Rhythmic Leaf Movements under Physical Loading of the Leaves

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The effect of physical loading on period and amplitude of the ultradian oscillating leaflets of *Codariocalyx motorius (= Desmodium gyrans)* and the circadian oscillating leaves of *Oxalis regnellii* was investigated. In *Codariocalyx* the leaflets were loaded with pieces of copper thread which were clamped to the leaflet. Weights of the loads varied from 3 to 12 mg, and a typical leaflet is 1.5 mg. In about 80% of the cases we recorded an increase of the period length due to the loading in the order of 10% (the period length is typically 3–4 min). The amplitude was lowered in all the cases, the magnitude depending on the size of the weight used. In most of the cases, when not too heavily loaded, both amplitude and period returned to (or close to) the previous value when the leaflets were unloaded. In *Oxalis* the leaves were loaded with metal clips fixed to the leaflets at the midrib position. All three leaflets on a stalk were loaded. The clips used weighed 390 ± 0.4, 640 ± 6 and 810 ± 4 mg respectively, and a typical leaflet is 91 ± 4 mg. For the heaviest weights at least, this resulted in a lowering of the amplitude, but the oscillations continued, and we could not observe any effect on the period of the rhythmic movements.

Introduction

In studies of leaf movements it is important to know whether the physical load on the leaf affects the movements. The physical pressure, P, contributes to the water potential of the motor cells regulating the leaf position. When the water potential gradients change, this might influence the leaf movement rhythms. In a classical study of the rapid, ultradian leaf rhythms in *Codariocalyx motorius* (= *Desmodium gyrans* [7]), Bose [1] applied different weights to the leaflets and recorded a qualitative influence on the period of the rhythmic movements. This experimental finding indicates that the water potential of the motor cells is part of the frequency determining system regulating the leaf movements.

If the loading of the leaflet affects the period of the movements it is, of course, important to avoid recording methods which implicate a physical strain on the leaves: Such methods would influence the period to be measured.

We have undertaken a quantitative study of the effect of physical loading on the rapid leaf movements of *Codariocalyx motorius*. Furthermore, we compare the results with corresponding measurements on a circadian, nyctinastic leaf movement system. We have chosen the trifoliate *Oxalis regnellii* as the experimental material, since this plant has been used in earlier studies of circadian leaf movements [3].

The relationships between the water potential gradients and the circadian oscillator is of great interest and importance [2]. The water potential might be part of the circadian clock mechanism and should then influence the period of the leaf movements.

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Materials and Methods

Codariocalyx motorius (Houtt.) Ohashi

Leaflet stems with one or two leaflets showing ultradian rhythms with a period of some minutes were cut from *Codariocalyx motorius* plants (kindly provided by Prof. W. Engelmann, Tübingen). They were put into tap water and left in constant white light (Philips TL 20 W/32 and Osram L 20 W/20, 0.5 mW/cm^2) for at least half an hour until they gained a stable state of oscillation.

Recordings were done with the aid of a video recording method [5]. A vertical view field with the moving leaflet was chosen and the vertical position of the object was determined. The positional data were stored in an ATARI computer and later evaluated.

Leaflets were loaded with pieces of folded copper thread which were clamped to the middle part of each leaflet. Weight of the threads varied from about 3 mg to about 12 mg, while the fresh weight of one leaflet itself is typically about 1.5 mg.

The analysis was done by plotting the time of consecutive maxima, and in some cases by plotting the time between consecutive maxima (the period). Autocorrelation analysis was also done in order to quantify the results.

Oxalis regnelli, Mig.

Plants were cultivated and stalks with three leaflets cut away from the plants as described by Johnsson *et al.* [3].

Recording of leaf movements was done with the aid of photoelements (the shadow on the photoelements being a measure of the leaf position) as described in the paper cited.

The leaflets were loaded with metal clips, fixed to the outermost part of the leaflet at the midrib position.

The clips used weighed 390 ± 0.4, 640 ± 6; or 810 ± 4 mg respectively. An ordinary leaflet of *Oxalis regnellii* at the age used in the experiments had a weight of 91 ± 4 mg. All three leaflets on a stalk were loaded.

