Plant Physiol. (1986) 80, 23–26
0032-0889/86/80/0023/04/$01.00/0

Effects of Irradiance on Crassulacean Acid Metabolism in the Epiphyte *Tillandsia usneoides* L. (Bromeliaceae)[1]

Received for publication June 25, 1985 and in revised form July 11, 1985

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ABSTRACT

Spanish moss (*Tillandsia usneoides* L.) was collected in South Carolina, maintained in a greenhouse, then exposed to five levels of photosynthetic photon flux density (PPFD) for 3 weeks. Following this treatment, plants were sampled for chlorophyll concentrations, nocturnal acid accumulations, and photosynthetic responses to subsequent exposure at a range of PPFD. No acclimation to PPFD was observed; all plants exhibited similar patterns of nocturnal CO_2 uptake and acid accumulation regardless of initial PPFD treatment. These patterns revealed that at a PPFD level of approximately 200 micromoles per square meter per second (daytime integrated PPFD of 10 moles per square meter per day), CAM saturated or, in low-PPFD plants, was optimal. The results of this study indicate that adaptation to high PPFD is not necessarily a requirement of CAM.

Investigations of photosynthetic responses to light using CAM plants are few. This is not surprising given the methodological difficulties in determining the effects of daytime PPFD[2] on nighttime CO_2 uptake. Recent evidence indicates that most CAM plants examined thus far are limited by the quantity of light received, even at full sunlight. Light-saturation of nocturnal CO_2 uptake and concomitant acid accumulation in several desert succulents occurred at integrated daytime PPFD of 20 to 24 mol m^{-2} d^{-1}, or approximately full sunlight on a vertical surface at midsummer in the temperate zone (10–14, 17). On the other hand, results of several studies of CAM in *Tillandsia usneoides* L. (Spanish moss) indicate that this epiphyte may exhibit saturation of physiological activity at PPFD levels substantially below full sunlight (1, 6, 7, 9). Also, in a recent study of Spanish moss in South Carolina, levels of nocturnal acidification did not vary between plants collected *in situ* along a roadside and those collected inside the forest canopy, indicating that saturation of CAM must have occurred at low PPFD (7). Thus, preliminary findings emphasize the potential for differences in photosynthetic responses to PPFD between a CAM epiphyte and CAM desert succulents.

It is not inconceivable that Spanish moss may be more shade-adapted than desert CAM plants since this epiphyte can be found growing deep within tree canopies. The observation that Spanish moss also grows fully exposed to sunight at the outer edges of tree canopies and on telephone lines (2, 15) is suggestive of an ability of this plant to acclimate to a wide range of PPFD. Such acclimation ability has been observed in several C_3 and C_4 species (3), but never in a CAM plant. The objectives of the current investigation were to determine the potential of Spanish moss to acclimate to various PPFD levels, and to demonstrate whether or not findings of the effects of light on desert CAM plants can be extended to epiphytic CAM plants.

MATERIALS AND METHODS

Plant Material. Clumps of Spanish moss were collected from the lower, outer canopies of five trees (*Quercus virginiana* Miller) at least 50 m apart in a suburban area of Charleston, SC. Plants were maintained in the University of Kansas greenhouse for a minimum of 3 weeks prior to experimentation. Typical greenhouse conditions were: 30/25°C day/night air temperatures (measured with a Cu-constantan thermocouple), 60 to 70% air RH day and night (measured with a Vaisala humidity sensor), maximum PPFD of 1000 μmol m^{-2} s^{-1} incident on the plants (measured with a LI-COR LI-190SB sensor and LI-185B meter), and extension of the natural photoperiod to 16 h with fluorescent lamps. Calculations of daily integrated PPFD were precluded by the complex and variable patterns of shading from neighboring plants and nearby campus buildings. All plants were watered every other day and received a dilute nutrient solution (4) once or twice each month.

