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The Circadian Rhythm of Leaf Movement of *Coleus blumei* x *C. frederici*, a Short Day Plant. I. Under Constant Light Conditions^{1, 2}

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Abstract. A new instrument for the recording of leaf movement rhythm is described. *Coleus blumei* x *C. frederici*, a short day plant, exhibits a circadian rhythm of leaf movement. The period length of the free running rhythm is shortest in continuous darkness and is increased with an increase in the light intensity. The amplitude of the rhythm tends to damp in continuous bright light.

Circadian rhythms are those biological rhythms with a period length of approximately 24 hr in constant conditions of light and temperature; the period length is temperature compensated. Most of the current knowledge of circadian rhythms in higher plants comes from studies of the rhythm of leaf movement in day-neutral species of *Phaseolus* (1, 2, 6, 7, 11) and the petal movement of the short-day-plant *Kalanchoe blossfeldiana* (3, 4, 13). It is the purpose of this paper to report the circadian rhythm of leaf movement in a short-day-plant, *Coleus blumei* x *C. frederici*, under different light intensities.

Several techniques for recording leaf movement were tested by this author. These were the kymograph and leverage system (1, 2), time lapse photography (5, 7), and the strain gauge method (8). These methods were found unsatisfactory for the present study and therefore a new one was developed, which is presented below.

Materials and Methods

Method for Leaf Movement Recording. The height of the leaf is measured hourly by periodically raising a motor driven contact until it completes a circuit by touching an electrical contact mounted on the leaf. Synchronously, a second motor drives a pen which records the height at which contact was

made, on pressure-sensitive paper mounted on a rotating kymograph.

The height sensing unit (fig 1a), located inside the plant growth chamber, consists of 2 electrical contacts and a reversible motor. The motor rotates a 12-inch long vertical lead screw which raises a gold-foil electrical contact until it touches the second electrical contact, a 15 mg 0.002 inch diameter gold wire suspended at the tip of the leaf. The contact reverses the synchronous motor and causes the driven gold foil to descend to its original reference point. No electrical current passes to the leaf itself.

The recording unit located outside the growth chamber consists of a similar lead screw to which a pen is attached, a reversible motor, and a drum covered with pressure sensitive recording paper (fig 1b). The pen travels vertically along the lead screw and registers the distance on the recording paper. By changing the relative speed of the height-sensing and recording motors one can either amplify or reduce the height of the recording. The drum rotates once per week. In test records made at 1 min intervals a maximum difference of about 0.5 % of the average height of the leaf movement was observed. Figure 2 is a sample of leaf movement recordings; the shortest vertical line represents the minimum position of the leaf, whereas the longest one represents the maximum height of the leaf. The time of the minimum leaf position is used as the phase reference point in the circadian cycle; it can be determined accurately to ± 1.0 hr. Tracings of the original leaf movement recordings are used in further graphs.

Plant Material and Light Conditions. A clone of *Coleus blumei* x *C. frederici* was used (a gift from Professor Laibach). Plants propagated by cuttings were grown in the greenhouse under con-

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tinuous illumination; during the night, light was supplied by 500 watt incandescent bulbs. Plants were transferred to the growth chamber (Model E0025, National Appliance Company) when they reached the 4 to 6 internode stage (not counting unfolded leaves). Only one 12 to 14 cm-long leaf on each plant was used for recording; the leaf below was cut off to prevent interference with the recording (fig 1a).

A light-dark cycle of 12 hr light, 12 hr dark was maintained in the growth chamber for 3 to 4 days before subjecting the plants to the experimental conditions.

