Light-induced Changes in the Period of the Circadian Rhythm of Carbon Dioxide Output in *Bryophyllum* Leaves

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**Summary.** The period of the rhythm of carbon dioxide output from leaves of *Bryophyllum fedtschenkoi* R. Hamet et Perrier at 15°C was shorter in continuous white light than in darkness. The period was monitored in leaves exposed to narrow spectral bands of monochromatic radiation at an incident quantum flux density of $4.7 \times 10^{-11}$ einsteins cm$^{-2}$s$^{-1}$. Bands centred on 660, 600, 730 and 530 nm significantly shortened the period, the greatest effect being achieved at 660 nm and the smallest at 530 nm; those centred on 760 and 450 nm were without effect. None of the bands tested significantly lengthened the period. The period of the rhythm in leaves exposed continuously to monochromatic radiation at 660 nm decreased with increasing quantum flux density. The extent to which a quantum flux density of $4.7 \times 10^{-11}$ einsteins cm$^{-2}$s$^{-1}$ at wavelength 660 nm shortened the period depended on the ambient temperature. At 15°C a significant reduction of 4.4 h occurred compared with the dark control, while at 30°C no significant reduction was observed. The transient (the time from the initiation of the rhythm to the first peak) showed a greater dependence on temperature than did the steady-state period. No such difference could be detected in relation to the intensity or quality of irradiation. The reduction of the transient by the various irradiation treatments was, in general, proportional to the reduction of the period.

**Introduction**

Several studies have been made of the effects of continuous illumination on circadian rhythms in plants. At high radiant flux densities the rhythms are usually inhibited, but at low flux densities they can persist for several days and under such conditions modification of the period has been observed. Different plants appear to react in a different manner since there are reports that the period may be lengthened (Halaban, 1968), shortened (Hastings and Sweeney, 1959) and unaffected (Kleinhoonte, 1932) by exposure to white light.

The spectral composition of the light employed may, in part, be responsible for the different responses observed. For example, in *Phaseolus multiflorus*, the period of the leaf movement rhythm is lengthened by fluorescent light which is rich in red but deficient in far-red radiation, and is shortened by tungsten light which is rich in both red and far-red radiation (Lörcher, 1958). The use of broad-band transmission filters revealed the lengthening effect of red, and the shortening effect of far-red, radiation (Lörcher, 1958). On the other hand, the period of the leaf movement rhythm of *Coleus blumei* × *C. Frederici* is lengthened by continuous exposure to the blue region of the spectrum and shortened by exposure to the red region, far-red radiation being without effect (Halaban, 1969).

Leaves of the Crassulacean plant *Bryophyllum fedtschenkoi* exhibit a circadian rhythm in their rate of carbon dioxide output when kept in darkness, at a constant temperature and in an air stream initially free of carbon dioxide. This rhythm appears to be inhibited by high flux densities of radiation at wavelengths greater than 565 nm. A rhythm of CO$_2$ output can however be initiated in irradiated leaves provided they are subjected to a sufficiently large decrease in radiant flux density (Wilkins, 1960).

Using a different measuring technique Jones (1973) and Jones and Mansfield (1970, 1972) recorded a circadian rhythm in carbon dioxide compensation in *Bryophyllum* leaves continuously exposed to a relatively high intensity of white light. This rhythm was thought to depend upon the same basic oscillating system as the rhythm of CO$_2$ emission reported by Wilkins (1960). The compensation rhythm was, how-
ever, initiated either by a decrease in the radiant flux density or by a "light on" signal, and its period was relatively unaffected by the intensity of the continuous illumination.

After exposure to an initiating or phase-resetting signal a rhythm may attain a steady-state rapidly or after one or more transient cycles. In Bryophyllum, the CO₂-emission rhythm reaches a steady state by the time of the next peak of carbon dioxide output (Wilkins, 1960). The transient, or time from initiation to the first peak, is reported to be influenced to a greater extent than the free running period by light intensity (Jones and Mansfield, 1972) and temperature (Wilkins, 1962).

This paper reports a detailed investigation of the effects of the flux density and wavelength of continuous irradiation on the period and transient of the rhythm of carbon dioxide output from leaves of Bryophyllum at several temperatures.

Materials and Methods

The experimental plant material was Bryophyllum (Kalanchoë) fedtschenkoi R. Hamet et Perrier. The stock of plants had been derived vegetatively as cuttings from a single original plant. They were grown in a greenhouse and provided with supplementary irradiation from mercury-vapour lamps to give a photoperiod of at least 16 h. Experiments were carried out on single leaves detached from plants which had been transferred from the greenhouse to a controlled environment room and maintained for at least 7 d in an 8 h photoperiod. Irradiation was provided by a mixture of daylight and warm white fluorescent lamps giving a radiant flux density of 47.3 J m⁻² s⁻¹ at bench level. The temperature was 25 °C during the photoperiod and 15 °C during the dark period.

The rate of carbon dioxide output into initially carbon dioxide-free air was measured with Grubb-Parsons SB1 and SB2 infra-red gas analysers; full details of the procedure have been given by Wilkins (1973).

Leaf chambers were maintained at constant temperatures in water baths. Monochromatic radiation was provided by Bausch and Lomb, High-Intensity Grating Monochromators mounted vertically above the water baths. Spectral bands 25 nm wide were used. Two layers of Cinemoid Orange (No. 5) filter were inserted in the beam when the monochromators were set at wavelengths longer than 560 nm to eliminate overlapping blue light of other order spectra from the grating. The quantum flux density was adjusted in the different bands of the spectrum by varying the potential across the lamp of the monochromator.

The leaves were detached from the plants, weighed, placed in the chambers and transferred to constant conditions at 1600 h. In each experiment two leaf chambers were irradiated and one was kept in darkness as a control. The rate of carbon dioxide output of the leaves was calculated in μg CO₂ h⁻¹ g (fresh weight)⁻¹ and plotted hourly against the time of day. The period of the rhythm was taken as the mean time between successive peaks during the first four cycles in constant conditions. The time from the start of the experiment to the first peak of carbon dioxide emission was termed the transient.

Results

The Rhythm in White Light. Rhythms in the rate of carbon dioxide output were initiated by transferring the leaves from the controlled environment room to darkness or a low radiant flux density of white light at 15° C at the end of the normal photoperiod. Examples of rhythms in darkness, in radiant flux densities of 0.6 J m⁻² s⁻¹ from white fluorescent lamps and of 2.5 J m⁻² s⁻¹ from tungsten lamps are shown in Figure 1A–C. The period of the rhythm was reduced from approximately 24 h in darkness to 20.3 h and 19.1 h, and the transient from 26 h in darkness to 22.0 h and 19.3 h by the irradiation treatments respectively.

Effect of Wavelength. To determine which regions of the spectrum were responsible for the effects of white light on the period, and to assess whether any wavelengths lengthened the period, as had been found in other organisms, leaves were continuously irradiated at 15° C with a number of 25 nm-wide spectral bands at a quantum flux density of 4.7 × 10⁻¹¹ einsteins cm⁻² s⁻¹. The results are shown in Figure 2.

Continuous irradiation with spectral bands centred at 730, 660, 600, and 530 nm significantly reduced the period as compared with that in darkness. Maximum reduction occurred at 660 nm and 600 nm while least reduction occurred at 530 nm. None of the spectral bands tested significantly lengthened the period. The transient was also shortened by the 660,