PPFD Treatment. Due to limitations imposed by the growth chambers, plants exposed to full sunlight were spread horizontally on metal screens directly above pools of water on a building rooftop. Environmental conditions at midday were typically 35°C and 30% RH, while at night air temperatures declined to 18°C and RH increased to approximately 90%. Although variable throughout each day as a result of clouds and changing solar angles, maximum PPFD incident on the plants was 2000 μmol m^{-2} s^{-1} (14 h photoperiod). Plants from all five populations were exposed simultaneously and were thoroughly wetted every other day for 3 weeks.

For lower PPFD treatments, plants from all populations were spread horizontally on metal racks directly above trays of water in a growth chamber for 3 weeks. All material was wetted every other day. Environmental conditions in the growth chamber were maintained at 32/22°C day/night air temperatures and a constant RH of approximately 52%. Photoperiods were always 14 h; PPFD incident on the plants was 500, 250, 125, or 65 μmol m^{-2} s^{-1} (25.2, 12.6, 6.3, or 3.3 mol m^{-2} d^{-1}), depending on the experiment. PPFD levels were altered by changing the distance between plants and lamps.

Physiological Responses to PPFD. Following exposure to a specific PPFD level, plants were sampled for acid fluctuations and Chl concentrations. The remaining plants were cleaned of nonliving tissue and dark-treated 3 d to reduce tissue carbohydrate reserves. After this, some of these plants were placed in another growth chamber while the remaining plants from the

[1] Supported in part by a University of Kansas Undergraduate Research Award to C. A. E. and by Biomedical Sciences Support Grant RR07037 to C. E. M.

[2] Abbreviations: PPFD, photosynthetic photon flux density; DW, dry weight.

five populations were pooled and sealed in the gas exchange chamber. Growth chamber conditions (same for the dark treatment) were 29/23°C day/night air temperatures and 72/62% day/night air RH. Photoperiod was 14 h and PPFD incident on the plants was 450, 330, 145, 95, or 53 μmol m^{-2} s^{-1}, depending on the distance from lamps to plants. After 5 d of exposure to the various PPFD levels, samples were taken for acidity analyses.

Chlorophyll Analysis. Several strands from each population of Spanish moss were frozen at −70°C following the 3 week PPFD treatment, ground with a mortar and pestle in 80% aqueous acetone (containing $MgCO_3$), and centrifuged at 12,000*g* for 3 min. All procedures were carried out at approximately 5°C and in dim light. Absorbance of the supernatant was determined spectrophotometrically at 645, 663, and 720 nm. Absorbance at 720 nm was subtracted from each value to minimize effects due to impurities. Procedures and equations used to determine Chl *a* and *b* concentrations were taken from Šesták (16). The pellet was recovered and dried at 85°C for at least 3 d, cooled in a desiccator, and weighed.

Titratable Acidity Analyses. Strands were collected 30 min before lights-on and 30 min before lights-out and frozen as above following both the 3 week treatment and the subsequent exposure to a range of PPFD. Plants were ground with a mortar and pestle in deionized H_2O and titrated to pH 7.0 with 0.01 N NaOH. The extract was evaporated, dried at 85°C, cooled in a desiccator, and weighed.

CO_2 Exchange Analyses. The gas exchange system used here has been described previously (8). Mean environmental conditions within the gas exchange chamber were: 30.0°C (SD = 0.4°C, n = 121 determinations) daytime air temperature, 20.0°C (SD = 0.7°C, n = 70 determinations) nighttime air temperature, 17.0°C (SD = 0.5°C, n = 238 determinations) day and night air dew point, and 345 μl l^{-1} (SD = 15 μl l^{-1}, n = 175 determinations) CO_2 concentration. After the dark exposure described above, plants were pooled from the five populations and placed in the chamber. CO_2 exchange was then monitored continuously for 6 d. PPFD (14 h photoperiod) was 40 to 60 μmol m^{-2} s^{-1} (range inside chamber) for d 1 and 2, 70 to 100 μmol m^{-2} s^{-1} for d 3, 160 to 260 μmol m^{-2} s^{-1} for d 4, 400 to 600 μmol m^{-2} s^{-1} for d 5, and 750 to 1500 μmol m^{-2} s^{-1} for d 6 and 7. Plants were removed on the 7th day and analyzed for Chl concentration as described previously (8). On each day of the experiment the plants were removed from the chamber, wetted thoroughly, and replaced. An apparent loss of CO_2 resulted in all cases (Fig. 4).

