Endogenous Rhythmic Activity of Photosynthesis, Transpiration, Dark Respiration, and Carbon Dioxide Compensation Point of Peanut Leaves[1]

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ABSTRACT

At 14-hour day length, 25 C leaf temperature, 9 mm Hg vapor-pressure deficit, and 1.17 joules cm^{-2} min^{-1} irradiance, the diurnal change in daily photosynthesis of the cultivated peanut (*Arachis hypogaea* L.) is a result of an endogenously controlled circadian rhythm in net photosynthesis which peaks near noon and troughs near midnight. By resetting the day-night light regime, the rhythm rephased in continuous light. The free-running rhythm approximates 26 hours. Both transpiration and dark respiration show similar rhythmicity, with transpiration closely in phase with the rhythm in photosynthesis. The rhythm in carbon dioxide compensation point is approximately 12 hours out of phase, peaking at midnight and troughing at midday. Endogenous changes in stomatal aperture seemed to be the major control of the rhythm in photosynthesis. The activity of ribulose-1,5-diphosphate carboxylase increased during the normal photoperiod, leveling off after 12 hours; however, the activity was not correlated with the rhythmic change in photosynthesis.

Diurnal changes in transpiration and net photosynthesis of cotton, bell pepper, soybean, and peanut in a constant environment have recently been reported (12). When that paper was written, very little literature was available on the subject of diurnal change; however, in the interim three additional reports have been published. They indicate that diurnal change also exists in the photosynthesis of alfalfa (16) and *Chenopodium rubrum* (4), and confirm its existence in soybean (24). Neither we nor the workers cited, however, proved that the rhythm in net photosynthesis of these higher plants was endogenous and circadianlike. In the studies reported here the periodicity of the photosynthetic rhythm was shown to persist under constant environmental conditions and the potential for phase shift was examined. Since the sinusoidal nature of the transpiration and

photosynthesis curves suggested endogenous rhythmic change in diffusive resistance or biochemical activity, or both, measurements of CO_2 compensation point, dark respiration, and RuDP carboxylase activity were carried out for synchronization with the rhythmic change in net photosynthesis and transpiration.

MATERIALS AND METHODS

For measurements of net photosynthesis, dark respiration, and CO_2 compensation, plants of *Arachis hypogaea* L. cv. Florigiant were grown in a fertilized peat-vermiculite medium in 5-inch plastic pots, under a controlled environment. This type media, because of its high water holding capacity (approximately 500%), minimized plant-soil-water stress as a factor in these studies. For RuDP carboxylase and transpiration studies, a large population was grown in the same medium but in deep wooden flats. Type 1 chambers (13) were programmed for 25 C, 60% relative humidity, and 350 μl CO_2 $liter^{-1}$ air for 14-hr photoperiods, and 20 C, 90% relative humidity, and 400 μl CO_2 $liter^{-1}$ air for 10-hr nyctoperiods (LD:14,10).[4] The light source for growth and experimentation consisted of a bank of VHO cool white fluorescents supplemented with incandescents with light intensities of 1.17 joules cm^{-2} min^{-1} of total radiation, of which 49% was infrared. Usually plants were used when 3 or 4 weeks old. Except for temperature response studies, all net photosynthesis, dark respiration, and CO_2 compensation point measurements were made at constant leaf temperatures of 25 C. Night temperatures of 25 C were maintained to assure soil temperature equilibrium. Vapor-pressure deficit was maintained at approximately 9 mm Hg.

The net photosynthetic or dark respiration rates of plants were determined by measuring the amount of carbon dioxide supplied or removed per time interval to maintain CO_2 at 300 $\mu l/1$ air in a sealed plant chamber. Windspeed in the chamber was >25 m/min. This semiclosed compensating system is described elsewhere (19). A special airtight Plexiglas chamber was fabricated to enclose the aboveground portion of 2-, 3-, or 4-week-old peanut plants. The tops of two plants were used simultaneously. The plants were sealed into the chamber with caulking gum around the stems. To prevent changes in leaf position due to "sleep" movements and to minimize self-shad-

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[4] Abberivations: A repetitive cycle of x hr of light and y hr of darkness will be denoted LD:x,y (where the period of the cycle = $x + y$); P_n: net photosynthesis.
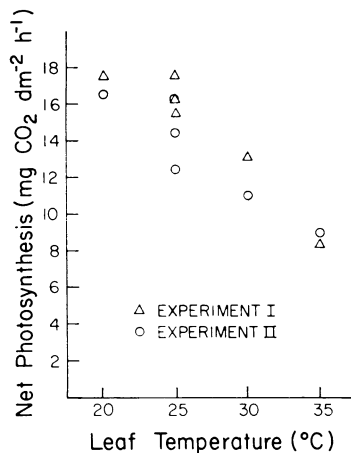
FIG. 1. Average hourly photosynthetic rate of peanut leaves as influenced by leaf temperature.

ing, we laced the leaves with a fine network of nylon thread. The top and sides of the transparent chamber were fastened directly to a metal plate underlayed by water-cooled coils acting as heat exchangers.

