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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 4.7×10^{-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 4.7×10^{-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 as 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 blumeii* x *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, however, 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 CO_2-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 CO_2 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,

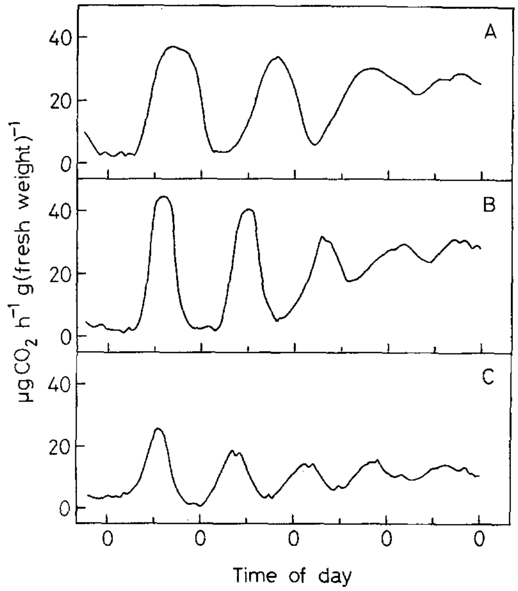

Fig. 1A–C. The circadian rhythm of carbon dioxide output in *Bryophyllum* leaves at 15° C in (A) darkness, (B) 0.6 J $m^{-1}s^{-1}$ from white fluorescent lamps and (C) 2.5 J $m^{-2}s^{-1}$ from tungsten lamps. 0 midnight

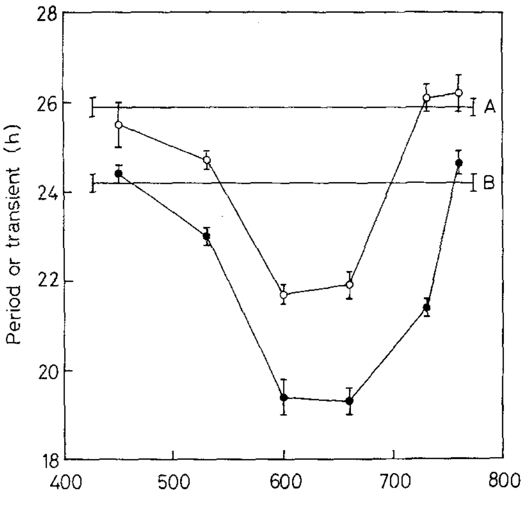

Fig. 2. The effect of continuous exposure to an incident quantum flux density of 4.7×10^{-11} einsteins $cm^{-2}s^{-1}$ in 25 nm-wide spectral bands of radiation on the period (closed circles) and the transient (open circles) of the rhythm of carbon dioxide output in *Bryophyllum* leaves at 15° C. The values of the transient and of the period in darkness are shown respectively by lines A and B. The vertical lines are 2 × the standard errors of the means

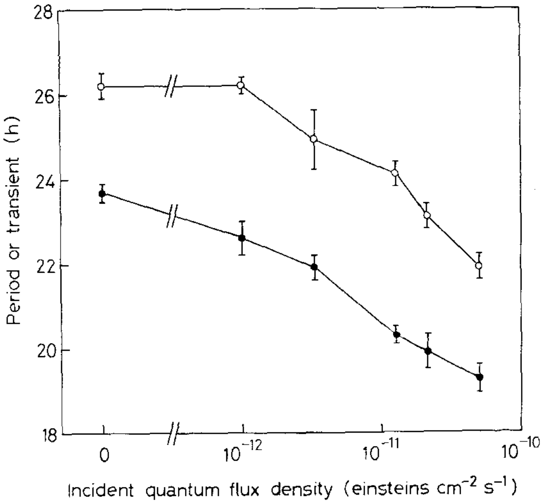

Fig. 3. The effect of the incident quantum flux density of a 25 nm-wide spectral band of radiation centred at 660 nm on the period (closed circles) and transient (open circles) of the rhythm of carbon dioxide output from leaves of *Bryophyllum* at 15° C. The vertical lines are 2 × the standard errors of the means

600 and 530 nm spectral bands by an amount proportional to the reduction of the period. However, while the spectral band centred at 730 nm reduced the period, it did not reduce the transient. These results establish that radiant energy in the red region of the spectrum is primarily responsible for the modification of the period of the rhythm observed in white light.

Effect of Flux Density. Leaves were continuously exposed at 15° C to 5 quantum flux densities of monochromatic radiation in the spectral band centred at 660 nm which had been found to be most active in shortening the period. The results of this series of experiments are shown in Figure 3. All the quantum flux densities employed significantly reduced the period as compared with that observed in darkened leaves, the magnitude of the reduction having an approximately linear relationship to the logarithm of the quantum flux density. The transient also decreased with increasing quantum flux density; the magnitude of the reduction was approximately proportional to that of the period for a given flux density. The lowest of the quantum flux densities used, however, significantly reduced the period but not the transient. There was also an approximately linear relationship between the magnitude of the reduction of the transient and the logarithm of the quantum flux density.

