THE EFFECT OF PHOTOPERIOD DURATION UPON THE RESPIRATORY ACTIVITY OF THE ROOTS OF WHEAT SEEDLINGS

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Summary

The root respiration rate of wheat seedlings was continuously measured (in terms of CO_2 output) in a controlled environment. The effects of varying photoperiods of high intensity light, incident on the leaves, upon the rate of root respiration were studied. It was demonstrated that increases in root respiration rate occurred in response to the light treatments. The fluctuations in root respiration rate, induced by the photoperiods used, had a pattern over the 24-hr cycle, which included two peaks—when the photoperiod was of 6 or 12 hr duration. These phenomena are discussed in relation to the hypothesis that root respiration rate fluctuates in response to a varying flow of assimilates from the leaves to the roots. No evidence was obtained of an endogenous (or circadian) rhythm of root respiration rate.

I. Introduction

It has been demonstrated that the root growth rates of leafy seedlings of *Acer pseudoplatanus* and *A. saccharinum* (Richardson 1953*a*, 1953*b*; Wassink and Richardson 1961) and also of tomato plants (Albert and Wilson 1961) are directly related to the quantity of radiant energy falling upon the leaves of these plants. It may be reasonably inferred, therefore, that the amount of leaf photosynthesis may determine the root growth rates of intact plants. Consequently it might be expected that the normal diurnal cycle of light and dark would affect root growth rates and root metabolism. Head (1965) has demonstrated a diurnal fluctuation† in the growth rate of the roots of cherry trees growing naturally in soil; and Huck, Hageman, and Hanson (1962) examined the respiratory activity of the roots of three species of plant growing in sand and nutrient culture solution and concluded that diurnal fluctuations in root respiration rate did occur.

However, with one exception, the experiments of Huck, Hageman, and Hanson (1962) were not done in a controlled environment, and root temperatures differed by 5 degC from day to night. Also the contribution of microorganism respiration in the root containers was not estimated, although the plants in one experiment were in the same container for 28 months.

The aim of the experiments reported here was to ascertain the effects of photoperiod duration, presented to the leaves, upon the rate of CO_2 evolution of the

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† The term "diurnal fluctuation", rather than "circadian fluctuation", is used throughout because the term "circadian" when applied to biological rhythms has, without etymological foundation, acquired an implicit meaning of "endogenous diurnal rhythm" (Bünning 1964; p. 1). The term diurnal fluctuation is therefore less specific, referring to fluctuations that may be endogenous (continues in a non-fluctuating environment), or exogenous (dependent upon the maintenance of environmental fluctuations).

roots of wheat seedlings held under controlled conditions. The periodic gas-sampling technique that Huck, Hageman, and Hanson (1962) used in the measurement of root respiration rates was replaced by a continuous gas-sampling method, in which a CO_2 infrared gas analyser was used.

II. Materials and Methods

(a) *Plant Material*

The root respiration rates of wheat seedlings (cv. Olympic) were measured when the seedlings were 20–25 days old.

The grain was surface-sterilized (Ferguson 1963), sown in heat-sterilized moist sand, and germinated for 4 days at $25 \pm 1 \cdot 0$°C in the dark. The containers were then transferred to a growth room (12-hr photoperiod, 24-hr cycle, temperature $16 \cdot 5$°C, and light intensity 650 f.c. at the base of the plants). After a further 6–8 days the seedlings were transferred to aerated and sterile nutrient culture solutions [half-strength Hoagland's No. 1 (Hoagland and Arnon 1950), with Fe-EDTA as the iron source], in which they were maintained for 10–12 days. The seedlings' roots were then washed three times in sterile distilled water and the plants were sealed into an opaque Perspex dish, in which there were three holes, with cotton-wool impregnated with Colophony wax. This dish was then sealed onto the ground-glass flanges on the rim of an opaque vessel containing 600 ml of aerated nutrient solution. The pH of this solution was stabilized with a mixture of the Ca^{2+} and H^+ forms of the ion-exchange resin, Amberlite IRC 50 (Hageman *et al.* 1961).

(b) *Conditions under which Root Respiration was Measured*

After 2 days, these special containers were transferred to a $20 \pm 1 \cdot 0$°C environment and placed in a light chamber with reflecting walls, in which illumination was provided by a single 400-W Philips HPLR fluorescent-coated Hg vapour lamp. This lamp was connected to a time switch to give the required photoperiod, and provided an intensity of *c.* 2000 f.c. at the base of the plants. The vessel containing the roots was connected to a CO_2-free, humidified air supply and placed in a water-jacket, through which air was also bubbled. The roots were in total darkness. The temperature of the solution in which the roots were immersed was normally maintained at $20 \pm 0 \cdot 5$°C, and has been recorded with root respiration rates in Figures 1, 2(*a*), 2(*b*), and 3(*b*).