RESULTS

After exposure for 3 weeks to a range of PPFD levels, Spanish moss exhibited the greatest acid fluctuations (Fig. 1) as well as the highest Chl concentrations at 125 μmol m^{-2} s^{-1} (Fig. 2). In both cases, exposure to higher or lower PPFD resulted in lower levels of acid or pigments. Chl *a*/*b* ratios varied only slightly in response to PPFD, being lower at the two highest PPFD levels (Fig. 2).

Following the 3 week exposure to full sunlight (maximum PPFD of 2000 μmol m^{-2} s^{-1}), no differences in morning titratable acidities were observed from 53 to 450 μmol m^{-2} s^{-1} PPFD (Fig. 3A). Similarly, evening acidities did not vary significantly with PPFD except acidity was higher at the lowest PPFD level. Thus, nocturnal increases in acidity increased from 53 to 95 μmol m^{-2} s^{-1} but did not change thereafter. Nearly identical results were obtained with Spanish moss exposed to 500 μmol m^{-2} s^{-1} for 3 weeks, then exposed 5 d to a range of PPFD (Fig. 3B). Plants treated 3 weeks with 250 μmol m^{-2} s^{-1} then 5 d of various PPFD showed even fewer differences (Fig. 3C). Although overall acid fluctuations were lower in the plants exposed 5 d to 53 μmol m^{-2} s^{-1} PPFD, as above, the evening acidity was not significantly different from two of the other means. No significant differences in responses of morning or evening tissue acidity to the 5 d

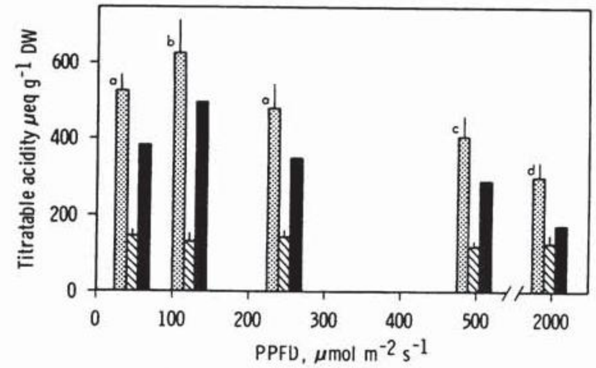

FIG. 1. Response of mean morning (dotted bars) and evening (slashed bars) tissue acidity to PPFD for Spanish moss grown 3 weeks at the same PPFD. Solid bars represent differences between the morning and evening means. Lines indicate 1 SE. Only means with different letters are significantly different ($p < 0.05$; one-way analysis of variance and Student-Newman-Keuls test). n = 12–15.

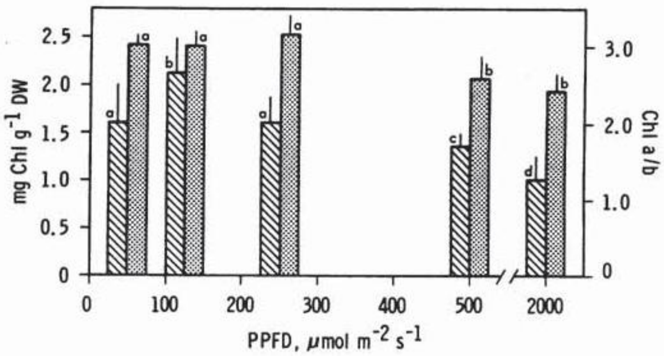

FIG. 2. Response of mean total Chl concentration (slashed bars) and a/b ratio (dotted bars) to PPFD for Spanish moss grown 3 weeks at the same PPFD. Lines indicate 1 SE. Only means with different letters are significantly different ($p < 0.05$; one-way analysis of variance and Student-Newman-Keuls test). n = 15.