Results

Codariocalyx motorius

In roughly 80% of the experiments a lengthening of the period was observed as a consequence of the loading. The period of the leaflet movements is 3–4 min, and the loading resulted in a period lengthening in the order of 20 sec (between 5 and 40 sec observed), which is a roughly 10% increase in the period length. Fig. 1 shows a typical recording where the leaflet was loaded for about 80 min. Fig. 2 presents the period as a function of the period number in the same experiment. In most cases the period returned to the previous value, or close to the previous value, when the plant was unloaded.

In accordance with Bose [1], we found a smaller amplitude and a lowering of the average position as a consequence of the loading.

Immediately after onset of loading the oscillations often almost halted, but then the amplitude increased again. A stable state of oscillation was regained after a few periods, the time depending on the size of the weight and the leaflet.

When the plants were unloaded, the leaflet movements were often quite irregular. Some leaflets showed irregular oscillations for up to 2 h before they regained a stable state of oscillation.

Those leaflets which did not show the irregular oscillations described, achieved their period changes within the first or second period after the treatment, but the period was quite unstable after the loading (*cf.* Fig. 2).

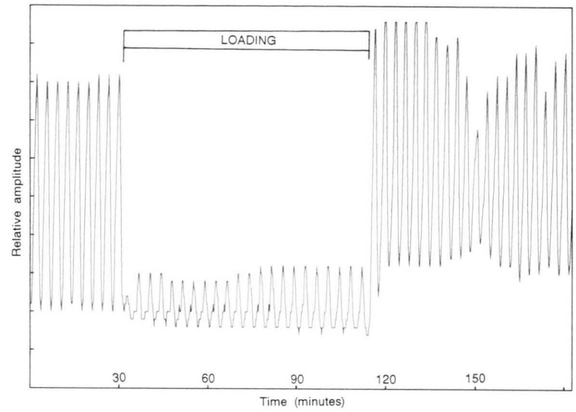

Fig. 1. A typical recording of the leaf movements in *Codariocalyx motorius*. The leaflet was loaded with a 6.9 mg load after about 30 min and then unloaded again about 80 min later, as indicated. Peak to peak value of the leaf movement before the loading was roughly 100 degrees.

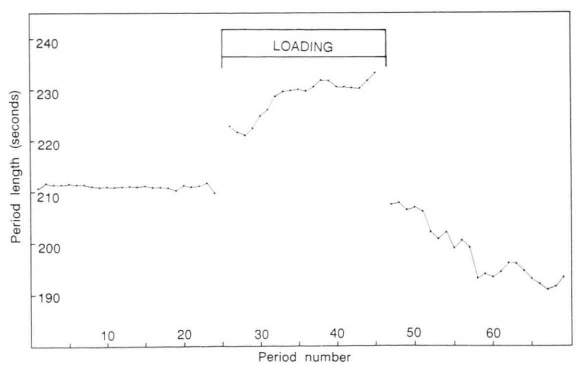

Fig. 2. The period as a function of period number in the experiment shown in Fig. 1. The period was found by averaging over four consecutive periods.

In some cases, especially when heavily loaded, a leaflet could go into a non-oscillating position (up position) when being unloaded. No leaflets were observed to start oscillating again from this position (at least not within 3–4 h).

Finally, in a few cases a shortening of the period due to the loading was observed.

Oxalis regnellii

Fig. 3 shows some typical recordings of the leaf movements.

The upper curve, 3a, presents movements of a non-loaded control plant. The lower curves, 3b–3d, show typical recordings where the time for loading has been denoted by the arrow.

After the seismonastic reactions immediately after the loading, the circadian movements resumed. The amplitude was changed but there was no indication of a change in period. Data analysis (direct analysis of timing for maxima, also TIMESDIA analysis [4]) revealed no difference in period between controls and loaded plants.

The period before and after loading was calculated. The control plants (n = 39) usually show a slightly increased period with age. This increase amounted to 0.3 ± 0.2 h for the time intervals investigated. For the loaded plants the lumped data (n = 29) showed a period increase of 0.1 ± 0.7 h. We could not see any differences in the results between plants loaded with 0.4 g (n = 6), 0.6 g (n = 10) or 0.8 g (n = 13). There might thus be a slight (non-significant) shortening as compared to the controls. A 10% increase in period length as found for *Codariocalyx* would have meant roughly 2.5 h period increase.

