Plant Physiol. (1980) 66, 463–465
0032-0889/80/66/0463/03/$00.50/0

Quantum Requirement for Photosynthesis in *Sedum praealtum* during Two Phases of Crassulacean Acid Metabolism[1]

Received for publication January 2, 1980 and in revised form April 3, 1980

MARTIN H. SPALDING[2] AND GERALD E. EDWARDS
Horticulture Department, University of Wisconsin, Madison, Wisconsin 53706

MAURICE S. B. KU
Biochemistry Department, University of Georgia and United States Department of Agriculture, Science and Education Administration, Richard B. Russell Agricultural Research Center. P. O. Box 5677, Athens, Georgia 30604

ABSTRACT

The quantum requirement (QR) for photosynthesis in *Sedum praealtum*, a Crassulacean acid metabolism plant, was compared with that of wheat, a C_3 plant, and maize, a C_4 plant, at 30 C. During the deacidification phase in *S. praealtum*, approximately 16 moles quanta were absorbed per mole malate consumed. This is equivalent to 16 moles quanta per mole CO_2 fixed, assuming 1 mole CO_2 is assimilated per mole malate decarboxylated. This QR for Crassulacean acid metabolism is similar to that of the C_3 or C_4 plant under atmospheric conditions, even though there are considerable differences in the biochemistry of photosynthesis. During late-afternoon C_3-like fixation of atmospheric CO_2 in *S. praealtum*, the QR was relatively high with values of 41 under 21% O_2 and 19 under 2% O_2. During the deacidification phase in *S. praealtum*, the relatively low QR can be accounted for by the repression of photorespiration and saturation of photosynthesis from the elevated CO_2 concentration in the leaves during malate decarboxylation.

Higher plants are divided into three groups based on differences in photosynthetic carbon assimilation: C_3, C_4, and CAM. Depending on the specific biochemical pathway, the QR[3] for photosynthesis (the number of quanta required to reduce one molecule of CO_2) in C_3 and C_4 plants varies and responds differently to environmental factors such as CO_2, O_2, and temperature (2, 4–7). In C_3 plants, O_2 inhibits photosynthesis and increases the QR. For example, at 30 C and atmospheric CO_2 concentrations, increasing O_2 from 2 to 21% increases the QR from approximately 12 to 17. C_4 plants overcome O_2 inhibition of photosynthesis by a CO_2-concentrating mechanism. Nevertheless, they have a QR (17–19 mol quanta/mol CO_2 fixed) similar to that of C_3 plants under atmospheric conditions, apparently due to the additional energy needed to drive the C_4 cycle.

Depending upon the conditions, CAM plants are capable of either fixing CO_2 released from malate decarboxylation analogous to C_4 plants or directly fixing atmospheric CO_2 analogous to C_3 plants. Often these species will fix CO_2 from malate decarboxylation behind closed stomata during the early part of the day and then directly fix atmospheric CO_2 through a C_3-like pathway in the latter part of the day (10, 11). The responses of these processes to light level have long been observed (10) but have not been examined in detail. In the present study, the QR for photosynthesis of *S. praealtum* was determined during these two phases of carbon assimilation and the results are discussed in relation to CAM.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. *S. praealtum* D.C. was grown under a 10-h photoperiod and a 30 to 15 C, day to night temperature regime. The quantum flux density at plant level was about 50 nmol/cm^2·s (400–700 nm). The plants were watered approximately every 3rd day and fertilized every 2 weeks. Under these conditions, this species exhibits CAM with considerable CO_2 fixation and malic acid accumulation in the dark followed by malic acid depletion in the light behind closed stomata (11). In the latter part of the photoperiod, when the malic acid is depleted, the stomata open and atmospheric CO_2 is assimilated directly.

Plants of *Triticum aestivum* L., a C_3 species, and *Zea mays* L., a C_4 species, were grown under a 16-h photoperiod, a 30 to 25 C, day to night temperature regime, and a quantum flux density of about 40 nmol/cm^2·s (400–700 nm). The plants were watered on alternate days with tap water and nutrient solution.

Quantum Requirement Measurements. To determine the QR for photosynthesis by individual leaves, the absorption of light by leaves was determined as previously described using a quantum sensor measuring wavelengths between 400 to 700 nm (Lambda Instruments, Lincoln, NE) (5). The absorption is defined as $\alpha = 1 - (T + R)$ where T is the fraction transmitted and R the fraction reflected by the leaves. The quantum absorption for *S. praealtum* leaves was about 0.82 (0.14 reflected and only 0.04 transmitted) and was 0.76 and 0.80 for *T. aestivum* and *Z. mays* leaves, respectively.

