anticipated to act on a starch grain in a gradient ( $\Delta(H^2/2)$ ) of the order of  $10^6$ – $10^7$  Oe<sup>2</sup>/cm or  $10^6$ – $10^7$  Gauss<sup>2</sup>/cm).

The measured magnetic flux values in our setup were 30 to 150 mT over a distance of 0.25 cm (corresponding to about 600 and 3000 Gauss, respectively). This means that the gradient in ( $\Delta(H^2/2)$ ) could be estimated to be about  $4 \cdot 10^7$  Gauss<sup>2</sup>/cm or

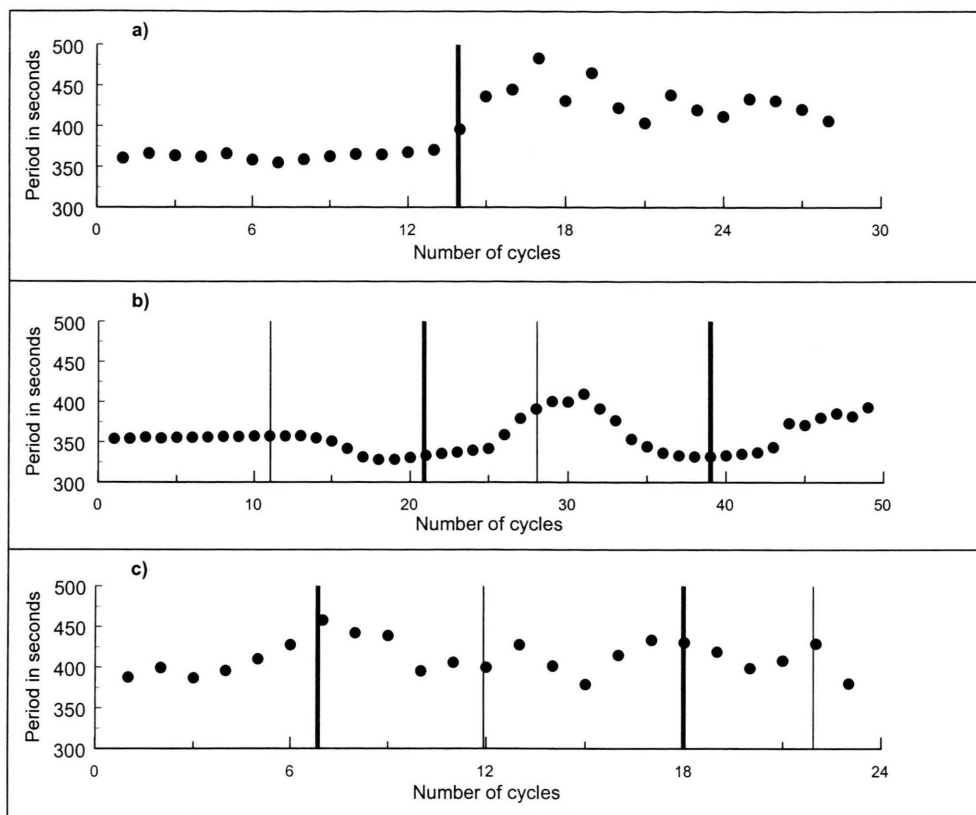


Fig. 2. Plot of period vs. cycle number for recordings shown in Figure 1. The period length increases after the magnetic field is applied as shown by the higher values following the introduction of the magnetic field, i.e. after the thick vertical lines. Correspondingly, the period decreases when the magnetic field is removed. Figures a, b and c illustrates the period lengths recorded in 1a, b and c respectively. The curves show that the period change is starting already during the first period after introduction of magnetic field; however, a steady state is attained only after a few cycles.

less. Following the arguments by Kutznetsov and Hasenstein (1996) such a gradient would cause a force of the order of about  $10^{-3}$  times the gravitational force on a diamagnetic starch grain. We regard it as unlikely that inhomogenous magnetic fields in between the magnets would play any role in the present experiments and no diamagnetic (or paramagnetic) particles are known that could be redistributed in the pulvinus to cause any observable effects on the leaflet movements in such a short time.

We, therefore, prefer to ascribe the effects on the rhythmic period of the leaflets to homogeneous fields. However, effects of inhomogenous fields cannot be completely ruled out for the time being. This can also be analogous to the basic mechanism which has been described in

the effects of magnetic fields on the circumnutations of tendrils.

Circumnutations of cucumber tendrils have been found to be more rapid in the presence of a static magnetic field (Ginzo and Décima, 1995).

The magnetic flux density of the static magnets used in the experiments was of the order of 1–15 mT. As the tendrils passed the static magnets, the speed of the tendril movement was significantly increased; field direction, however, did not influence this effect. The effect was hypothesised to be via osmotic reactions, via moving electric charges or other mechanisms.

The rhythmic leaflet movements in *Desmodium* represent oscillatory processes that are about 50 times more rapid than the circumnutations mentioned.

The cellular changes in electric potentials and currents that can be anticipated to accompany the movements should, therefore, also be more rapid with the same factor.

Electrophysiological methods have unraveled the basis of the ultradian leaflet movement rhythms in *Desmodium*. When microelectrodes were placed on the surface of the pulvinus or within the apoplast, the electric potentials were found to change periodically. The leaflet position and the extracellular electrical potentials were found to oscillate in tandem. Simultaneous extra- and intracellular recordings of the pulvinus indicate that the oscillations in the extracellular potentials are generated by periodic changes in the membrane potentials of abaxial motor cells (Antkowiak *et al.*, 1992). These results show that

there are electrical potentials generated in the pulvinus and strongly indicate that currents are flowing. Although other possibilities can not be ruled out, we suggest that the period changes in the presence of a static magnetic field may be caused by an interaction between ion fluxes and the static magnetic field. As an example, calcium channel activation has been shown to be inhibited in GH3 cells by 120 mT static magnetic fields (Rosen, 1996).

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