(c) *Measurement of Root Respiration Rates*

Humidified air (CO_2-free) was passed through the vessel containing the wheat roots at constant and measured flow rates in the range 50–150 ml/min. The CO_2 content of the effluent airstream was measured with a direct-reading CO_2 infrared gas analyser, which was calibrated with gas mixtures of known CO_2 content obtained by the method of Bierhuizen and Slatyer (1964). Root respiration rates for the three plants together were calculated from the recorder trace of the output signal from the analyser and the current flow rate, and have been expressed on a single-plant basis. Root respiration rates of the same plants were continuously followed over two to six 24-hr cycles, the nutrient solution being renewed periodically.

(*d*) *Contamination with Microorganisms*

Despite the precautions described above, CO_2 continued to be evolved for several hours from solutions from which the plants had been removed. This residual biological activity was eliminated by the addition of cyanide, and was not present in freshly made-up solutions before the plant roots were added. The magnitude of microorganism respiration was therefore measured in five of the experiments by removing the plants from the solution at various phases of the 24-hr cycle, and these results are described below. It is concluded that the CO_2 from microorganisms in the rooting medium did not account for the observed fluctuations in root respiration rate.

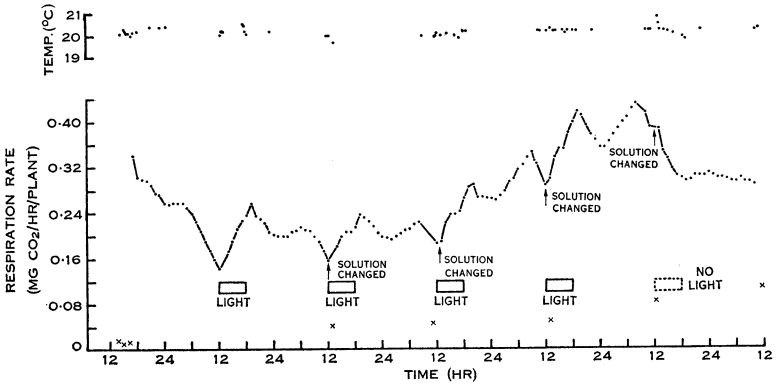

Fig. 1.—Fluctuations in root respiration rate under a 6 hr light–18 hr dark cycle. × Residual biological activity (i.e. the amount of CO_2 evolved by the solution from which the plants have been removed). For the sake of clarity, a line has not been drawn through those experimental points which are in close proximity to each other. The temperature of the nutrient solution is shown at the top of the figure.

III. Results

(*a*) *Evidence for the Existence of Diurnal Fluctuations in Root Respiration Rate which are Dependent upon Photoperiod*

The first experiments, in which plants were maintained under a 6 hr light–18 hr dark cycle, demonstrated (Fig. 1) that diurnal fluctuations in the rate of CO_2 evolution did occur. This was repeatedly observed, as indicated in the results of two further similar experiments [Figs. 2(*a*) and 2(*b*)]. These fluctuations were unrelated to the small changes in the temperature of the nutrient solution that did occur [Figs. 1, 2(*a*), and 2(*b*)]. However, the residual biological activity of the solutions, after the removal of the plant roots, was appreciable [Figs. 1, 2(*a*), and 2(*b*)]. In order to establish that the observed fluctuations of CO_2 output were root respiration effects, the residual biological activity of the solutions was repeatedly measured at the times when experience had shown peaks in the rate of CO_2 evolution to occur. It was demonstrated that the peaks [Fig. 2(*b*)] and troughs [Fig. 2(*a*)] in the rate of CO_2 output of the plants in nutrient solutions were not due to fluctuations of a

similar magnitude in the output of CO_2 by the solutions (which contained micro-organisms) alone. Therefore it was concluded that there were real fluctuations in the root respiration rate.