Measurements for late afternoon CO_2 fixation in *S. praealtum* were made between 6 to 9 h into the light period (10-h day). Malic acid concentration remains relatively constant during this period and does not contribute any net CO_2 through decarboxylation. Measurements for CO_2 fixation in the C_3 and C_4 plants were made in the middle of the light period (16-h day). Photosynthesis and transpiration were determined as previously described using a Barnes multispec IR CO_2 and H_2O vapor analyzer (Barnes Engineering Co., Stamford, Conn.) in an open circuit system (5) at a leaf temperature of 30 C. A Clark-type O_2 electrode (YSI, Yellow Springs, Ohio) was incorporated into the gas analysis system for measurement of O_2 concentration. The photosynthesis measure-

[1] This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by National Science Foundation Grant PCM 77-09384 to G.E.E.

[2] Present address: Department of Agronomy, University of Illinois-Urbana, Urbana, Ill. 61801.

[3] Abbreviations: QR: quantum requirement; Mops; 3-(*N*-morpholino)-propanesulfonic acid.

Table I. *Quantum Requirement for Photosynthesis in Plants Representing Three Photosynthetic Groups*
Photosynthesis was measured at 30 C and other conditions as indicated.

Species	Conditions			Stomatal Resistance to CO_2[a]	Internal $[CO_2]$[a]	Quantum Requirement
	Time	External $[O_2]$	External $[CO_2]$			
		%	μl/l	s/cm	μl/l	*mol quanta/ mol CO_2 fixed*
T. aestivum (C_3)	Mid-day	2	315	1.6–2.0	275–306	12
	Mid-day	21	315	1.3–1.6	292–309	17
Z. mays (C_4)	Mid-day	2	315	2.4–3.0	273–307	17
	Mid-day	21	315	2.5–2.7	269–308	17
S. praealtum (CAM)	Morning	21[e]	370	>100[b]	~4000[c]	16[d]
	Late afternoon	2	315	3.4–5.0	263–285	19
	Late afternoon	21	315	5.6–9.2	277–291	41

[a] Calculated from simultaneous measurements of photosynthesis and transpiration at light levels between 3 and 12 nmol quanta/cm^2·s used for QR determination. The internal $[CO_2]$ decreased slightly with increasing light intensity.
[b] Measured with diffusive resistance meter.
[c] Data from Spalding *et al.* (11).
[d] Measured as mol quanta absorbed/mol malate consumed. See text for measurement details.
[e] Internal $[O_2]$ of 22 to 26% (11).

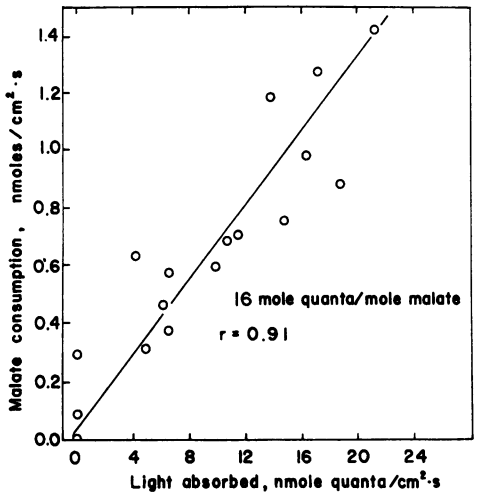

FIG. 1. Rate of malate consumption at varying levels of light absorption by leaves of *S. praealtum* at leaf temperatures of 30 to 32 C between 2 to 4 h into the photoperiod.

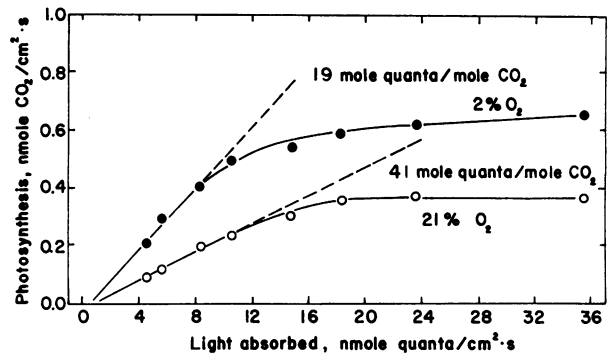

FIG. 2. Rate of CO_2 uptake at varying levels of light absorption by leaves of *S. praealtum* at 2 and 21% O_2. Measurements were made between 6 to 9 h into the photoperiod at a leaf temperature of 30 C and an ambient CO_2 concentration of 315 μl/l.

ments proceeded from high to low quantum flux densities after reaching steady state photosynthesis at each condition.

