Plant Physiol. (1982) 70, 1718–1722
0032-0889/82/70/1718/05/$00.50/0

Light-Stimulated Burst of Carbon Dioxide Uptake following Nocturnal Acidification in the Crassulacean Acid Metabolism Plant *Kalanchoë daigremontiana*[1]

Received for publication April 1, 1982 and in revised form June 30, 1982

KLAUS WINTER AND JOHN D. TENHUNEN
Lehrstuhl für Botanik II, Universität Würzburg, 8700 Würzburg, West Germany

ABSTRACT

CO_2 exchange characteristics were studied during the light-stimulated burst of CO_2 uptake (MB) immediately following a period of nocturnal CO_2 fixation in the Crassulacean acid metabolism plant *Kalanchoë daigremontiana*. During the early parts of the MB, stimulation of net CO_2 uptake by low ambient O_2 concentration (1.5%) was small, and leaves showed the capacity for net CO_2 uptake at low ambient CO_2 partial pressure (30 microbars) and when the MB was interrupted by darkness. During the later phase of the MB, stimulation of net CO_2 uptake by 1.5% O_2 was increased, and net CO_2 loss was recorded both at 30 microbars CO_2 and during dark interruptions. These results suggest that CO_2 fixation during the MB occurs simultaneously via phosphoenolpyruvate carboxylase (predominant during the early phase of the MB) and via ribulose bisphosphate carboxylase (predominant during the later phase of the burst). The magnitude and duration of the MB was increased by a reduction in the length of the dark period and by low (15°C) compared to high (30°C) leaf temperatures.

Plants with CAM exhibit a burst in CO_2 uptake at the onset of illumination following a nocturnal period of CO_2 fixation and malic acid synthesis. This light-stimulated MB[2] occurs under laboratory conditions when plants are exposed to abrupt transitions from dark to light periods and has also been observed under natural conditions when irradiance gradually increases at dawn (3, 10). The MB links the phase of nocturnal malic acid synthesis and storage with the phase of malic acid mobilization and degradation in the light and, therefore, represents an important period with respect to the regulation of primary carboxylation reactions via PEP carboxylase and RuBP carboxylase. CO_2 fixation during the MB may also contribute substantially to total carbon gain (12). Carbon metabolism during the MB is, however, not well understood (9). In the studies of Osmond and Allaway (7), malate was the main product of $^{14}CO_2$ fixation initially labeled during the MB of *Kalanchoë daigremontiana* leaves, suggesting CO_2 fixation via PEP carboxylase. Subsequent labeling experiments, using *K. daigremontiana* leaf discs, demonstrated CO_2 fixation via RuBP carboxylase as well, particularly during the later parts of the MB (6). In the study presented here, the characteristics of CO_2 exchange of attached *K. daigremontiana* leaves were observed in order to obtain information about the carboxylation reactions operating during the MB.

MATERIALS AND METHODS

Growth of Plants. *Kalanchoë daigremontiana* was grown in a glasshouse at the Botanical Garden in Würzburg during summer. Mercury vapor lamps provided PAR of at least 0.2 mmol photons m^{-2} s^{-1} for 12 h (07.00–19.00) per day. The PAR increased with illumination from outside and reached approximately 1 mmol photons m^{-2} s^{-1} at noon on bright days. Day and night temperatures were 30 and 20°C, respectively, and RH varied between 55 and 80%. Plants were cultivated in 2-L pots containing soil. They were watered daily and received 200 ml nutrient solution (14) containing 24 mM NO_3^- at 3- to 4-d intervals. Experiments were performed with recently expanded leaves (4th or 5th leaf pair from top) of plants about 3 months old.