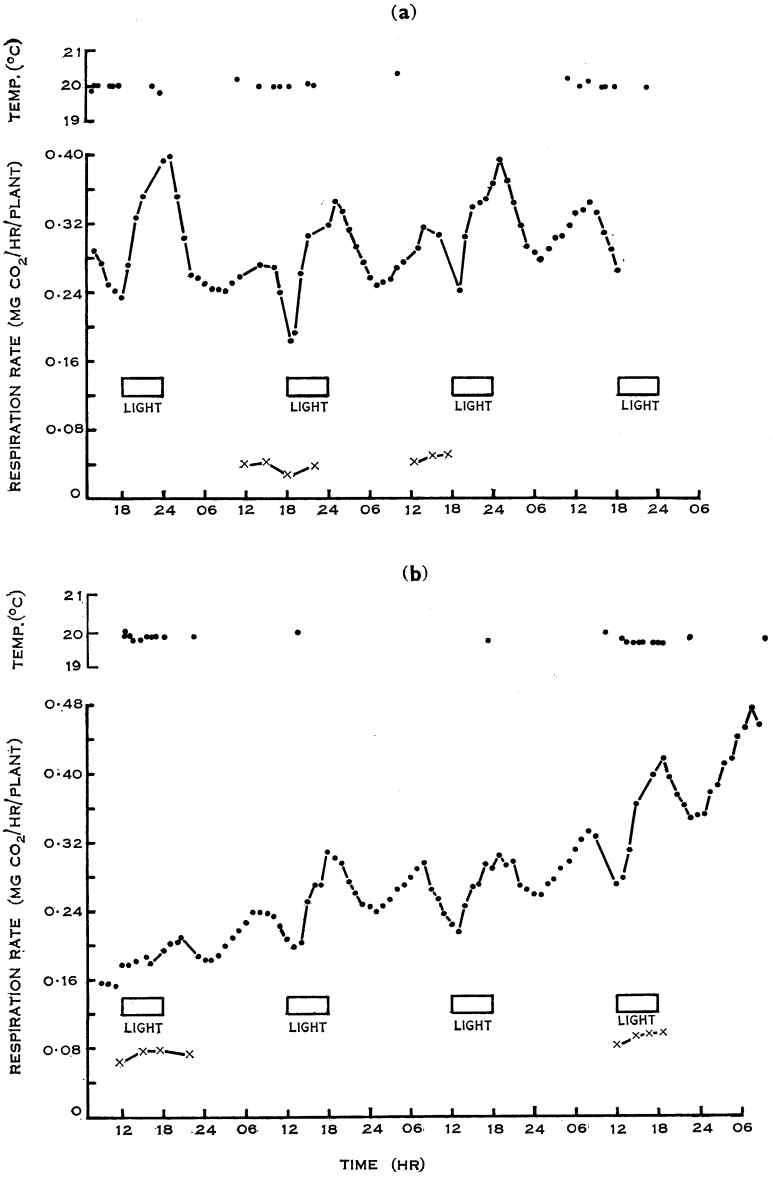

Fig. 2.—As for Figure 1 but with the residual biological activity (×) measured (*a*) during the second "trough" and (*b*) during the first "peak" of root respiration rate. The temperatures of the nutrient solutions are indicated.

These fluctuations in the rate of CO_2 output by the roots were related to the timing of the photoperiod during the 24-hr cycle. In all the experiments in which a 6-hr photoperiod was used there were two characteristic peaks, and two troughs

within each 24-hr cycle [Figs. 1, 2(*a*), and 2(*b*)]. The times at which these peaks and troughs appeared in experiments I, II(*a*), and II(*b*) are given in Table 1, from which it may be seen that peak 1 occurs 7·2±0·3 hr and peak 2 19·6±0·2 hr after the beginning of the photoperiod. The troughs occur 12·9±0·5 and 24·2±0·2 hr

TABLE 1

TIMING (HR AFTER THE BEGINNING OF THE PHOTOPERIOD OF EACH CYCLE) OF THE TWO PEAKS AND TROUGHS IN ROOT RESPIRATION RATE OF WHEAT SEEDLINGS GROWN UNDER A 6-HR PHOTOPERIOD

Each cycle is considered to begin with the 6-hr photoperiod. Data from Figures 1, 2(*a*), and 2(*b*), corresponding to experiments I, II(*a*), and II(*b*), respectively