PPFD treatment were found with Spanish moss exposed 3 weeks to either 125 μmol m^{-2} s^{-1} (Fig. 3D) or 65 μmol m^{-2} s^{-1} (Fig. 3E).

In general, levels of tissue acidity measured immediately after the 3 week PPFD treatments were comparable to levels measured after the dark treatment and 5 d PPFD exposure (compare Fig. 1 with Fig. 3). This comparison is not possible with plants exposed to full sunlight since the growth chambers were incapable of producing this PPFD level. Greatest tissue acid fluctuations were found in those plants treated 3 weeks with a PPFD of 125 μmol m^{-2} s^{-1} (Figs. 1 and 3D).

After Spanish moss was exposed 3 weeks to full sunlight, nocturnal CO_2 uptake increased from a daytime PPFD of 40 to 60 μmol m^{-2} s^{-1} to 160 to 260 μmol m^{-2} s^{-1} then saturated thereafter (Fig. 4A). Daytime CO_2 losses decreased in a similar fashion. Nearly identical patterns of CO_2 exchange were obtained with plants exposed to 500 μmol m^{-2} s^{-1} for 3 weeks (Fig. 4B), though nocturnal CO_2 uptake rates were higher and daytime losses lower. The same trend of increasing nocturnal CO_2 uptake with increases in daytime PPFD from 40 to 60 to 160 to 260 μmol m^{-2} s^{-1} was observed with plants exposed 3 weeks to 250, 125, and 65 μmol m^{-2} s^{-1}, but higher PPFD resulted in progressively decreasing CO_2 uptake rates (Fig. 4, C, D, and E).

Integration of 24-h net CO_2 exchanges (Fig. 5) emphasizes three findings: (a) plants exposed 3 weeks to 250 μmol m^{-2} s^{-1} exhibited the greatest rates of nocturnal CO_2 fixation, (b) plants exposed 3 weeks to high PPFD lost CO_2 on a daily basis at low PPFD, and (c) Spanish moss treated 3 weeks with high PPFD exhibited saturation-type responses to increasing PPFD whereas