In *S. praealtum,* rate of consumption of malic acid was used as a measure of donation of CO_2 to the C_3 pathway during the decarboxylation phase of CAM. Previous studies with these species indicated that the rate of decrease of malate between approximately 2 to 4 h in the light period could be used as an estimate of the rate of photosynthetic CO_2 assimilation during the same period (11). The CO_2 concentration in the leaves is relatively constant during this period and loss of CO_2 is prevented by stomatal closure. Thus, the rate of consumption of malate through its decarboxylation is a reasonable estimate of rate of carbon donation to the C_3 pathway. The amount of malate consumed was measured between approximately 2 to 4 h in the light period in the growth chamber at leaf temperatures of 30 to 32 C. Various light levels were obtained by inserting cheesecloth screens between the leaves and the light source. Zero light levels were obtained by placing plants inside lightproof cardboard boxes inside the growth chamber.

Leaf pairs receiving identical light levels were chosen and, at approximately 2 h into the photoperiod, three 7-mm diameter discs were cut from one leaf of each pair with a cork borer and immediately placed in 5 ml boiling ethanol. The second leaf of each pair was similarly sampled at approximately 4 h into the photoperiod. Time of sampling was recorded, so the elapsed time between sampling for each pair was known. The discs were left boiling for an additional 30 min, after which the ethanol was evaporated at room temperature and the samples were stored at −10 C until assayed for malate content. Before assay, the samples were resuspended in 1 ml of water. Malic acid was assayed using chicken liver NADP-malic enzyme (Sigma) by spectrophotometrically determining the change in *A* at 340 nm due to NADP reduction by malic acid. The assay was performed at 25 C in a medium containing 1 unit malic enzyme, 50 mM Mops (pH 7.4), 1 mM NADP, 5 mM $MgCl_2$, and 10 μl of the sample in a final volume of 1.2 ml.

RESULTS AND DISCUSSION

As shown in Figure 1, there was a linear increase in malate consumption by leaves of *S. praealtum* with increasing quanta

absorbed giving a QR of 16 mol quanta/mol malate used. This is similar to the QR for photosynthesis in C_3 plants and in C_4 plants under atmospheric conditions at 30 C (16–19 mol quanta/mol CO_2 fixed; Table I; refs. 4 and 5). The photosynthetic metabolism during deacidification in CAM is more nearly analogous to C_4 photosynthesis than to C_3 photosynthesis. Measurements of both internal $[CO_2]$ and $[O_2]$ during the deacidification phase in CAM plants indicate a large increase in the $[CO_2]$ in the leaf and in the CO_2/O_2 ratio compared to the atmosphere (3, 11). In *S. praealtum* during deacidification behind closed stomata, the internal $[CO_2]$ and CO_2/O_2 ratio in the leaf is about 10-fold higher than in the atmosphere (11). Under these conditions, the O_2 inhibition of photosynthesis and its influence on QR would be minimized. In a similar way, the $[CO_2]$ and CO_2/O_2 ratios are proposed to increase in bundle sheath cells of C_4 plants during photosynthesis (1). In C_4 plants, each mol CO_2 fixed and donated to the C_3 pathway by malate decarboxylation requires 5 mol ATP and 2 mol NADPH for conversion to the level of triose-P (3 mol ATP/2 mol NADPH in the C_3 pathway; 2 mol ATP required for the C_4 cycle). In CAM plants, such as *S. praealtum*, decarboxylating malate by malic enzyme, the theoretical requirements for carbon assimilation to the level of triose-P are 6 mol ATP and 2 mol NADPH (3 mol ATP/2 mol NADPH·mol CO_2 fixed in the C_3 cycle and 3 mol ATP for converting pyruvate to triose-P). The additional NAD(P)H required for pyruvate conversion to triose-P would be generated by malic enzyme. These similarities in the theoretical energy requirement for photosynthesis in C_4 and CAM plants, where there is little or no O_2 inhibition of photosynthesis, can account for the similar QR between *S. praealtum* (during the deacidification phase) and maize.

