

NATURAL AND FORCED CIRCADIAN OSCILLATIONS IN THE LEAF OF *TRIFOLIUM REPENS*

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Abstract

The leaves of white clover (*T. repens*) exhibit typical circadian characteristics with an endogenous period, τ , of 25–27 hr. Corresponding oscillations are observed of the torque exerted by leaflets prevented from closing. The mean variation in torque during a cycle was 339 ± 13 dyne cm. When leaves were subjected to light-dark cycles at constant temperature, entrainment was obtained for periods from 0.7 to 1.4 τ . Phase shifts were observed ranging from a lag of 40° for 0.7 τ to an advance of 130° for 1.4 τ . The pulvinus was shown to be both the photoreceptor and the site of the endogenous timer.

Similar responses to those for light were observed in leaves under constant illumination which experienced rhythmic temperature variations between 8 and 18°C, and between 15 and 22°C.

A mathematical model is considered of a simple oscillatory system whose natural oscillations and phase responses to forcing oscillations resemble those of the leaf. It is argued that the basic oscillator is linear, producing damped sinusoidal oscillations, and that the non-linearities which introduce higher harmonics in the observed leaf oscillation occur in the coupling between oscillator and output.

I. INTRODUCTION

In recent years, much attention has been given to rhythmic phenomena in living organisms. While these rhythms are often associated with periodic changes in the environment (e.g. diurnal, annual, or tidal), it can readily be shown in many cases that they are not merely passive responses to these changes since the rhythm persists with approximately the same period of oscillation in an unchanging environment. Other biological oscillators have periods which differ from those of any known environmental oscillations. For example, oscillations having periods within the range 4–20 min (depending on temperature) are observed in the bioelectric field of broad bean roots (Scott 1957; Gunther and Scott 1966).

Notwithstanding Brown's (1962) arguments in support of an exogenous timing system, there appears to be little doubt that the primary cause of many rhythmic biological changes is endogenous, although there may be close associations with environmental characteristics (such as light and temperature) that normally also vary rhythmically. Innate oscillatory behaviour is so widespread in living organisms that further studies may reveal it to be a fundamental characteristic of life. Recent reviews of rhythmic phenomena in plants are given by Sweeney (1969) and Cumming and Wagner (1968).

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It is remarkable that the many extensive investigations that have been undertaken have revealed so little about the basic mechanism of the timer or biological clock. Sweeney (1969) discusses the considerable amount of largely negative evidence that has been accumulated. Part of the difficulty arises because not all investigators have recognized that studies of the mode of interaction between a light-dark (L : D) cycle and a biological oscillation do not give direct information about the basic timer, which continues to operate in continuous light (L : L) or continuous darkness (D : D). For example, diurnal plant rhythms are often accounted for in terms of Bünning's "photophile-skotophile" hypothesis (Bünning 1950), or the "hour-glass" hypothesis (described by Cumming and Wagner 1968), yet these do not by themselves imply the endogenous periodicity needed to account for persistence of the oscillation in L : L or D : D. Preoccupation with photo-reactions including photo-periodicity may have directed attention away from the basic oscillator, although it is obvious that any model of an oscillator which interacts with light must take this interaction into account.

The present paper describes preliminary aspects of an investigation having the ultimate aim of describing the underlying causes of biological oscillations. The system chosen for study is the leaf of white clover, *Trifolium repens*, whose movements exhibit typical *circadian* characteristics (i.e. in constant environmental conditions the leaf continues to open and close with a period of about a day). The leaf movements occur because of the alternate swelling and compression of the cells on either side of the pulvinus at the base of each leaflet. This material was chosen because it apparently has not been studied before and because the oscillatory changes being observed ("hands of the clock") can be described, at least approximately, in terms of a single parameter, the angular position of the terminal leaflet with respect to the petiole. Furthermore the material can readily be subjected to various biological, chemical, and physical treatments to ascertain the effect of these on the biological oscillator.

The interaction of the biological oscillator with light and temperature oscillations was studied as a means of investigating the basic oscillator itself and not primarily to determine the nature of the interaction.

II. MATERIAL AND METHODS

The leaf of *T. repens* consists of one terminal and two lateral leaflets each attached to the common petiole by a short pulvinate stalk.

The terminal leaflet was chosen for study as its nyctinastic movements are simpler and more readily observable than those of the other leaflets. As the three leaflets under normal conditions perform these movements simultaneously, the state of the terminal leaflet and its pulvinus was taken as indicative of the leaf as a whole.

The experimental material was grown from cuttings (in some cases from seed) in small individual plastic pots filled with a mixture of peat-moss, sand, and fertilizer. Light from a combination of fluorescent and tungsten sources at an intensity of about 2200 lux was used in a 24-hr cycle of 12 hr light and 12 hr dark (denoted by 12L : 12D). Temperature was not closely controlled during growth and was about 20°C with the lights on and about 10°C in the dark.

For some later experiments, plants were grown in a growth cabinet which was divided into two compartments, one of which was dark and the other lit by fluorescent and tungsten lamps (intensity 2200 lux). The plants were arranged at regular intervals around a horizontal circular platform which was partly in each compartment and rotated about a vertical axis. Rotation of

the platform in steps of 15° each hour resulted in each plant spending 12 hr in darkness and 12 hr in light. The phasing of the L : D cycle for each plant depended on its position on the platform. Thus at any time a plant could be selected which was at any desired stage of its physiological cycle. An identical platform was constructed which could be rotated at different rates so that the effects of cycles of different periods could be examined.