To monitor CO_2 compensation, leaves were enclosed in a confined atmosphere (polyvinyl chloride bag or Plexiglas chamber coupled to a model 315A Beckman[5] infrared gas analyzer) where near steady state CO_2 concentrations were established. They were maintained under the normal, lighted, growth chamber conditions for several days while checking for fluctuation in the CO_2 compensation concentration. Leaf temperature was maintained at 25 C during the experimental period with the aid of a water-cooled, Plexiglas chamber and water filters containing $CuSO_4$, or with convective cooling by circulating fans when bags were used.

One leaflet from each of three fully expanded tetrafoliates from 3- to 4-week-old plants were used for the RuDP carboxylase enzyme assay. The tissue was finely cut up and then ground in a cold mortar containing acid-washed sand in 5 ml of the following medium: 2 mM sodium nitrate, 2 mM EDTA, 5 mM $MgCl_2$, 5 mM dithiothreitol, and 50 mM HEPES-NaOH buffer at pH 7.8. Tissue homogenates were then filtered through a 30-μm nylon net.

To assay for RuDP carboxylase (4.1.1.39), aliquots of cell extracts were added to a medium containing 50 mM HEPES-NaOH buffer at pH 7.8, 5 mM dithiothreitol, 10 mM $MgCl_2$, and 20 mM $NaH^{14}CO_3$. After 4 min of incubation at 30 C, 1 mM RuDP was added to make a total volume of 300 μl. Forty-μl aliquots were removed at specific time intervals (a zero-time aliquot was taken after 3.5 min incubation before addition of RuDP), and the reaction was stopped by addition of 50 μl of 20% trichloroacetic acid. After addition of 10 ml of 2,5-tert-butylbenzoxyzalythiopene scintillation fluid, ^{14}C uptake was measured by liquid scintillation counting. ^{14}C incorporation was linear for about 2 min.

Transpiration was measured continuously on one flat of each population used for RuDP carboxylase measurements. This was accomplished by weighing water loss on an automatic, sensitive recording balance (5).

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[5] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the United States Department of Agriculture.

RESULTS

Before we attempted to determine diurnal net photosynthetic trends in cultivars of Arachis hypogaea, leaf temperature optima for photosynthesis were established. Since net photosynthesis under normal conditions generally plateaued between 0900 and 1500 hr, the effect of leaf temperature on net photosynthesis was studied during those hours. One hr of net photosynthesis measurements were made at a reference temperature of 25 C, followed by 1 hr at the prescribed leaf temperature, followed by at least 1 more hr at 25 C. Since studies on cotton had indicated that changes in vapor-pressure deficit could measurably affect photosynthetic rate (14), vapor-pressure deficit was maintained relatively constant in these experiments at approximately 9 mm Hg. Although seemingly the rate at 20 C equaled that at 25 C, as shown in Figure 1, following the 20 C treatment, a 50% drop in P_n was noted for several hours after returning to the reference temperature of 25 C. Since a series of similar experiments showed the phenomenon to be reproducible, it was decided that subsequent net photosynthesis measurements for these studies would be made at leaf temperatures of 25 C.