During the late afternoon, net CO_2 fixation in CAM plants occurs predominantly through the C_3 pathway (10) and the QR, therefore, might be expected to be similar to that of C_3 plants. The QR of *S. praealtum* during the late afternoon under 21% O_2 was 41 mol quanta/mol CO_2 fixed (Fig. 2; Table I), more than twice that for *T. aestivum* (Table I) and other C_3 plants (4, 5). In C_3-like photosynthesis under 21% O_2, the QR increases as $[CO_2]$ decreases (4). Due to high stomatal resistance, *S. praealtum* has a consistently lower substomatal $[CO_2]$ than *T. aestivum* at any given light intensity and external concentration of gases (Table I). The differences in intercellular concentration of CO_2 seem too small to account for the higher QR in *S. praealtum*. Reducing the $[O_2]$ from 21 to 2% in *S. praealtum* caused the QR to decrease from 41 to 19 (Fig. 2), which qualitatively is like the response of C_3 plants. Under 2% O_2, the QR of 19 in *S. praealtum* is still considerably higher (1.6 times) than that of C_3 plants and is similar to that of C_4 plants (Table I; refs. 4 and 5). A possible explanation for the relatively high QR in *S. praealtum* during the late afternoon C_3-like phase would be that a significant proportion of the CO_2 assimilated is first fixed via P-enolpyruvate carboxylase into malate which is, in turn, decarboxylated to release the CO_2. This would result in a futile cycle which utilizes energy but results in neither net CO_2 assimilation nor increased internal $[CO_2]$, thus increasing the QR. Evidence that such a futile cycle may operate during this phase of CAM was discussed by Osmond (10).

Rather high quantum requirements have been reported for CAM plants (*Agave deserti* with a QR of 46, ref. 9; *Ferocactus acanthodes* with a QR of 68, ref. 8). Calculation of QR in these cases was based on the relationship between total quanta received over an entire preceding day and the nocturnal CO_2 uptake rather than on the photosynthetic CO_2 assimilation during a particular phase of daytime CAM activity. The results here indicate that CAM plants have an efficiency similar to both C_3 and C_4 plants in utilizing energy for assimilating CO_2 during the deacidification phase of CAM. However, due to the limited storage of malic acid and the inefficiency of energy utilization outside this deacidification phase, over the entire photoperiod CAM plants have a greater QR than either C_3 or C_4 plants.

LITERATURE CITED

1. Berry J, G Farquhar 1978 The CO_2 concentrating function of C_4 photosynthesis. A biochemical model. *In* DO Hall, J Coombs, TW Goodwin, eds, Proc 4th Int Congr Photosynthesis The Biochemical Society, London, pp 119–131
2. Campbell WH, CC Black 1978 The relationship of CO_2 assimilation pathways and photorespiration to the physiological quantum requirement of green plant photosynthesis. Biosystems 10: 253–264
3. Cockburn W, IP Ting, LO Sternberg 1979 Relationship between stomatal behavior and internal carbon dioxide concentration in CAM plants. Plant Physiol 63: 1029–1032
4. Ehleringer J, O Björkman 1977 Quantum yields for CO_2 uptake in C_3 and C_4 plants. Dependence on temperature, CO_2, and O_2 concentration. Plant Physiol 59: 86–90
5. Ku SB, GE Edwards 1978 Oxygen inhibition of photosynthesis. III. Temperature dependence of quantum yield and its relation to O_2/CO_2 solubility ratio. Planta 140: 1–6
6. Ku SB, GE Edwards 1980 Quantum requirement of photosynthetic processes. *In* CC Black, A Mitsui, eds, CRC Handbook of Biosolar Resources, Vol 1, Fundamental Principles. CRC Press, Ohio. In press
7. McCree KJ 1972 The action spectrum, absorptance, and quantum yield of photosynthesis in crop plants. Agric Meteorol 9: 191–216
8. Nobel PS 1977 Water relations and photosynthesis of a barrel cactus, *Ferocactus acanthodes*, in the Colorado desert. Oecologia 27: 117–133
9. Nobel PS, TL Hartsock 1978 Resistance analysis of nocturnal carbon dioxide uptake by a Crassulacean acid metabolism succulent, *Agave deserti*. Plant Physiol 61: 510–514
10. Osmond CB 1978 Crassulacean acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29: 379–414
11. Spalding MH, DK Stumpf, MSB Ku, RH Burris, GE Edwards 1979 Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2 concentrations in *Sedum praealtum* D.C. Aust J Plant Physiol 6: 557–567