Whole plants, excised leaves, and excised leaf portions were used in various experiments. The youngest fully expanded leaf on a shoot was usually chosen for study. The plant or excised leaf was transferred to a ventilated glass-fronted cabinet which could be controlled at temperatures in the range $8\text{--}30^\circ\text{C}$ to $\pm 0.5^\circ\text{C}$. Light was provided outside the cabinet by fluorescent and tungsten lamps so that the intensity at the plant was normally 1900 lux. For some experiments, a time clock was used to provide light-dark cycles of various periods both longer and shorter than 24 hr. In other experiments, two thermostats alternated as controllers of temperature in the box, producing cyclic temperature changes of a similar range of periods. In all the experiments described in this paper, the cycles comprised equal proportions of light and dark or hot and cold.

Time-lapse cameras (Vinten Scientific Mk III) were used in most experiments to record leaf movements, a photograph being taken every 5 min. Photography of the leaf during the "dark" phase was performed in the very dim illumination provided by a high-pressure mercury lamp and a Wratten No. 74 filter which passed only the green 546.1 nm line to which the leaf appeared to be insensitive. In some later experiments, dim white light (intensity about 5 lux) was used for illumination and photography during the "dark" phase as it did not appear to have a significant effect on the leaf cycle. The angular orientation of the terminal leaflet was estimated visually from the projected film to an accuracy of about $\pm 5^\circ$.

Other methods of studying leaf movements were used in some experiments. In one, the rotating leaflet turned a delicately pivoted arm whose angular position was tracked and recorded by means of a beam of light and a pair of phototransistors.

In another series of experiments, the torque exerted by a leaf which was prevented from moving was measured and recorded, using a simple feedback device adapted from a 1 mA moving-coil meter movement and shown schematically in Figure 1. Any slight movement of the leaf, *L*,

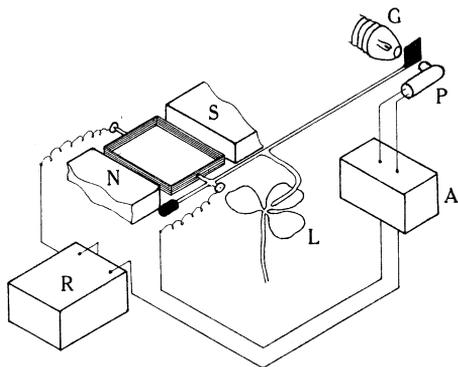


Fig. 1.—Schematic diagram of apparatus for measuring and recording the torque exerted by leaflet prevented from closing. See text for details.

exposes the phototransistor, *P*, to increased light from the small light globe, *G*, thereby causing a current (amplified in *A*) to flow in the coil which tends to be deflected in the magnetic field in opposition to the leaf movement. The current needed to prevent the leaf from moving, which is proportional to the torque it exerts, is recorded by *R* (Goerz Multiscript 3 dotting recorder). The instrument was calibrated by the application of known torques. Use of these non-visual methods of obtaining data was limited, however, because it was often found that a leaf experiencing other than the normal 24 hr environmental cycle moved in a complex fashion, with some movement and rotation about the main axis of the leaflet. This tended to introduce artifacts into the recorded data.

The leaflet angle was measured from the abaxial surface of the petiole to the abaxial surface of the leaflet. Thus a leaflet in the normal fully opened position is recorded as 90° , at half-closed it is 180° , and when in the normal fully closed position it is 270° .

III. RESULTS

(a) Leaf Position in 12L : 12D

Figure 2(a) shows a typical record of the angular position of the terminal leaflet in 12L : 12D (i.e. 12 hr light followed by 12 hr darkness every 24 hr). The temperature was kept constant for each experiment and in most cases was $20 \pm 0.5^\circ\text{C}$. It will be noted that the leaf commences to close before the light is switched off and commences to open before the light returns. This is a clear indication that the leaf does not move only as a direct response to a change in illumination. The angular velocity of the leaf is usually greatest when it is half-closed ($\theta = 180^\circ$) and in a 24-hr cycle this closely corresponds to the time of switching from light to dark or from dark to light. In 15 records, the maximum angular velocity was found to be 46.2 ± 2.3 deg/hr (the limits throughout this paper represent the standard error in the mean value).