Interaction of Light and Temperature. In continuous darkness, the period of the rhythm in *Bryophyllum* leaves shows a small but significant dependence on the ambient temperature (Wilkins, 1962). The period of the rhythm in leaves continuously irradiated was compared with that in leaves held in darkness throughout the experiment over a range of temperatures to determine whether or not the effectiveness of red radiation in shortening the period and transient was influenced by temperature. Since preliminary experiments had shown the carbon dioxide output of *Bryophyllum* leaves at 10° C or 35° C to be arhythmic, the present experiments were confined to the 15–30° C range. Rhythms in leaves in darkness and in those irradiated with 4.7×10^{-11} einsteins $cm^{-2}s^{-1}$ of monochromatic radiation at 660 nm were recorded at 4 ambient temperatures.

The periods and transients under these conditions are shown in Figure 4. The periods of rhythms in both darkened and irradiated leaves increased between 15° C and 25° C and decreased between 25° C and 30° C. However, while irradiation of the leaves reduced the period at 15° C by 4–5 h the magnitude of this reduction decreased with increasing temperature until at 30° C it was no longer significant.

The transient decreased with increasing temperature over the range 15–30° C whether the plants were in constant light or in darkness. Whether or not the transient was shortened by irradiation also depended on the ambient temperature in a manner similar to that observed for the reduction in the period. The magnitude of the reduction progressively decreased between 15° C and 25° C while at 30° C no reduction was detectable. Although the transient showed a

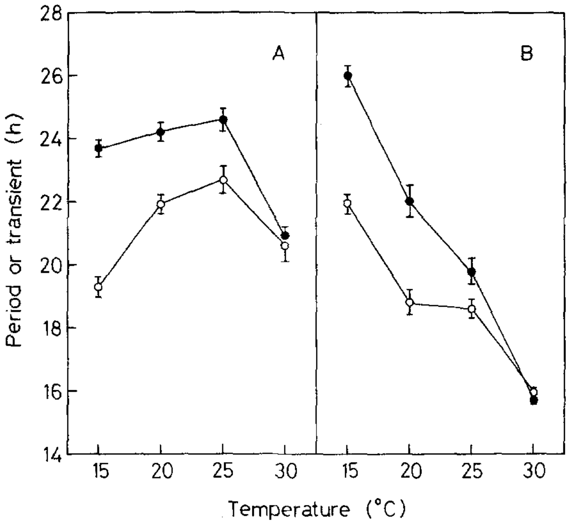

Fig. 4A, B. The effect of temperature on (A) the period and (B) the transient of the rhythm of carbon dioxide output from leaves of *Bryophyllum* in darkness (closed circles) or irradiated with 4.7×10^{-11} einsteins $cm^{-2}s^{-1}$ in a 25 nm-wide spectral band (open circles) of radiation centred on 660 nm. The vertical lines are 2 × the standard errors of the means

greater dependence than the free-running, steady-state period of the rhythm on the ambient temperature, the reduction of the transient induced by irradiation was approximately proportional to the reduction of the period.

Discussion

The period of the circadian rhythm of carbon dioxide emission from leaves of *Bryophyllum fedtschenkoi* is shorter in continuous light than in darkness. Increasing the light intensity causes a progressive decrease in the period. In this respect the rhythm in *Bryophyllum* is similar to those of luminescence in *Gonyaulax polyedra* (Hastings and Sweeney, 1959) and of petal movement in *Kalanchoë blossfeldiana* (Bünsow, 1953). On the other hand, the period of the leaf movement rhythms in *Coleus blumeii* × *C. frederici* (Halaban, 1968) and *Pharbitis nil* (Bollig, 1974) increase with increasing light intensity, while that of the leaf movement rhythm in *Canavalia ensiformis* remains unchanged (Kleinhoonte, 1932).

Although some exceptions have subsequently been found (Hoffman, 1965), Aschoff (1960) deduced a general rule relating the period to the incident light intensity from investigations of animal rhythms. This stated that when exposed to continuous illumination the period of rhythms in diurnal animals decreased, while that in nocturnal animals increased, with increasing light intensity. Furthermore, the changes in the period were, for the most part, linearly related to the logarithm of the light intensity. While the two response-types found in animals obviously have no counterpart in plants, the present findings with the *Bryophyllum* rhythm agree closely with Aschoff's (1960) rule with respect to the log-linear nature of the relationship between the period and quantum flux density.

Exposing leaves of *Bryophyllum* to high flux densities in the red region of the spectrum had previously been shown to inhibit the rhythm of carbon dioxide emission (Wilkins, 1960). The greatest modification of the period has also been obtained with continuous exposure to lower flux densities of radiation in spectral bands centred on 660 nm. The detailed spectral dependence of this response differed slightly from that found for the induction of a phase shift in the rhythm by a single 4-hour exposure to radiation (Wilkins, 1973) in that the spectral band centred on 730 nm modified the period but did not induce a phase shift. The longer exposure and larger total quantum dose of radiation given in the present experiments on period control may account for the activity of the 730 nm band, especially since a photostationary state may be slowly established in the phytochrome system in which a small amount of Pfr is maintained throughout the experiment. However, the 730 nm spectral band has also been found to be active in entrainment experiments in which leaves were irradiated each day for only 15 min. In such experiments a complete reversal of the effects of 660 nm spectral band by far-red radiation has been achieved (Harris and Wilkins, unpublished). There can thus be little doubt that phytochrome is the primary photoreceptor involved in the control of the period of the rhythm in *Bryophyllum*.