	First Peak			First Trough			Second Peak			Second Trough		
Expt. No.:	I	II(*a*)	II(*b*)	I	II(*a*)	II(*b*)	I	II(*a*)	II(*b*)	I	II(*a*)	II(*b*)
Cycle 1	7·5	7	8·5	14	15	13	18	20	19·5	24	24·5	25
Cycle 2	7·5	7	5·5	14	12·5	12·5	20	19·5	19·5	24	24·5	25
Cycle 3	8·5	6·5	6·5	13	12·5	12·5	21	19	19·5	24	24	23·5
Cycle 4	9·0	—	6·0	12	—	10·0	20	—	—	30·5*	—	—
Cycle 5	None	—	—	None	—	—	None	—	—	None	—	—
Mean time (hr), all experiments	7·2±0·3			12·9±0·5			19·6±0·2			24·2±0·2		

* Delayed because no photoperiod given in fifth cycle (expt. I).

respectively after the beginning of the photoperiod. The consistency of timing of these peaks and troughs in root respiration rate is indicated by their small standard error (Table 2).

TABLE 2

AMPLITUDE OF THE FLUCTUATION IN ROOT RESPIRATION RATE OF WHEAT SEEDLINGS GROWN UNDER VARIOUS PHOTOPERIODS

Expt. No.	Photoperiod (hr)	Plant Age* (days)	Amplitude between Peak and Subsequent Trough (mg CO_2/hr/plant)				Mean Amplitude
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	
I	6	24	0·114	0·080	0·102	0·129	0·106
II(*a*)	6	25	0·164	0·164	0·071	0·146	0·136
II(*b*)	6	22	0·037	0·090	0·071	0·146	0·086
III(*a*)	3	20	0·041	0·031	0·062	—	0·045
III(*b*)	3	20	0·055	0·053	—	—	0·054
IV	12	21	0·115	0·120	—	—	0·118

* As at beginning of experiment.

Huck, Hageman, and Hanson (1962), who made no measurements of the effects of the respiratory activity of microorganisms in the rooting medium and who used a greenhouse environment without controlled temperatures in all except

one of their experiments, also concluded that light-controlled fluctuations in root respiration rate occurred in *Derris elliptica* (Wall), *Zea mays* (L.), and *Glycine max* (L.). However, they give only one plot of root respiration rates, continuously recorded, and this was for plants growing in a greenhouse under natural photoperiods, when in two out of the three 24-hr cycles followed, there was only a single peak in the root respiration rate. In the third cycle there is evidence for a double peak which they do not comment upon.

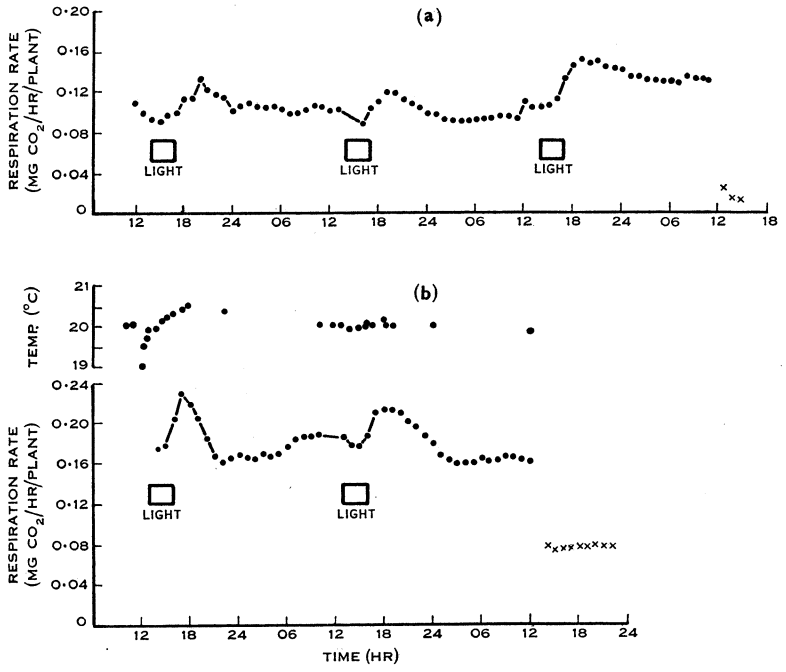

Fig. 3.—(*a*) and (*b*) Root respiration rate of seedlings grown in 3 hr light–21 hr dark cycles in two separate experiments. The temperature of the nutrient solution is indicated for the second experiment only. × Residual biological activity.