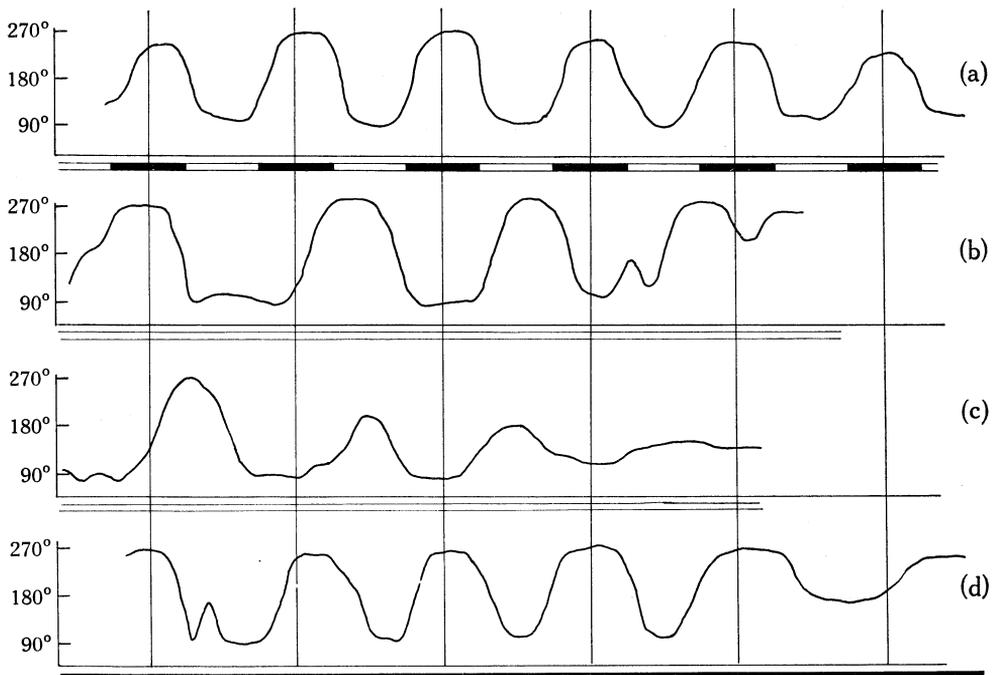


Fig. 2.—Angular position of freely moving leaf in 12L : 12D (a), in L : L (b) and (c), and in D : D (d). Temperature was constant at $20 \pm 0.5^\circ\text{C}$. Light condition indicated in strip below trace. Vertical lines denote midnight local time. When the leaf is closed the angle is large.

The motion of the leaf is periodic but non-sinusoidal, and shows some variation from cycle to cycle and from leaf to leaf. Fourier analysis of typical oscillations showed that the angular position could be described approximately by the equation

$\theta = [105 \sin(2\pi t/T) + 18 \sin(6\pi t/T) + 3 \sin(10\pi t/T)]$ degrees, where T is the period of oscillation. This function is shown graphically in Figure 3.

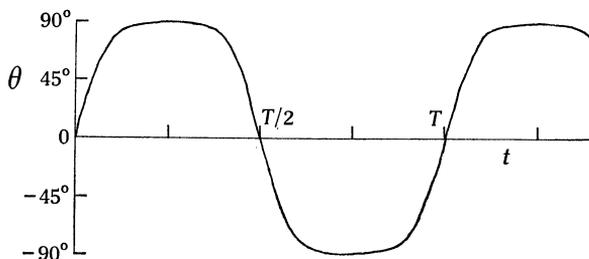


Fig. 3.—Graph of the function $\theta = 105 \cos(2\pi t/T) + 18 \sin(6\pi t/T) + 3 \sin(10\pi t/T)$.

The predominant component is therefore the fundamental oscillation (in this case of period 24 hr) with the 3rd harmonic (period 8 hr) and 5th harmonic (period 4.8 hr), having amplitudes 17 and 3% respectively of that of the fundamental. In a few records, however, the higher frequency oscillations are much more prominent, and rapid oscillatory changes having periods of from 2 to 6 hr are observed superimposed on the basic oscillation.

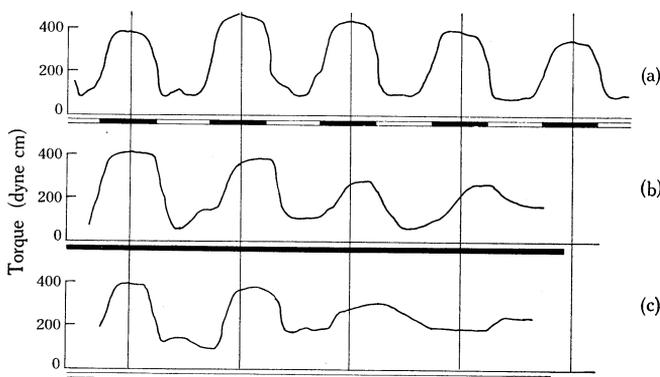


Fig. 4.—Torque exerted by leaf clamped in open position in 12L : 12D (a), in D : D (b), and in L : L (c). Temperature was constant at $20 \pm 0.5^\circ\text{C}$.

Figure 4(a) is a typical record showing the torque exerted by the terminal leaflet when it is prevented from closing. This record is very similar in form to those denoting angular changes of unclamped leaves [Fig. 2(a)]. It can therefore be concluded that either the angular position of the leaf or the torque exerted when clamped is a suitable index of the rhythmic pulvinal behaviour. During a cycle the torque exerted by a clamped leaflet was found to fluctuate through a range of 339 ± 13 dyne cm in records for eight leaves. This torque is far greater than is needed to support and move it in the Earth's gravitational field, which for a leaflet of mass 17 mg centred 7 mm from the pulvinus would require a torque of about 12 dyne cm.

It will be noted that the oscillations of both angle and torque are closely in phase with the 12L : 12D cycle. If the phase is reckoned from the difference between the mid-time for which the leaf is closed and the mid-time of the dark interval, there is a phase lag of (6.1 ± 5.8) deg in observations on eight leaves.

(b) Observations in Constant Environmental Conditions

Rhythmic leaf movements persist for a number of cycles when a plant is transferred to an environment in which light and temperature are unchanging. Corresponding torque oscillations are also observed. The form of the oscillation is very similar to that found in 12L : 12D. In some cases, such as illustrated in Figures 2(b) and 2(d), there is little sign of damping for four or five cycles although the rhythmic component may become less pronounced and random aperiodic movements more noticeable in the latter stages. In other cases such as Figures 2(c), 4(b), and 4(c), the oscillation is clearly damped. The average decrement (i.e. the ratio of the amplitudes of successive cycles) of the natural oscillations is about 1.5 and seldom exceeds 2.0.