In addition to light-dark cycles, the following conditions were studied: 1) Constant darkness; 2) Constant darkness + 4% w/v sucrose and a trace of Phaltan, a commercial fungicide. The solution of sucrose + Phaltan (200 ml) was added to each pot (7 inches in diameter) on the first day in constant dark; 3) Constant dim light of 10 ft-c; 4) Constant dim light of 30 ft-c; 5) Constant bright

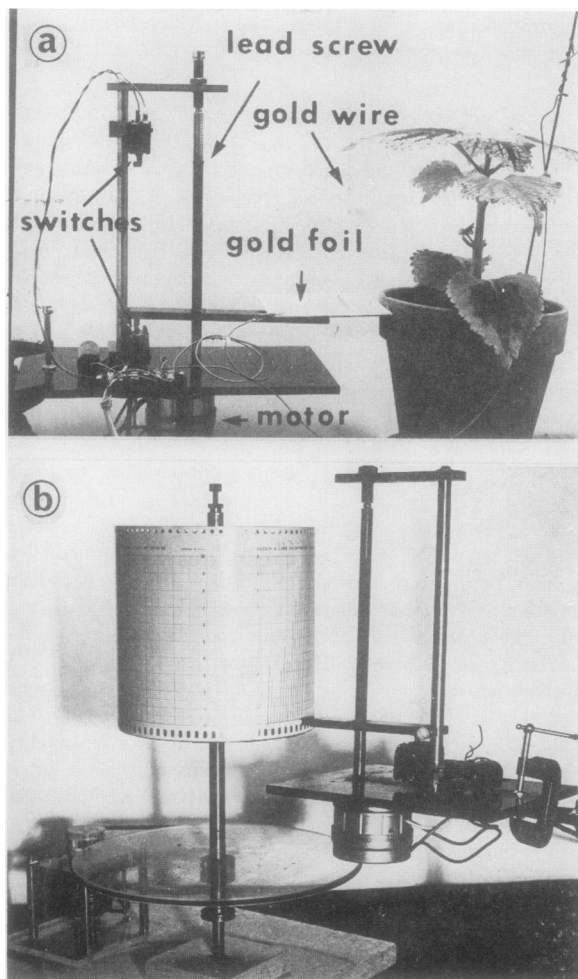


FIG. 1. The leaf movement recording apparatus: a) A photograph of *Coleus blumei* x *C. frederici* and its height sensing unit; b) The recording unit. For further details see text.

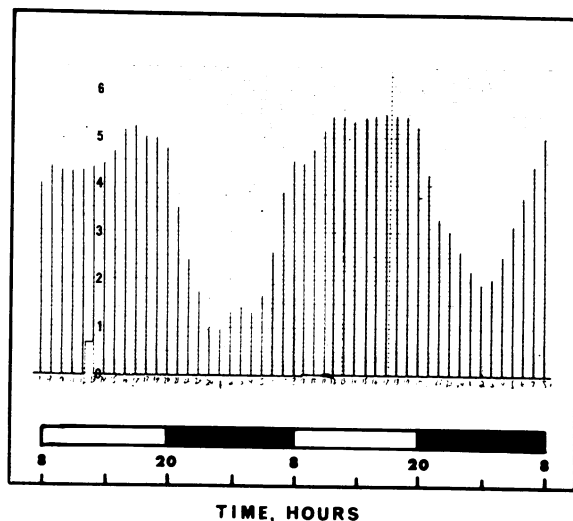


FIG. 2. A sample of actual leaf movement recording of *Coleus* under light-dark conditions. Distance between vertical numbers is 13 mm. Each black bar on the abscissa represents a 12 hr dark period ending at 0800 eastern standard time.

light of 1300 ft-c. The light intensity, as measured with a foot-candle meter (Weston illumination meter Model 756, Daystrom, Incorporated), was approximately 1300 ft-c at the plant level during the main light period. The lamps used were 32 cool-white fluorescent bulbs (Sylvania F9612/CW/VHO/L) and 12 60-watt incandescent bulbs, all separated from the plants by heat-reducing transparent plastic sheet. Spectral energy distribution curve for the cool-white fluorescent bulbs is given by Thomas and Dunn (12). The lights were turned on in the light-dark regime at 0800 eastern standard time (EST) and went off at 2000 EST. The transition from dark to light and *vice versa* occurred in stages and took about 30 min.

Light intensity of 30 ft-c was obtained by reducing the number of operating bulbs in the growth chamber and by the addition of a darkened cheesecloth below the bulbs. Light intensity of 10 ft-c was obtained by using a 20 watt fluorescent bulb (Arc Ray).