Gas Exchange Techniques. Measurements of CO_2 exchange were made on whole, attached leaves enclosed in a gas exchange cuvette (Walz Mess- und Regeltechnik, Effeltrich) which was located in a walk-in growth chamber (Weiss Klimatechnik, Giessen). PAR during the 12-h standard light period (07.00–19.00) was 1 mmol photons m^{-2} s^{-2}. Unless stated otherwise, leaf temperature, leaf-air vapor pressure difference, and ambient CO_2 partial pressure entering the leaf cuvette were kept at 20°C, 8–10 mbar and 330 μbar, respectively. Temperature and RH in the growth cabinet were controlled to be approximately the same as those in the leaf cuvette.

Different CO_2 partial pressures and O_2 concentrations in the leaf cuvette were obtained by mixing CO_2 free air and nitrogen containing 1.5% O_2 with pure CO_2 using mass flow meters (Brooks, Veenendaal). Dew points of air entering and leaving the cuvette were measured with dew point mirrors (Walz Mess- und Regeltechnik). Any increase in humidity resulting from transpiration was compensated for by a variable rate of pumping of air through a closed loop bypass with a cold trap. Further details of the measurement procedures and details of calculation of data have been reported previously (4, 11).

Titratable Acidity. Leaf discs of known area were extracted in 20% (v/v) boiling ethanol for 15 min and the extracts were titrated with 5 mM NaOH to pH 6.5.

RESULTS AND DISCUSSION

Control Experiments. It is known for several CAM species including *K. daigremontiana* that uptake of external CO_2 in the late light period, following deacidification, is predominantly via RuBP carboxylase (9, 12). Therefore, CO_2 fixation is stimulated by low ambient O_2 concentration (1.5%) during this period of time (Fig. 1A). The CO_2 compensation point is around 50 μbar (1) and

[1] Supported by a grant from the Deutsche Forschungsgemeinschaft (Wi 641/1-1 and 1-2).

[2] Abbreviations: MB, morning burst (of light-stimulated CO_2 uptake following nocturnal acidification); PEP, phosphoenolpyruvate; RuBP, ribulose 1,5-bisphosphate.

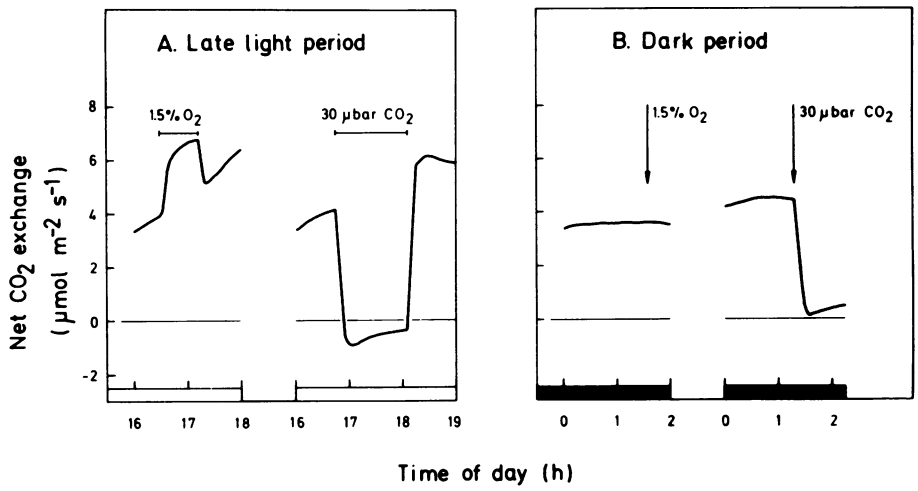

FIG. 1. Net CO_2 exchange in response to decreased ambient O_2 concentration and decreased ambient CO_2 partial pressure during uptake of external CO_2 in the course of the late light period (A) and the middle of the dark period (B). Plants were preadapted to 12-h light (07.00–19.00)/12-h dark cycles. Positive values refer to net CO_2 uptake, negative values to net CO_2 loss. ■, dark; □, light.