The short-term response of the leaf to change in environmental conditions depends on the time at which the change is made (cf. Pittendrigh 1965). In the present experiments, prolonged exposure to darkness was commenced at 1800 hr for plants grown in normal day-night conditions. Continuous light treatment commenced at 0600 hr. This appeared to minimize transient responses, and leaves usually adjusted to the new regime within 24–36 hr, subsequently oscillating at near-constant period.

The following tabulation shows the natural period of oscillation (in hours) for 23 plants in different conditions of light and temperature:

Temp. (°C)	Continuous light	Continuous darkness
8.5	25.3	
12	25.8, 26.0, 26.0, 27.0, 27.0, 28.5	23.2, 24.5
15	25.3, 27.3	23.4
16		25.7
18	27.7, 28.6, 29.1	26.5, 28.5
20		25.0, 25.0, 26.0, 26.7
21.5	27.5	
Mean period (±S.E. of mean)	27.0 ± 0.40	25.4 ± 0.74

At temperatures outside the range 8–22°C, leaf movement was usually sluggish and aperiodic. Each result is the average period for a leaf over at least two cycles. It will be noted that the average period of natural oscillations is significantly greater than 24 hr and is greater in continuous light than in continuous darkness. Furthermore, in either L : L or D : D there is an apparent small rise in period with temperature, regression lines having slopes of 0.20 hr/degC and 0.29 hr/degC for the L : L and D : D data respectively.

(c) Effects of L : D Cycles of Various Periods

Results described in Section III(a) show that although the natural period of oscillation of the leaf is not exactly 24 hr, it can be driven to oscillate at a 24-hr period by a 12L : 12D cycle. Further experiments were performed to test the effects of L : D cycles of other periods. Plants grown in 12L : 12D were transferred to the changed L : D environment at the beginning of the dark interval as this appeared to minimize transient effects. The temperature was kept constant to $\pm 0.5^\circ\text{C}$ and for most experiments was 20°C. It was found that within the range of periods from 18 hr (i.e. 9L : 9D) to 36 hr (i.e. 18L : 18D), the leaf could be driven to oscillate at the

period of the driving oscillation, although the entrainment was often less marked near these extremes of period, and more random leaf movement ("noise") was observed.

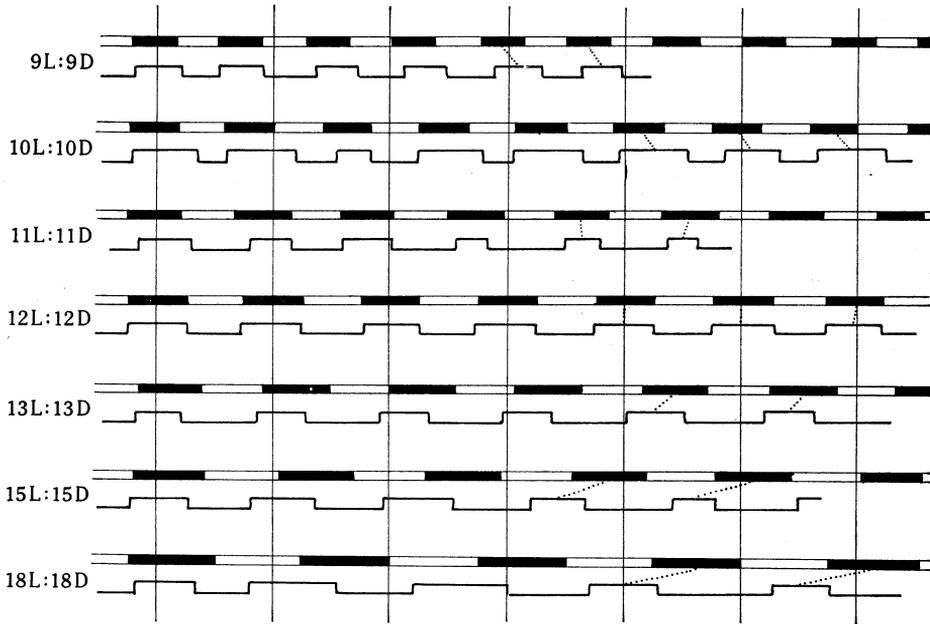


Fig. 5.—Leaves at constant temperature in L : D cycles of various periods. Crest of trace signifies "closed" leaf (i.e. angle $> 180^\circ$). Trough signifies "open" leaf (i.e. angle $< 180^\circ$). Dotted lines relate the mid-dark times to the corresponding mid-closed times for the last few cycles in each record.

Figure 5 shows typical results obtained at different L : D periods. For simplicity in this diagram, a leaf was regarded as "open" if the leaf angle was less than 180° and "closed" if the leaf angle was greater than 180° . After about 48 hr, it will be noted that the leaf has settled down to oscillate at the same period as the L : D oscillation. However, the phase of the leaf's oscillation is advanced for periods greater than 24 hr and retarded for periods less than 24 hr. This is shown in Figure 6 where the phase difference is plotted against period. The phase is determined from the interval between the mid-times of leaf closure and of dark. Each point is for a leaf of a single plant and the phase difference is averaged for at least two cycles after transient behaviour ceased. The graph includes data obtained from observations during several years. No seasonal effects were detected.