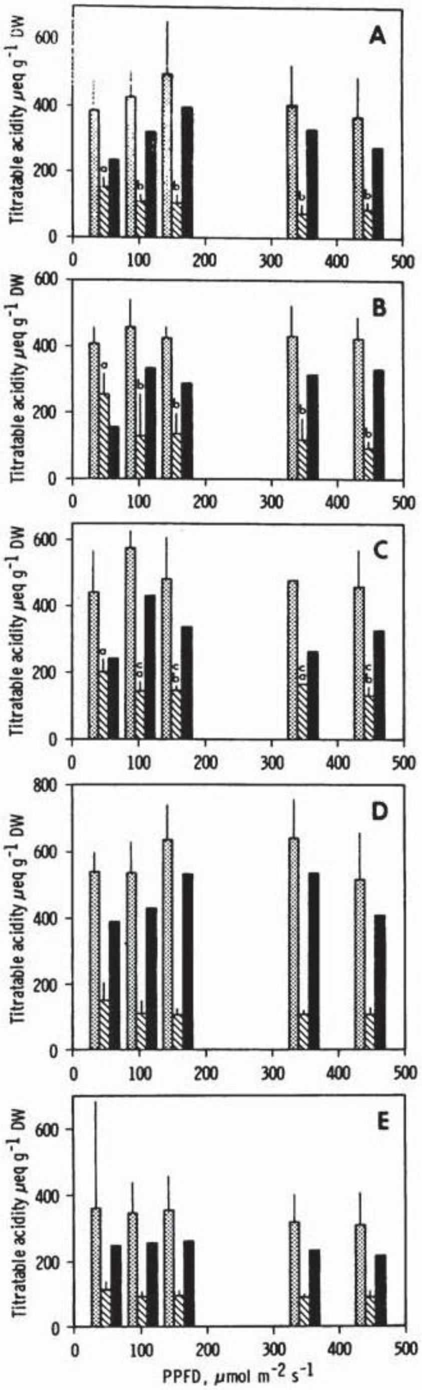

FIG. 3. Response of mean morning (dotted bars) and evening (slashed bars) tissue acidity to a 5 d PPFD treatment for Spanish moss after a 3 week exposure to full sunlight (maximum PPFD of 2000 μmol m^{-2} s^{-1}) (A), 500 μmol m^{-2} s^{-1} (B), 250 μmol m^{-2} s^{-1} (C), 125 μmol m^{-2} s^{-1} (D), and 65 μmol m^{-2} s^{-1} (E). Solid bars represent differences between the morning and evening means. Lines indicate 1 SE. Only means with different letters are significantly different ($p < 0.05$; one-way analysis of variance and Student-Newman-Keuls test). $n = 4$–5.

plants exposed to lower PPFD exhibited optimum-type responses.

DISCUSSION

Nobel *et al.* (10–14, 17) have shown conclusively that saturation of CAM in desert cacti with vertical stems does not occur, even at full sunlight. However, the results of the current study

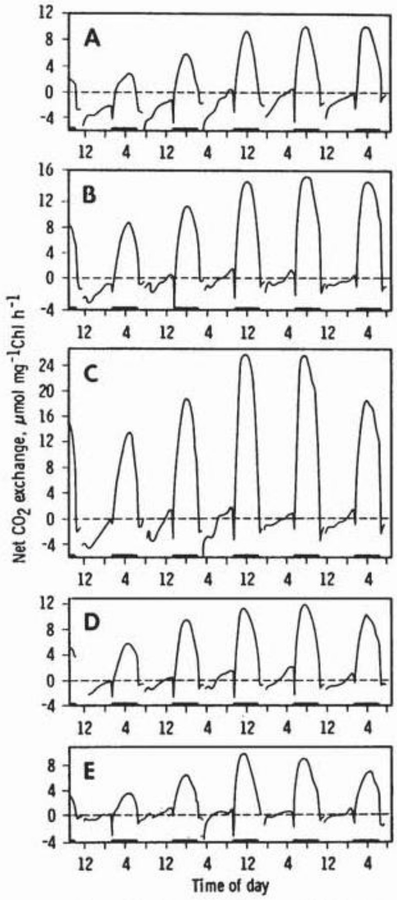

FIG. 4. Response of net CO_2 exchange to PPFD for Spanish moss after a 3 week exposure to full sunlight (maximum PPFD of 2000 μmol m^{-2} s^{-1}) (A), 500 μmol m^{-2} s^{-1} (B), 250 μmol m^{-2} s^{-1} (C), 125 μmol m^{-2} s^{-1} (D), and 65 μmol m^{-2} s^{-1} (E). PPFD levels during the gas exchange measurements were 40 to 60 μmol m^{-2} s^{-1} (range inside chamber) for the first complete day shown (d 2 in "Materials and Methods"), 70 to 100 μmol m^{-2} s^{-1} the 2nd d, 160 to 260 μmol m^{-2} s^{-1} the 3rd d, 400 to 600 μmol m^{-2} s^{-1} the 4th d, and 750 to 1500 μmol m^{-2} s^{-1} the 5th d. Other environmental conditions inside the gas exchange chamber were kept constant throughout and are given in "Materials and Methods". Plants from five populations were included in the chamber. Positive values indicate net CO_2 uptake. Black bars represent darkness.

have clearly shown that CAM in Spanish moss saturates at PPFD much lower than full sunlight and, depending on treatment PPFD, shows signs of photoinhibition at high PPFD. Nocturnal increases in the acidity in Spanish moss saturated at or below 95 μmol m^{-2} s^{-1}, regardless of the PPFD exposure prior to experimentation (Fig. 3). Similarly, the greatest acid fluctuations were observed in plants exposed 3 weeks to 125 μmol m^{-2} s^{-1} (Fig. 1). The CO_2 exchange data also indicate that photosynthesis in Spanish moss, unlike other CAM plants studied thus far, saturates at low PPFD. Saturation or an optimum level of nocturnal CO_2 uptake occurred at 160 to 260 μmol m^{-2} s^{-1}, regardless of the PPFD level during the 3 week treatment (Fig. 4). The slight discrepancy between the acidity and CO_2 exchange data may be attributed to differences between the two methods of quantifying CAM: (a) net CO_2 exchange includes respiratory losses at night which may change with PPFD (3) while acid accumulation reflects gross CO_2 uptake only (although a small amount of malate may provide substrate for respiration [5]); (b) the extent of internal recycling of respiratory CO_2 may vary with PPFD; (c) it is possible that patterns of acid fluctuations changed with PPFD such that the acid levels of plants sampled in the morning and evening under one PPFD regime did not coincide with the