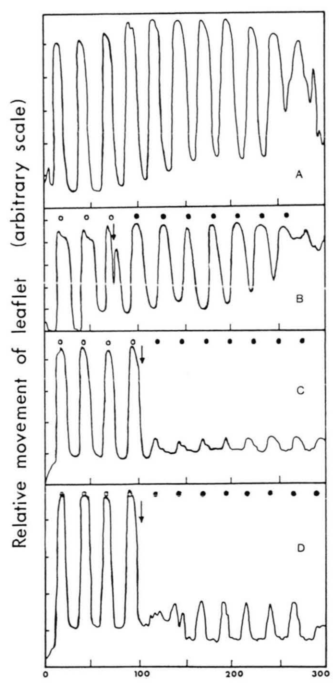

Fig. 3. Recordings of the leaf movements in *Oxalis regnellii.* A) Non-loaded control plant; B) plant loaded with 0.1 g at arrow; C) and D) examples of plants loaded with 0.8 g at arrows. In B, C and D points above the curves (●) indicate predicted timing of maxima (based on the rhythm before loading, ○). Period remains the same after loading, but phase shifts might occur (*e.g.* curve C).

Discussion

In the experiments by Bose [1] the load was increased with 5, 10 and 20 mg. The load changed both period and amplitude. The period seems to change in the ratio 1:1.06:1.06:1.16, as judged from Fig. 147 in the book cited. The amplitude of the pulsations decreased and was roughly halfed for a load between 5 and 10 mg. A 25 mg load is reported to halt the oscillations.

Loading of *Codariocalyx* leaflets has also been carried out by Dutt and Thakurta [6] who restricted their study to the effects of loading on the amplitude and on the load-work relations. They did not pay any attention to the effect on the period of the oscillations. It should be observed that their recording method influenced the loading conditions (as was also the case in Bose's studies).

We could confirm the qualitative observations by Bose and also add some further observations. The amplitude of the *Codariocalyx* leaflet movements is decreased by physical loading. The period changes transiently after loading, and it is therefore necessary to specify the period under steady state conditions. We recorded a period lengthening due to the loading in about 80% of the leaflets. In some cases the oscillations halted, and in some very few cases the period was slightly decreased.

In the nyctinastic movements of *Oxalis,* however, we could not find any period changes after rather heavy loading with 0.4, 0.6 or 0.8 g. Even if it caused a momentaneous seismonastic movement of the leaflets, the circadian movement resumed without period changes. In most cases the leaf position maxima of loaded plants occurred as predicted from the unperturbed (control) plants, in the others a phase shift occurred.

We must therefore conclude that in this circadian leaf movement system, the water potential is not part of the period determining structures. If it is, the increased pressure caused by the loading was insufficient to pass a threshold value to cause observable effects. Since the load corresponded to several times the mass of the leaflet, studies of leaf movements will be possible on, *e.g.*, a $3 \times g$ centrifuge without affecting the period of this circadian rhythm (even if amplitude changes might then occur).

The results point to a fundamental difference in the oscillators behind the ultradian *Codariocalyx* movements and the circadian *Oxalis* movements. In the *Codariocalyx* case the physical forces (the hydrostatic pressure) in the motor organ constitute a frequency determining part of the oscillatory system; in the *Oxalis* system this is not so. In discussions about the structures of the leaf movement rhythms and their origins [2], this is a principal difference to be taken into account. Since the water potential depends on the osmotic pressure (as well as on the hydrostatic pressure), the results indicate that the period of the *Codariocalyx* leaflet movements should depend strongly on the osmotic pressure in the motor cells. Furthermore, an osmotic effect on the period of *Oxalis* should be comparatively small, if it exists at all.

A case for further studies would be to see what happens to the circadian oscillating leaves of the *Codariocalyx* plant when being loaded. Do they follow the pattern of the ultradian oscillating leaflets from the same plant, or will they follow the same pattern as the leaves from the *Oxalis* plant? This experiment would reveal whether the difference between the two cases discussed in this paper is a consequence of the difference between ultradian and circadian rhythms or if it is a consequence of differences between the two species, *Oxalis* and *Codariocalyx*.

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