Despite the scatter of observations, the relationship between phase angle and period appears to follow an S-shaped curve such as that shown in Figure 6. The slope of the graph is greatest when the period of the L : D oscillation is 26–27 hr. At higher and lower periods, the graph is flatter, the phase having values of about $+130^\circ$ and -40° at the upper and lower extremes of period respectively. As noted before, the phase is near zero when the period is 24 hr. The significance of these observations will be considered in Section IV.

(d) Effects of Oscillations in Temperature

A leaf in constant conditions of light may oscillate at periods other than its natural period if it is subjected to oscillatory changes in temperature. Two series of experiments have been conducted, one in which the temperature alternated between the steady levels of 22 and 15°C, and the other between 18 and 8.5°C. The time taken for the transition from one temperature level to the other was about 30 min. Light intensity was kept constant at 1900 lux. As in the case of L : D oscillations, the range of periods of hot : cold (H : C) oscillations for entrainment was from 18 hr (9H : 9C) to 36 hr (18H : 18C).

For 12H : 12C, the leaf oscillation was closely in phase, the mid-time of closure corresponding to the middle of the cold interval. For H : C cycles of shorter period, the leaf oscillation lagged in phase, and led if the period was longer. The phase relationship is shown in Figure 7. It is seen to be very similar to the corresponding relationship for light. No significant difference between the effects of the two temperature ranges can be inferred from the available data.

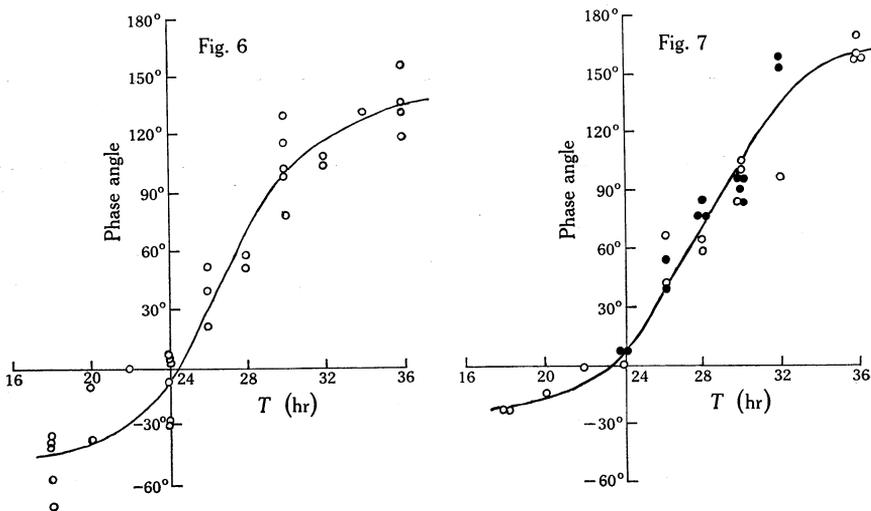


Fig. 6.—Phase relationships for leaves oscillating at constant temperature in L : D cycles of various periods. Phase is determined from mid-time of closure relative to the mid-dark time. Positive angles signify phase advance; negative angles signify phase lag. Each point is obtained from the record for a single leaf, averaging at least two cycles after entrainment.

Fig. 7.—Phase relationships for leaves in H : C cycles of various periods. Light intensity was kept constant at 1900 lux. Temperature ranges were 15–22°C (●) and 8.5–18°C (○). Phase is determined from the time of mid-closure relative to the mid-cold time.

(e) Localization of the Oscillatory Centre

Leaf movements occur because the pulvinal cells on either side of the leaflet base undergo periodic volume changes. Experiments were performed to locate the site in the plant of the biological clock which controls the changes in volume of pulvinal cells.

Isolated pulvini were kept in a humid atmosphere on moist filter paper in closed Petri dishes at 20°C. When subjected to a range of L : D cycles, they were found to respond in the same way as the pulvini in intact plants in similar L : D cycles. Photoreceptors are therefore located in pulvini themselves as has been shown for *Albizzia* (Koukkari and Hillman 1968). Furthermore, isolated pulvini in L : L or D : D continued to open and close rhythmically, apparently in response to an endogenous control located within them.

There appears to be no significant interaction between different leaves of the same plant. If part of an intact plant is in L : L or D : D and the remainder in a L : D cycle, or if different parts of the plant are in different L : D cycles, each leaf responds in a manner appropriate to its own lighting conditions and is unaffected by the behaviour of leaves in other parts of the plant.

It is concluded from these experiments that pulvini contain both biological clocks which control their movements and photoreceptors through which they respond to light.

Studies of pulvinal structure and of ionic and electric changes that are associated with rhythmic leaf movements will be described in a subsequent paper.

IV. DISCUSSION

The clover leaf is shown in this paper to have typical circadian characteristics. In an environment in which light and temperature are controlled, the leaf continues to oscillate with near-constant period. As is usual for circadian rhythms, the period is not exactly 24 hr and is in fact longer under most experimental conditions, increasing slightly with temperature. The reciprocal period (i.e. frequency) of the oscillation has a Q_{10} of about 0.9 over the temperature range 8.5–21.5°C. As the rates of physical and chemical processes involved in the timing mechanism are likely to rise with temperature, thus tending to reduce the period, compensatory processes appear necessary to account for the observed opposite trend. The small temperature dependence of circadian oscillators is well known (Sweeney and Hastings 1960) and a detailed model of the oscillator must take into account the factors which reduce or reverse the effects of temperature change.