Distinct diurnal trends in net photosynthesis of the variety Florigiant were shown consistently in the constant, controlled conditions of the semiclosed system (Fig. 2b). The amplitude of the one-half sine wave form depicting net photosynthesis was not as large as reported in earlier studies (12). This is probably because in the studies reported here, the nylon fretwork of the leaf chamber minimized leaf turgor movement, whereas the uncontrolled sleep movements of leaves in the earlier studies changed the angle of incidence of light and increased reflectance with increase in angle of incidence, thus more markedly reducing photosynthesis at the beginning and end of the day. Since sleep movements are undesirable in tracing the mechanism for changing daily photosynthetic rate, the nylon meshwork allowed net photosynthesis measurements in these studies to be made on leaves held perpendicular to light at all times. Several other cultivars of peanut, such as 'Early Runner,' 'Florunner,' 'Tifspan,' 'Argentine,' 'Tifton 8,' and 'NC-4,' were examined for diurnal change in net photosynthesis. All of the cultivars showed daily curves similar to that of Florigiant under constant conditions.
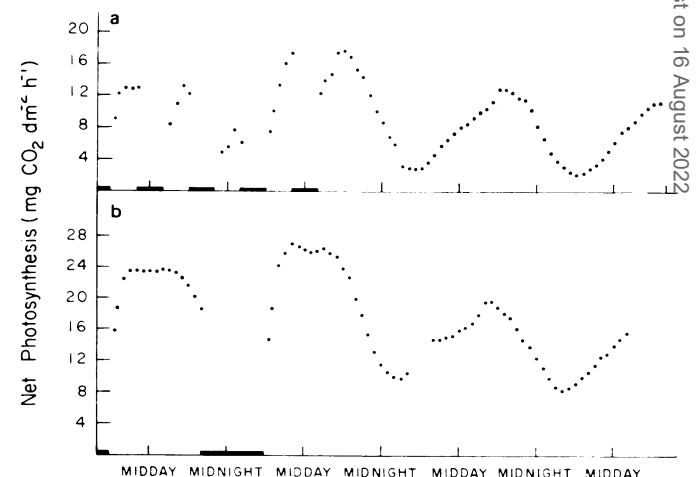

FIG. 2. Net photosynthesis in continuous light as related to the light and dark period. Each point represents the average hourly value. The closed bars on the abscissa indicate darkness. The free-running cycle after LD:4,4 (a); LD:14,10 (b).

To establish that peanut net photosynthesis was controlled endogenously, the following experiments were undertaken:

1. Normal day-night conditions were reversed. This reset the biological clock and resulted in the diurnal curve for photosynthesis exhibiting its peak during normal night hours, thereby minimizing the possibility of geophysical factors playing a role.

2. The normal light period was allowed to proceed uninterrupted for several days. Under this regime, definite maxima and minima in net photosynthesis were evinced (Fig. 2b). With time, the photosynthetic rate gradually declined. The natural period of the rhythm seemed to approximate 26 hr and was thus circadianlike.

3. The sensitivity to light and response to light on signal were tested as a function of the phase of the rhythm. Leaves were treated with 4-hr cycles of light and darkness for several days (LD:4,4). The dark treatment was terminated at a different phase of the rhythm (Fig. 2a) and then replaced by continuous light. By this treatment the rhythm of net photosynthesis was placed slightly out of phase (compare Fig. 2a with 2b).

Our earlier studies (12) indicated that changes in diffusive resistance, primarily stomatal resistance, closely paralleled the diurnal change in photosynthesis. Lack of complete synchrony between the photosynthetic and transpiration curves of peanut in those studies led us to believe that stomatal resistance was not solely responsible for the change in photosynthesis and that biochemical activity other than that involved in stomatal operation might play a role. Since peanut photosynthesis was increased under low O_2 concentration at given light levels (15) and the CO_2 compensation point was high, it was assumed that

the peanut was a C_3 plant. Since RuDP carboxylase mediates CO_2 fixation in plants with the Calvin cycle, and RuDP carboxylase activity had at one time been implicated with the circadian rhythm of photosynthesis in the marine plankton dinoflagellate *Gonyaulax polyedra* Stein (22, 23), RuDP carboxylase activity at selected time intervals was surveyed. Assays from peanut leaves in continuous light indicated an increase in activity upon illumination until a maximum rate was achieved after about 12 hr (Fig. 3). Differences in activity associated with longer illuminations were not significant.

Peanut CO_2 compensation under constant conditions shows a peak around midnight and a trough during midday (Fig. 4). The cycling of the CO_2 compensation point is almost completely out of phase with net photosynthesis or transpiration. The rhythms of net photosynthesis and transpiration seem to be closely correlated, more so than in the earlier studies (12), with both peaking at midday and troughing at midnight.

The circadian nature of dark respiration of peanut leaves is shown in Figure 5. After a normal photosynthesizing day, lights were turned off for 3 full days, followed again by a normal day-night regime. Just as net photosynthesis dropped off with time in continuous light, so did dark respiration in continuous dark. The original rate was re-established, however, after a normal 14-hr light period (Fig. 5).