Phytochrome has also been implicated in the photocontrol of the leaf movement rhythm in *Phaseolus multiflorus* in which far-red radiation shortens, and red radiation lengthens, the period (Lörcher, 1958). This finding differs from the present results, since in *Bryophyllum* we find no spectral band increases the period, far-red (730 nm) radiation having an effect similar to, though less marked than, red radiation (660 nm). In *Phaseolus* the situation is obviously more complex in that far-red radiation has an essentially different effect from red. This difference in the effectiveness of red and far-red radiation is also evident in the phase-response data for *Phaseolus* where red and far-red radiation give different phase-response curves and are effective only when absorbed by different parts of the plant (Bünning and Moser, 1966). In *Coleus* alternative or additional pigments must be involved since the red and blue regions of the spectrum are active and opposite in their effect; red shortens and blue lengthens the period while far-red is ineffective (Halaban, 1969).

Although at none of the temperatures tested was the period of the *Bryophyllum* rhythm increased by irradiation, the extent to which it was decreased depended upon the ambient temperature. Thus, an analysis of the effects of irradiation on the period of a rhythm must take account of the possibility that different responses may occur at different temperatures, and conversely of the possibility that the effect of temperature on the period may vary with the irradiance. The high degree of temperature compensation reported for the *Bryophyllum* rhythm in darkness (Wilkins, 1962) is borne out by the present results. The magnitude of the reduction in period observed between the lower and upper temperature limits at which the rhythm persisted in the present investigation is also very similar to that reported by Wilkins (1962). The effect of temperature on the period over part of the temperature range examined was slightly different from that reported earlier. A small but significant increase in the period between 15° C and 25° C was observed in the present investigation whereas previously a small decrease between 16° C and 26° C was observed (Wilkins, 1962). The pattern of change in the period with temperature reported here is somewhat similar to that found in the rhythm of luminescence in *Gonyaulux polyedra* (Hastings and Sweeney, 1957). The absence of a significant modification of the period by irradiation reported in an earlier study (Wilkins, 1960) may have been due to the shorter periods obtained in darkness at 26° C, a temperature at which, on the basis of the present results, only a relatively small reduction of the period by radiant energy would be expected.

The water balance within a plant has been reported to modify the period of circadian rhythms. Bünning and Moser (1968) found that withholding water from *Phaseolus* seedlings had an effect on the leaf movement rhythm similar to that obtained with red light in that the period could be lengthened, and the phase shifted. In this investigation some loss of turgor was noted in detached *Bryophyllum* leaves after several days at 30° C and to a lesser extent at 25° C, but not at lower temperatures. The sharp reduction in the period between 25° C and 30° C might therefore be attributed, at least in part, to a loss of turgor. Differences in the resistance to water loss, particularly between leaves grown under different conditions, may also account for the slight difference in the results of this investigation, and that of Wilkins (1962). The effects of water stress on the circadian rhythm in *Bryophyllum* leaves require further investigation.

The changes in the transient with ambient temperature are much larger than those in the steady-state period, as had been reported previously (Wilkins, 1962). The changes in the transient induced by irradiation were, in general, proportionally related to those in the period for a particular quantum flux density and spectral band. Two exceptions to this generalisation were noted; the period but not the transient was shortened (a) by the lowest of the quantum flux densities used in the spectral band centred at 660 nm, and (b) by the spectral band centred at 730 nm. The first of these exceptions suggests a difference in the sensitivity of the controlling mechanism to the radiant flux density during the transient on the one hand, and during the steady state of the rhythm on the other, the sensitivity being greater when the steady state of the rhythm has been established. The second of the exceptions cannot be due solely to a difference in the sensitivity to the radiant flux density since spectral bands which are less effective than that centred at 730 nm are, unlike the 730 nm band, also effective in reducing the transient. Jones and Mansfield (1975) have also reported a difference in the response of the transient and the period to radiant flux density. In contrast to the present results, they found the transient was the more sensitive, decreasing with increasing flux density while the free-running period was virtually unaffected.

Modification of the time from the initiation of the leaf movement rhythms in *Phaseolus* (Lörcher, 1958) and *Coleus* (Halaban, 1969) seedlings to the first phase reference point, in these cases the first minimum leaf position, does not show the same relationship to the change in period length as was found for the rhythm in *Bryophyllum*. In *Phaseolus* this time was virtually unaffected by wavelengths which either lengthen or shorten the period. The first minimum in *Coleus,* however, occurred earlier when plants were irradiated with all wavelengths tested, irrespective of their effect on the period, than when they were maintained in darkness.

The molecular mechanism by which continuous exposure to light modifies the period and transient of the rhythm in *Bryophyllum* leaves cannot be elucidated until the biochemical events involved in the generation of the rhythm have been identified.

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