In previous papers (Scott 1957, 1962; Jenkinson and Scott 1961) it is argued that the most probable mechanism for a biological oscillator involves overcorrection in a negative feedback pathway. Feedback interactions are commonly found in living organisms and it is well known that most feedback systems can become oscillatory if the open loop gain exceeds a limiting value. If the system is oscillatory, the differential equation which describes it must be of at least second order. The period of the oscillation depends largely on the time delays which occur in the feedback pathway, and in a biological system these could readily lead to oscillations of about 1 day in period. Discussion of another biological oscillator, including analysis of it in feedback terms and discussion of stability criteria is given in an earlier paper (Jenkinson and Scott 1961).

A common method of study of an oscillatory system, whose components cannot readily be isolated for individual analysis, is to subject it to forcing oscillations having a range of periods. The response of the system to these external oscillations can provide information about the nature of the basic oscillator and of its mode of coupling to the environmental factor being varied.

Some problems arise in applying this approach to test whether the oscillatory behaviour of the clover leaf in response to environmental oscillations conforms to that expected of a feedback oscillator.

The first problem relates to the form of the input oscillation of light or temperature. Mathematical analysis is made much simpler if this oscillation is sinusoidal. What is not known is how light or temperature should be varied in the environment in order to subject the plant to a physiological stimulus which varies sinusoidally. In the case of light, for example, the relationship between intensity and stimulus may be linear at low intensities, more nearly logarithmic at higher intensities and may saturate when the intensity is very high.

To avoid the uncertainties associated with the unknown nature of this dependence, a square wave input was used in the experiments described in this paper, the input quantity changing quickly from one steady level to another, and back again half a cycle later. The stimulus is now dependent only on the input levels before and after each step and not on a response to intermediate levels. In some cases the stimulus may depend only on the *change* of input and not on the input levels themselves. For example, the responses to temperature oscillations over two separate ranges do not differ significantly [Section III(*d*)].

The response of a damped sinusoidal oscillator to a square wave forcing oscillation is discussed in the Appendix. It is shown that the steady-state response is quasi-sinusoidal. Furthermore, the amplitude and phase relationships are not greatly different from those for a sinusoidal input, provided that the period of the input oscillation does not exceed about 150% of the natural period.

A further complication in the analysis is due to the non-sinusoidal nature of the changes in leaf angle. Although the predominant component of the motion is the fundamental sinusoidal oscillation [Section III(*a*)], it cannot be assumed that the higher harmonics have no effect on the response of the system to forcing oscillations. Techniques for analysing the steady-state responses of a system of this kind are also discussed in the Appendix.

In seeking a model of the leaf oscillator which corresponds to the observed responses to oscillatory stimuli, most attention has been given to phase relationships. The relationship between the amplitude of leaf oscillation and the amplitude of the forcing oscillation of light or temperature is complex and has not been studied, although it is apparent that the system is most responsive to forcing oscillations with periods close to the natural period, τ . This can be regarded as a resonance effect which is to be expected for any oscillatory system. In the present system, satisfactory entrainment was possible only for periods in the range $0.7-1.4\tau$.

The phase of the leaf oscillation changes from about 40° lag for 9L : 9D to about 130° advance at 18L : 18D, being near zero at 12L : 12D, and changing most rapidly at a period of about 26 hr which is close to the natural period. The maximum slope of the graph is 20° phase change per hour change in period. The phase responses for temperature oscillations are very similar.

Previous studies with other circadian oscillators have shown phase changes with period which are qualitatively similar to clover. These include *Calendula arvensis* (Stoppel 1910), *Kalanchoë blossfeldiana* (Bunsow 1953), *Pseudosmittia arenaria* (Remmert 1955), and *Gonyaulax* (Hastings and Sweeney 1959).

In all cases, the phase advance increases (or the phase lag decreases) as the forcing period is increased. In these examples, the maximum rate of change of phase with period is less than that for clover. In some instances, the total range over which the phase varies is greater than 180° , although this was found for a wider range of periods than was possible for entrainment in clover.

Attempts have been made to match the observations on clover with those of possible models of the endogenous oscillator. The simplest system whose natural oscillations decrease in amplitude and whose phase responses to forcing oscillations resemble those for clover is a damped sinusoidal oscillator of the form

$$x = A \exp(-kt) \cos(\beta t + \phi).$$

The characteristics of this oscillator are described in the Appendix. The phase relationships for both sinusoidal and square wave inputs are shown to be very similar, with a *total phase shift* (i.e. the total change of phase when the forcing oscillation is varied from very short to very long periods) of 180° , in agreement with the experimental data. The maximum slope occurs close to the natural period, again in agreement with observation. The maximum slope depends on the relative values of k and ω and is in good agreement with the observed data, provided $k = \omega/10$ (see Fig. 8).