(*b*) *Effects of Photoperiod Duration upon the Fluctuation of Root Respiration Rate*

In addition to these 6-hr photoperiods, the results of which treatment have been described above, root respiration rate was followed in plants subjected to continuous light and to photoperiods of 12 and 3 hr. It is apparent that photoperiods of 3 hr [Figs. 3(*a*) and 3(*b*)] and 12 hr (Fig. 4) also induce fluctuations in root respiration rate. The amplitudes of the fluctuations induced by the three photoperiods used are given in Table 2, from which it is apparent that the amplitude is markedly less when the leaves are exposed to light for 3 hr contrasted with the longer photoperiods. It is noteworthy that there are also two maxima of respiration rate, in each 24-hr cycle, for plants growing under photoperiods of 12 hr (Fig. 4), whereas there are

only single maxima in the 3-hr photoperiod treatment [Figs. 3(*a*) and 3(*b*)]. Thus root respiration rate appears to have two peaks in each 24-hr cycle, for the 6 and 12-hr photoperiods; but only a single peak in 3-hr photoperiods.

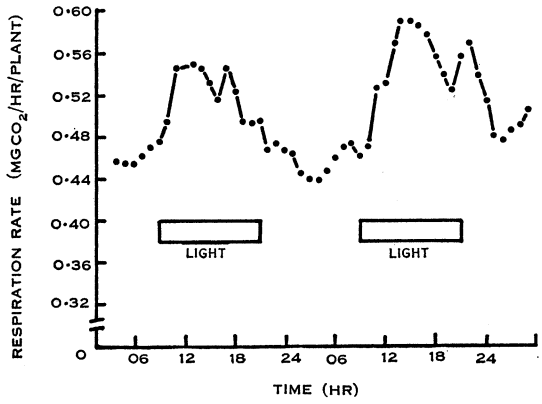

Fig. 4.—Root respiration rate of seedlings grown in 12 hr light–12 hr dark cycles.

(*c*) *Evidence of the Absence of Endogenous Fluctuations in Root Respiration Rate*

Endogenous (or circadian) fluctuations in root respiration rate were looked for by subjecting the plant to continuous light (Fig. 5), or to continuous dark at the end of an experiment with 6-hr (Fig. 1) photoperiods. Under continuous light (Fig. 5)

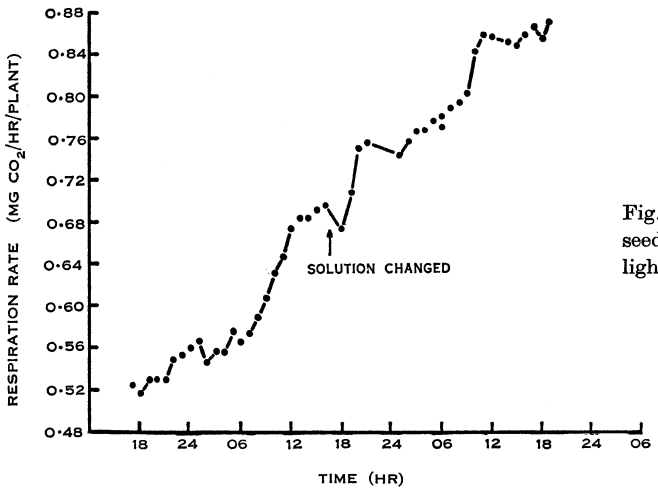

Fig. 5.—Root respiration rate of seedlings grown under continuous light.

there were small fluctuations in respiration rate, but these did not have the regular periodicity (Table 1) of those of other experiments with light/dark cycles of a fixed period. Also when the light period was omitted in an experiment with 6-hr photoperiods (Fig. 1), the periodicity in the root respiration rate disappeared. It is concluded that these rhythmic fluctuations are exogenous, or controlled by current environment fluctuations.

IV. Conclusions and Discussion

The main conclusion from these experiments is that the root respiration rate of wheat seedlings is markedly stimulated by a photoperiod after darkness, and that this stimulation cannot be attributed either to root temperature fluctuations or to the microorganism respiration in the nutrient culture.

Previous evidence for a connection between leaf photosynthesis on the one hand and root growth and respiration rates on the other has been mentioned in the Introduction to this paper. In addition, Davidson (1963, and personal communication) has demonstrated that the root respiration rate of *Dactylis glomerata* L. falls rapidly (within 24 hr) of partial defoliation.

Subsidiary conclusions from the experiments reported in this paper are:

(1) Root respiration rate begins to increase soon after the beginning of photoperiods of 6 and 12 hr [Figs. 1, 2(*a*), 2(*b*), and 4], but this response is delayed when a 3-hr photoperiod is used [Figs. 3(*a*), 3(*b*)].