Temperature in the growth chamber was maintained constantly at $21 \pm 1^\circ$.

Table I. Values of the Free Running Period (τ) of Leaf Movement Under Different Light Conditions

Light conditions	$\tau \pm$ Standard deviation of the slope
Constant darkness (DD)	22.67 \pm 0.06
Constant darkness + sucrose	22.33 \pm 0.06
Constant dim light (10 ft-c)	23.06 \pm 0.02
Constant dim light (30 ft-c)	23.86 \pm 0.08
Constant light (1300 ft-c)	24.79 \pm 0.01

Results

Tracings of the pattern of the movement of an individual leaf of 1 plant in each of the above listed conditions are represented in figure 3a to f. Regression lines were fitted to the data (fig 4) to estimate the period of the circadian rhythm under the different conditions and table I tabulates these values.

A statistical analysis of the slopes of the regression lines (table II) shows that the slope of the regression lines in constant dark compared to constant dark + sucrose and of 2 different runs in constant light of 1300 ft-c are not significantly different. The purpose of supplying sucrose to plants kept in constant darkness was to prolong the leaf movement rhythm for more than 2 days by providing a source of metabolic energy. However, as the sample in figure 3c indicates, the pattern of the rhythm is highly erratic, and, furthermore, the period length for individual plants is inconsistent, varying between 21 and 26 hr. The addition of sucrose to the pot changes the pattern of the leaf movement (perhaps by raising the osmotic pressure in the pot) and, therefore, is not similar to constant darkness alone. The slopes of regression lines for constant darkness, constant dim light of 10 and 30 ft-c and bright light are significantly different from each other.

Table II. Statistical Test for the Significance of Period Differences in Different Light Conditions

The coefficients of regression lines fitted to time of minimum leaf position on successive days (fig 4) are the measure of the period length. Student's t-values are calculated for the conditions indicated in the table.

Treatment	t-values
DD + sucrose vs. DD	0.930
DD vs. dim LL 10 ft-c	2.477 ¹
DD vs. LL 30 ft-c	3.070 ²
DD vs. LL 1300 ft-c	5.700 ²
dim LL 10 ft-c vs. dim LL 30 ft-c	4.850 ²
dim LL 10 ft-c vs. LL 1300 ft-c	-11.220 ²
dim LL 30 ft-c vs. LL 1300 ft-c	4.480 ²
LL 1300 ft-c vs. repeat LL 1300 ft-c	1.028

- 1 Periods significantly different at the 95 % confidence level.
- 2 Periods significantly different at the 99 % confidence level.

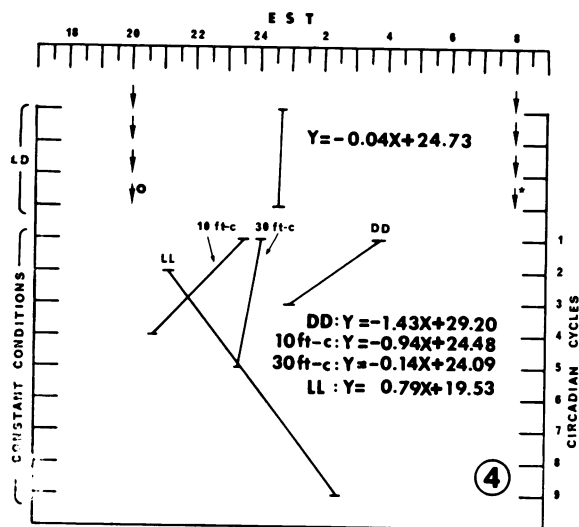
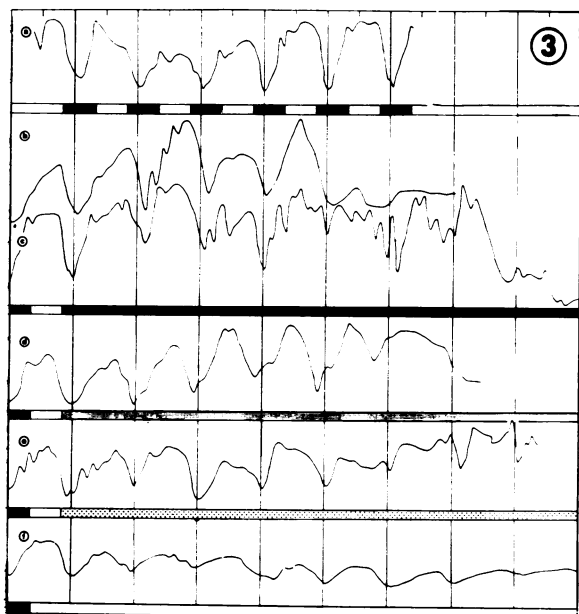