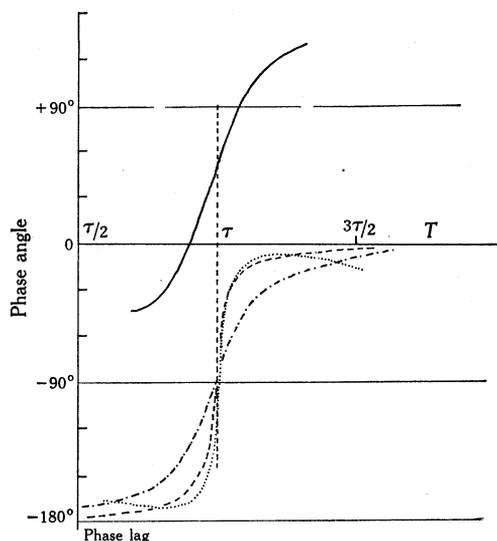


Fig. 8.—Phase of various oscillators responding to forcing oscillations of period T , which is varied above and below the natural period τ .
 - - - Sinusoidal oscillator and square wave input $k = \omega/30$.
 - · - · Sinusoidal oscillator and square wave input $k = \omega/10$.
 · · · · Oscillator with higher harmonics and square wave input.
 The full line gives the phase of the leaf oscillator for a L : D input.

This corresponds to a decrement of about 1.8, which is in reasonable agreement with the observed average value of 1.5 which is found for the natural oscillator [Section III(b)].

Attempts have been made to refine the model by considering a basic oscillator whose natural damped oscillations include higher harmonics, described by an equation of the form

$$x = Ae^{-kt}(\sin \beta t + \alpha_3 \sin 3\beta t + \alpha_5 \sin 5\beta t).$$

Values of α_3 and α_5 suggested in Section III(a) are 0.17 and 0.03 respectively. Even with these small coefficients the higher harmonics have the effect of steepening the phase graph by a factor of about 4 (Fig. 8). A reduction in slope could be achieved by increasing the ratio k/ω (and hence the damping), but to bring the maximum slope back to the value observed experimentally would require a decrement far greater than is found for the natural oscillation. It therefore appears that a feedback loop in which the natural oscillations are of the non-sinusoidal form exhibited by angle and torque cannot account for the observed phase response to forcing oscillations of light and temperature.

If this is the case, the observed output quantity (angle or torque) would not be a direct component of the loop, but would be linked to it through a non-linear coupling. The higher harmonics would arise in this coupling rather than in the feedback loop itself.

A detailed model of the leaf oscillator must account for the absolute phase of the leaf oscillation and not only for the phase changes which occur with period of the forcing oscillation. Phase differences occur within the feedback loop itself and phase shifts may arise in the coupling between the input and loop, and between loop and output. It is noteworthy that the leaf response is closely in phase with forcing oscillations of light or temperature having periods of 24 hr. Presumably this has provided an evolutionary advantage.

Further study of oscillator models is proceeding. This includes the prediction by them of transient responses and the effects of forcing oscillations with unequal proportions of L and D or H and C. More complex models such as those considered by Wever (1965) have some of the characteristics of the clover leaf oscillator, including prediction of the 180° total phase shift.

It is not yet possible to speculate on the components of the proposed feedback loop. Experiments to be described in a subsequent paper show that the cells on the swollen side of the pulvinus have a higher potassium concentration than those on the opposite side. The cause of leaf movement may therefore be an intermittent pump which transports potassium ions into cells, first on one side of the pulvinus and then the other. Water would follow the potassium passively to reduce the osmotic imbalance, thus causing cellular volume changes. Satter, Marinoff, and Galston (1970) have recently suggested a similar mechanism to account for leaf movements in *Albizzia*.

The results described in this paper show rhythmic responses in pulvinal cells to two distinctly different environmental oscillations, light and temperature. It is well known that both of these stimuli affect a wide range of circadian systems. Furthermore, recent experiments with *Mimosa* and *Albizzia* show pulvinal cells respond to phytochrome changes induced by red or far-red light (Fondeville, Borthwick, and Hendricks 1966; Satter, Sabnis, and Galston 1970).

It needs to be emphasized, however, that any explanation of leaf movement based entirely on direct responses to external stimuli cannot account for the endogenous periodicity. Furthermore, it cannot explain how the response can be in phase with the environmental cycle when its period is 24 hr, yet be almost completely out of phase when the period is 36 hr. These phase differences are readily accounted for

through interactions between the external stimuli and the basic oscillator responsible for the endogenous rhythm. The nature of the interaction has yet to be elucidated, as has the mode of action of the endogenous timer itself.

V. ACKNOWLEDGMENTS

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APPENDIX

STEADY-STATE RESPONSES OF DAMPED OSCILLATORS TO SINUSOIDAL AND SQUARE WAVE INPUTS

(a) Sinusoidal Input to Damped Sinusoidal Oscillator

The differential equation

$$\ddot{x} + 2k\dot{x} + \omega^2x = 0$$

describes a damped sinusoidal oscillator, provided k is positive and less than ω . The solution of this equation is

$$x = A \exp(-kt) \cos(\beta t + \phi),$$

where A and ϕ are constants and $\beta = (\omega^2 - k^2)^{\frac{1}{2}}$. The natural period of the oscillation $\tau = 2\pi/\beta$ and the decrement (i.e. the amplitude ratio for successive cycles) is

$$\exp(k\tau) = \exp(2\pi k/\beta).$$

When subjected to a sinusoidal input, $K \cos pt$, the forced response, after transient terms have become negligible, is

$$x = A \cos(pt + \epsilon),$$

where A and ϵ are functions of the period of the forcing oscillation and are given by

$$A = K / [(p^2 - \omega^2)^2 + 4k^2p^2]^{\frac{1}{2}}$$

and

$$\epsilon = \text{artan}[(p^2 - \omega^2)/2kp] + 90^\circ.$$

The oscillator resonates when the forcing period is close to the natural period. The oscillations are in phase for very slow oscillations but as the forcing period is shortened the response lags behind, the lag approaching 180° when the period is very short. The most rapid change of phase with period occurs close to the natural period of the oscillator (see Fig. 8).