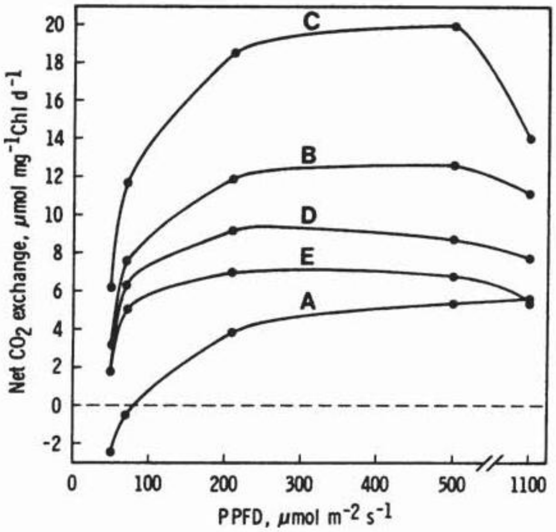

FIG. 5. Response of integrated 24-h net CO_2 exchange to a 5 d PPFD treatment for Spanish moss after a 3 week exposure to full sunlight (maximum PPFD of 2000 μmol m^{-2} s^{-1}) (A), 500 μmol m^{-2} s^{-1} (B), 250 μmol m^{-2} s^{-1} (C), 125 μmol m^{-2} s^{-1} (D), and 65 μmol m^{-2} s^{-1} (E). Positive values indicate net CO_2 uptake. Data were integrated from Figure 4.

maximum and minimum acid levels, but did coincide in plants sampled under another PPFD regime; and (d) plants were exposed to slightly different environmental conditions in the gas exchange chamber and growth chamber (see "Materials and Methods").

In spite of these potential problems, it is clear that Spanish moss exhibits the greatest level of CAM at a minimum of approximately 200 μmol m^{-2} s^{-1}, or 10.1 mol m^{-2} d^{-1} which is about half the total PPFD received by a vertical surface at midsummer in the temperate zone (10–14). This value represents the PPFD at which saturation of CAM was observed in plants previously exposed to high PPFD and the optimum PPFD for plants exposed to low PPFD for 3 weeks. This response to PPFD is not unlike that found in typical shade plants (3), although it is difficult to compare photosynthetic responses to PPFD in CAM plants with non-CAM plants. The results of the present study corroborate the results of field research on Spanish moss in South Carolina where levels of nocturnal acid accumulation did not change with increasing exposure *in situ* (7). Also, the results of this study are in agreement with past studies indicating that nocturnal CO_2 uptake in Spanish moss saturates at integrated PPFD between 10 and 20 mol m^{-2} d^{-1} (1, 6, 7, 9).

Spanish moss collected in South Carolina and grown a minimum of 3 weeks in a greenhouse exhibited an inability to acclimate photosynthetically to a range of PPFD levels during 3 week exposures. In all cases, plants responded similarly. The greater Chl concentrations at lower PPFD indicate that the 3 week period was long enough to allow adjustment of pigments to PPFD (the lowest PPFD was apparently too low to maintain a high Chl concentration; Fig. 2).

The results of this study have shown for the first time that a CAM plant does not require high PPFD. They have also provided an explanation for the ability of the epiphyte Spanish moss to grow in the interior of tree canopies.

Acknowledgments—We are grateful to J. Eades for assistance in the collection of Spanish moss, M. Smith for improving the quality of the manuscript, and J. Elder, S. Hagen, and C. Spencer for their excellent assistance in preparation of the manuscript.

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