DISCUSSION AND CONCLUSIONS

When the photosynthetic capacity of peanut leaves was examined under constant conditions of illumination, leaf temperature, and vapor-pressure deficit, the diurnal change previously reported (12) appeared as a portion of a circadian rhythm. The phase of the rhythm was changed by the previous dark period, as is evident by comparing Figure 2a with 2b. The very short day-night regime of (LD:4,4) did not distort the curve but did reset and delay the phase about 4 hr. Apparently, the rhythm in photosynthesis is endogenously controlled. Some dampening of photosynthetic capacity within the rhythm with time can be noted. This is due to possible buildup of photosynthetic products (*i.e.*, carbohydrates) (24). However, changes in amount of soluble carbohydrates cannot fully explain such cyclic trends. Nitrate reductase may be implicated. Nitrate reductase activity in *Capsicum frutescens* seems to account for the marked diurnal periodicity in the incorporation of ^{14}C from photosynthetically fixed $^{14}CO_2$ into amino acids (21). The activity or incorporation resembles the diurnal variation in photosynthesis of *Capsicum* (12). The strong persistence of the
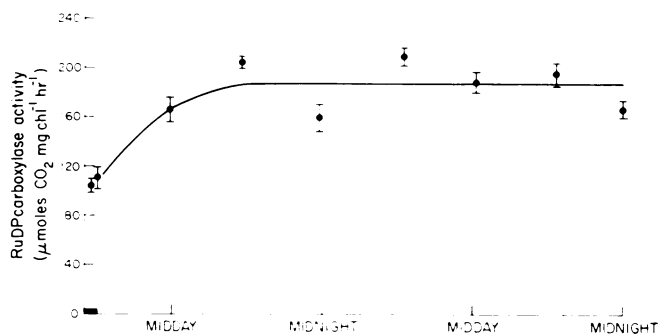
FIG. 3. RuDP carboxylase activity in peanut leaves as a function of continuous light. Each point represents the mean of three determinations; each determination was made on separate leaves. The standard errors of the means are shown by the vertical lines.
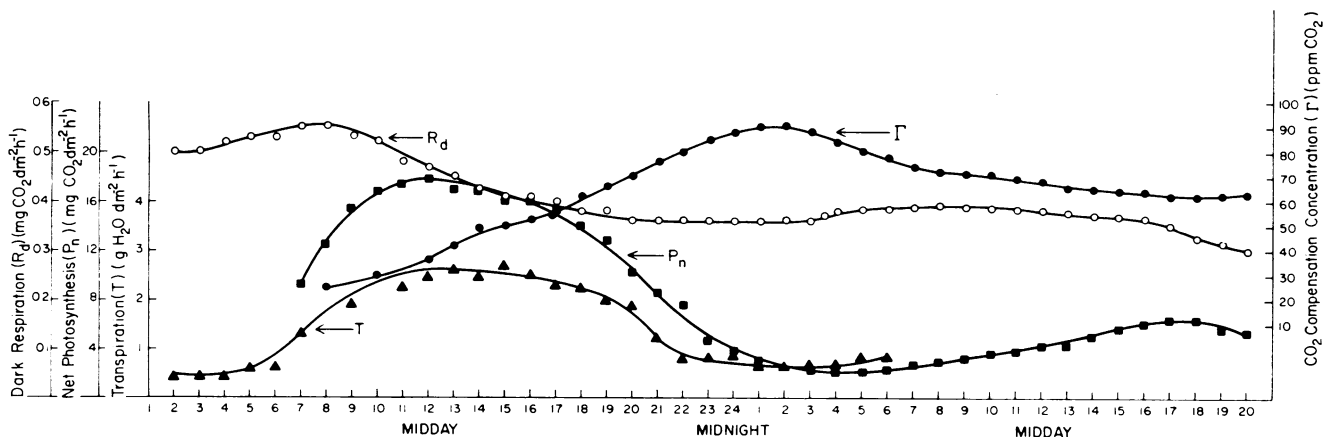
FIG. 4. Photosynthesis, transpiration, dark respiration, and carbon dioxide compensation of peanut leaves in continuous light.
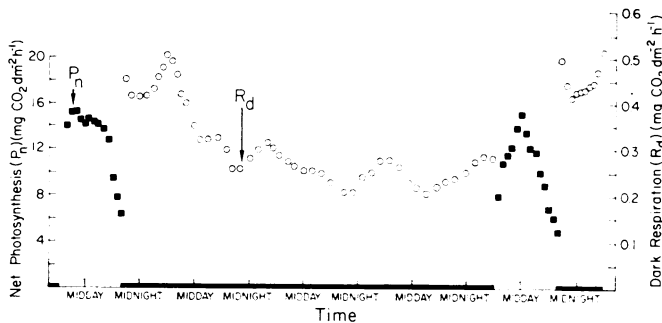
FIG. 5. Photosynthesis and dark respiration of peanut leaves. Heavy bar on abscissa indicates dark period.

rhythmic endogenous nature of photosynthesis in peanut leaves in constant light is similar to reports on algae (25).