(2) The amplitude of the fluctuations in root respiration rate are greatest after 6- and 12-hr photoperiods, and least after 3-hr photoperiods (Table 2).

(3) Root respiration rate falls (over a period of approximately 6 hr) immediately at the end of a 6-hr photoperiod, but then rises to a second maximum during the dark period. Then it again falls and reaches a minimum at the end of the 24-hr cycle (Figs. 1 and 2).

The simplest and most obvious hypothesis to account for the increase in root respiration, which resulted from the exposure of the leaves to light, is to propose that the flow of assimilates from the leaves commences with the onset of photosynthesis and that this induces an increase in root respiration. Diurnal fluctuations in the concentration of sugars in the phloem have been reported by Mason and Maskell (1928), Huber, Schmidt, and Jahnel (1937), and Zimmermann (1958). Using wheat seedlings, Doodson, Manners, and Myers (1964) showed that 20% of the assimilated ^{14}C label (presented to the third leaf of 25-day-old plants) was translocated to the roots within 3 hr from the beginning of the presentation of $^{14}CO_2$ to the illuminated leaf. Similar rapid transport of leaf assimilates to the roots has been reported in many plants: rye (Mayer and Porter 1960); rye grass (Marshall and Sagar 1965); tobacco (Jones, Martin, and Porter 1959; Porter and Bird 1962); sugar-cane (Hartt *et al.* 1963), and soybean (Thaine, Ovenden, and Turner 1959). The root exudation of some amino acids and amides is also reduced by a decrease of light intensity incident upon the leaves (Rovira 1959). If the rapid increases in root respiration rate, evident within 1 hr of the exposure of the leaves to light (Figs. 1 and 2), are to be attributed to leaf assimilates reaching the roots, then an approximate velocity of assimilate flow from leaves to roots can be calculated. As the maximum distance from leaf to root tips of the wheat seedling used was 45 cm, then the velocity of assimilate flow required by this hypothesis is not greater than many estimates of the "velocity of translocation" which lie between 50–100 cm/hr [Mortimer 1965(*a*), 1965(*b*)].

A further requirement of the hypothesis, as proposed above, is that there should be a light-controlled fluctuation in the concentration of assimilates in the roots.

However, although this effect has been sought (reviewed by Huck, Hageman, and Hanson 1962) there is no consistent evidence for a diurnal fluctuation in the concentration of reducing sugars in plant roots. This does not invalidate the hypothesis because, as has been reported for leaves (Yemm 1935; Porter and Bird 1962), there may be no simple relationship between root respiration rate and the concentration of any one sugar. If assimilate flow is responsible for fluctuations in root respiration rate, then it is to be expected that the amplitude of these fluctuations would be least under short photoperiods of 3 hr, contrasted with those of longer duration (Table 2).

A further complication arises because root respiration rate has a second maximum in the dark period after a photoperiod (Figs. 1 and 2). Under the hypothesis proposed above, this requires that there shall be a continued flow of assimilates to the roots during the dark, and that this flow should fluctuate in quantity. There is a considerable evidence that assimilate flow from a leaf, after a "pulse" of $^{14}CO_2$ assimilation, continues whether the leaf continues to be illuminated (Jones, Martin, and Porter 1959; Doodson, Manners, and Myers 1964; Mortimer 1965*a*), or placed in the dark (Nelson and Gorham 1957; Porter and Bird 1962). Porter and Bird (1962) showed that 52% of the total ^{14}C assimilated over 3–6 hr was translocated from a tobacco leaf during 17 hr darkness following the assimilation of $^{14}CO_2$. The fluctuations in root respiration rate in the dark period might be due to the changeover from the translocation of current assimilates to stored carbohydrates, or to an effect of light on leaf transpiration rates—effects both reported by Peel and Weatherley (1962) to influence the rate of sucrose exudation from the phloem of willow stems, via aphid stylets. The separation of the effects of light upon transpiration rates on the one hand and upon the induction of a flow of assimilates to the roots on the other might be achieved by measuring the root respiration rates of illuminated plants in which translocation to the roots has been impeded by a low temperature "block" of the type used by Thrower (1965).

Although endogenous (circadian) rhythms in the rate of CO_2 evolution have been reported for excised leaves of three species of *Bryophyllum* (Wilkins 1960), there was no conclusive evidence in our experiments suggesting that such rhythms occurred in wheat roots (Figs. 1 and 5).

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