FIG. 3. a to f. Nyctinastic leaf movement tracings of *Coleus* under different light conditions. Vertical lines are 24 hr apart and indicate hr 2400 eastern standard time. Each recording is a typical representative from a group of 5 plants. a) Under light-dark cycles of 12 hr light (empty bars) and 12 hr dark (black bars). b) Plant entrained under 12 hr light and 12 hr dark then released to constant darkness. c) Plant released to constant darkness; sucrose was added to the pot. d) Plant released to constant dim light of 10 ft-c. e) Plant released to constant dim light of 30 ft-c. f) Plant released to constant bright light of 1300 ft-c.

FIG. 4. Calculated regression lines for different light conditions fitted to the minimum leaf positions in successive days (indicated by the vertical divisions). Numbers on top scale indicate eastern standard time (EST) in hr. LD = light dark cycles of 12 hr light and 12 hr dark, with light turned off at 2000 EST (arrows on the left), and turned on at 0800 EST (arrows on the right). LL: Plants were transferred from the light-dark regime to constant bright light of 1300 ft-c which started at

0800 EST (arrow at right that is marked by an asterisk). DD: Plants were transferred from the light-dark regime to constant dark which started at 2000 EST (arrow at left marked by an open circle). Regression line for DD + sucrose was not significantly different from DD, and therefore was omitted. 10 ft-c and 30 ft-c: Plants were transferred from the light-dark regime to constant dim light of either 10 ft-c or 30 ft-c, starting at 2000 EST (arrow marked by an open circle).

Discussion

Table I indicates that the endogenous rhythm of leaf movement in *Coleus blumei* x *C. frederici* persists in constant light as well as in constant dark with a free running period (τ) of about 24 hr. It also shows that the value of the free running period (τ), as calculated from the regression lines, depends on the light intensity; it increases with increasing light intensity. The effect of light intensity on leaf movement is species specific rather than general. The rhythm in *Phaseolus* persists under continuous light of different intensities without any damping effect or significant changes in period length (7, 11). *Kalanchoe* petal movement gradually damps under increasing light intensities whereas the free running period shortens (3). The rhythm of leaf movement in *Canavalia ensiformis* damps under continuous illumination of high intensity with no significant difference in the period from that in constant darkness (10). Persistence of leaf movement rhythm under light of high intensity was also observed in *Portulaca grandiflora* (9).

The tracing of the leaf movement in *Coleus* shows another feature (fig 3, b to f): the amplitude of the rhythm is greatest in constant darkness and damps with increasing light intensities.

From both the tracings of leaf movement and the regression lines for constant darkness and constant dark + sucrose (fig 3, b to c, and 4), it is evident that there is a phase delay of 3.6 and 2.5 hr, respectively, in the position of the minimum point on Day 1 compared to Day 0 (these values are achieved by subtracting the average time of minimum leaf position on Day 0 from that on Day 1). The same calculation, applied to conditions of constant light of 1300 ft-c, shows that there is a phase advance of 3.1 hr, which is achieved by the second cycle in constant light conditions (fig 3f and 4). One can suppose, therefore, that in a rhythm entrained by a light-dark cycle, the "light-off" signal initiates a phase delay of 3 hr (the average value of the 2 constant darkness regimes) which is followed, after the "light-on" signal, by a phase advance of 3 hr.

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