No significant rhythmic change in RuDP carboxylase activity of peanut leaves was found. During the light period, RuDP carboxylase activity increased for approximately 12 hr, before leveling off at 80% above the levels found at the end of the preceding 10-hr dark period. The longtime increase during the photoperiod is substantiated in two other recent reports. In *Euglena*, RuDP carboxylase activity increased 150% during a 10-hr photoperiod (25), whereas in barley, RuDP carboxylase activity increased 10% in 12 hr of light (17). Presumably, the increase in RuDP carboxylase activity in light could be due to a light-activated *de novo* synthesis of the enzyme (17).

The increase in RuDP carboxylase activity during morning hours could help explain the increase in apparent photosynthesis but offers no logic for explaining the normal fall-off in photosynthesis in late afternoon or its continuous cyclic nature in constant light. Our analysis of RuDP carboxylase activity in peanut leaves agrees with the report of Bush and Sweeney (3) on the dinoflagellate *Gonyaulax*, in which it seems that the photosynthetic rhythm is not related to a change in the amount of RuDP carboxylase activity.

The closest approximation to control of the rhythm in photosynthesis is that of change in stomatal diffusion resistance. Figure 4 depicts transpirational change of a segment of a plant population used in RuDP carboxylase measurements as measured by a recording balance. The peaks and dips closely coincide in time with those of photosynthesis in the same figure. Our earlier report (12), showing a correlation of daily stomatal movement in certain species with diurnal change in photosynthesis, has been substantiated for soybeans by Upmeyer and Koller (24).

Now the total endogenous change in peanut photosynthesis seems to be correlated with stomatal movement. Endogenous change in stomatal aperture of a number of species has been described (10, 11, 20). Possibly the photosynthetic rhythm is not dependent on stomatal movement alone. A biochemical clock must operate any endogenous part of stomatal rhythm. This timing device operates in continuous light as in these studies, or in continuous dark as found in previous studies (11). Since chronologically maxima and minima in dark respiration approximate those of photosynthesis and transpiration (Fig. 4), one can deduce that either a fluctuation in energy requirement is associated with the phenomenon or *de novo* enzymatic synthesis occurs, or both. Recent work presents logical argument for consideration of energy cycling as the basic oscillator in circadian rhythmicity (2).

The circadianlike endogenous change in dark respiration of peanut leaves varied between 0.5 and 0.2 mg CO_2 dm^{-2} hr^{-1}

(Fig. 5). Endogenous rhythm in the rate of carbon dioxide output has been reported to exist in the leaves of several species (4, 6, 26). Circadianlike changes in dark respiration of roots, tubers, and germinating and ungerminated seed are also known to exist (1, 2, 7).

The CO_2 compensation point of the cultivar Florigiant shows rhythmic change throughout a 24-hr light period (Fig. 4). The range for this plant amounted to 30 $\mu l/l$; however, the range between maxima and minima of CO_2 compensation points of other plants has been greater and lesser than 30 $\mu l/l$. The rhythm is out of phase with photosynthesis, dark respiration, and transpiration. The timing of the peak and trough in these studies is similar to that observed for the CO_2 compensation point of coffee leaves (8). Circadian rhythm in the level of the CO_2 compensation point has also been reported for *Bryophyllum* (9). The inverse nature of the CO_2 compensation curve to that of photosynthesis suggests that CO_2 efflux increases as photosynthesis slows down. This also provides circumstantial evidence for biochemical processes affecting an endogenous change of net photosynthesis. The increase of the CO_2 compensation point (Γ), during what would be normal night hours, could come about by the lowering in photosynthetic efficiency, which in turn would lower the recycling potential for CO_2 produced at the photorespiratory center that is refixed in the chloroplast. On the other hand, Γ could be paced by changing either the rate of photorespiration (L) or the physical and chemical resistance (r_m) between the photosynthetic and photorespiratory centers, since $\Gamma = L \cdot r_m$ (18).

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