(b) Square Wave Input to Damped Sinusoidal Oscillator

In this case, the input is constant for each half-cycle and during this time the oscillator executes its natural damped oscillation about a value appropriate to this constant input. At the end of the half-cycle, the oscillator receives a stimulus in the form of a step to a new steady input and the system responds by oscillating about a new equilibrium level.

The differential equation in this case is

$$(\ddot{x} + 2k\dot{x} + \omega^2x) = f(t),$$

where

$$f(t) = +a \text{ when } nT < t < \frac{1}{2}(2n+1)T,$$

$$f(t) = -a \text{ when } \frac{1}{2}(2n+1)T < t < (n+1)T,$$

where T is the period of the square wave oscillation and n is an integer.

For the half-cycle $0 < t < +\frac{1}{2}T$, the solution is

$$[x - (a/\omega^2)] = A \exp(-kt) \cos(\beta t + \phi).$$

When the oscillation is in a steady state, the values of A and ϕ may be determined by noting that successive half-cycles must be identical except inverted relative to one another. Thus

$$(x)_{t=0} = -(x)_{t=\frac{1}{2}T} \quad \text{and} \quad (\dot{x})_{t=0} = -(\dot{x})_{t=\frac{1}{2}T}$$

Substitution of these conditions gives the following equations which determine ϕ and A :

$$\tan(\phi + \gamma) = -\sin \frac{1}{2}\beta T / (\exp \frac{1}{2}kT + \cos \frac{1}{2}\beta T),$$

where

$$\gamma = \arcsin(k/\omega),$$

and

$$A = -(2a/\omega^2) / [\cos \phi + \exp(-\frac{1}{2}kT)\cos(\frac{1}{2}\beta T + \phi)].$$

Turning points occur when $\dot{x} = 0$ and it is readily shown that these occur at the time or times within the half-cycle for which $\sin(\beta t + \phi + \gamma) = 0$.

A typical graph of the steady state response of the damped sinusoidal oscillator to a square wave input is shown in Figure 9. It is found that the response is quasi-

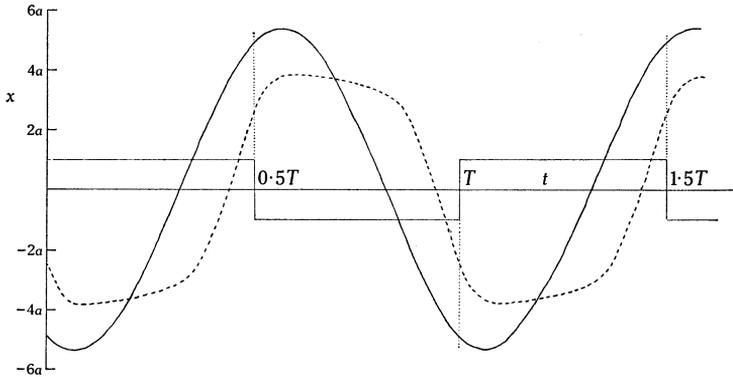


Fig. 9.—Steady-state response of damped sinusoidal oscillator (solid line) and damped oscillator with higher harmonics (dotted line) to square wave input. Data calculated for $T = 0.95\tau$. Note in each case that the values of x at the beginning and end of each half-cycle are equal (but opposite in sign), as are the values of the slope, \dot{x} .

sinusoidal provided that the natural oscillation is lightly damped ($k \ll \omega$) and the period of the square wave is not more than about 150% of the natural period. For periods within this range, the amplitude and phase of the quasi-sinusoidal response are found to be very similar to those for a sinusoidal input. The phase response is shown in Figure 8.

(c) *Square Wave Input to a Non-sinusoidal Oscillator*

No analytical solution could be found when a square wave input was applied to a damped oscillator whose natural oscillations contained higher harmonics besides the fundamental. However, the steady-state solution could be readily obtained in a particular case by a graphical technique. For example, suppose the natural oscillation

contains the first, third, and fifth harmonics and is described by an equation of the form

$$x = Ae^{-kt}(\sin \beta t + \alpha_3 \sin 3\beta t + \alpha_5 \sin 5\beta t).$$

Then for a particular set of values of k , β , α_3 , and α_5 , graphs of x and \dot{x} against t are plotted, choosing an arbitrary value for A . Use is now made of the steady-state requirement that the values of \dot{x} at the beginning and end of each half-cycle are equal but opposite in sign. For each value of $\frac{1}{2}T$, a segment of the \dot{x} graph is located whose end values satisfy this requirement. The corresponding segment of the x graph is located, and this must be positioned relative to the zero line so that the other requirement for the steady state that

$$(x)_{t=0} = -(x)_{t=\frac{1}{2}T}$$

is also satisfied. Thus both the form of the steady-state response and the square wave input responsible for it can be shown graphically. The response for a typical oscillator whose natural oscillations are similar to those of the leaf is shown in Figure 9. From graphs such as this, the amplitude and phase relationships can be determined.

The phase response is shown in Figure 8. It is noted that phase changes much more rapidly with period in the vicinity of resonance than is the case for a simple sinusoidal oscillator which has the same damping coefficient, k .