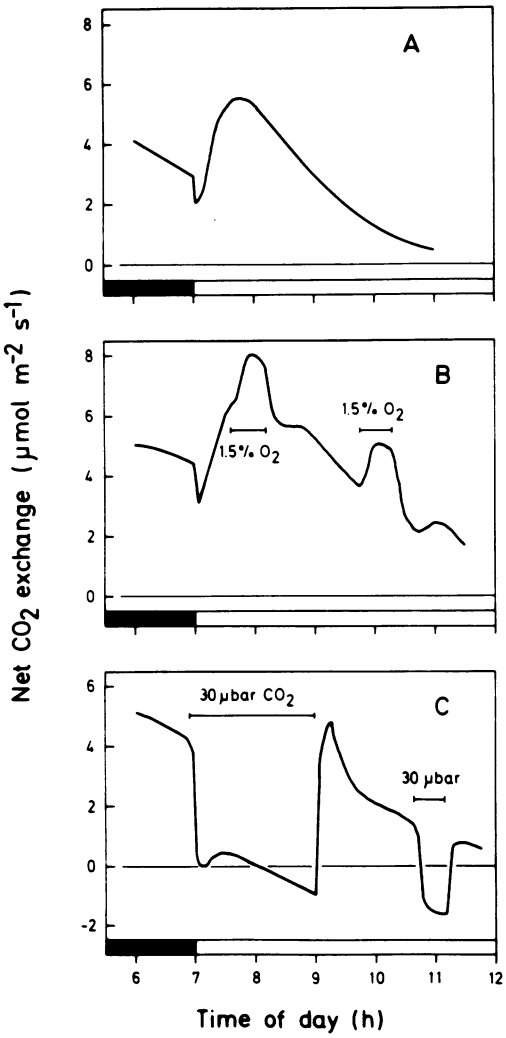

FIG. 2. Net CO_2 exchange during the early 12-h light period following a 12-h dark period (A), and the effects of decreased ambient O_2 concentration (B) and decreased ambient CO_2 partial pressure (C).

hence, at 30 µbar external CO_2, net CO_2 evolution occurs (Fig. 1A). Dark CO_2 fixation is not affected by low external O_2 and at 30 µbar CO_2, net CO_2 uptake occurs (Fig. 1B) because at night the CO_2 compensation point is in the vicinity of 0 µbar due to the operation of PEP carboxylase (12). These results were used to characterize the primary carboxylation reactions occurring during the MB of CO_2 uptake.

Standard 12-Hour Light/12-Hour Dark Cycle. Figure 2A shows the MB obtained following a standard 12-h dark period. A change in the ambient O_2 concentration from 21 to 1.5% stimulates net CO_2 uptake (Fig. 2B). The percentage degree of stimulation increases from 27% at the peak of the MB to 43% at later times of the MB. This result suggests that RuBP carboxylase is functioning. Measurements of transpiration indicated that the stimulation of CO_2 uptake at any point in time was not due to an increase in leaf conductance. At 30 µbar external CO_2, net CO_2 uptake still occurs immediately after onset of illumination (Fig. 2C). Thus, PEP carboxylase is operating as well.

About 1 h after start of the light period and at 30 µbar external CO_2, net CO_2 evolution occurs, which is probably largely due to internal production of CO_2 by decarboxylation of malic acid. Net CO_2 loss under these conditions does not rule out CO_2 fixation via PEP carboxylase. Nevertheless, CO_2 fixation via PEP carboxylase seems to occur primarily during the initial phase of the MB, whereas CO_2 fixation via RuBP carboxylase becomes predominant during the later part of the MB. This conclusion is supported by the data shown in Figure 3 where the MB was interrupted by short periods of darkness. When darkness occurs during the early phases of the MB, leaves still exhibit dark net CO_2 uptake. When darkness occurs after CO_2 fixation during the MB has exceeded its maximum value, substantial loss of CO_2 from the leaf is observed (Fig. 3, C and D).

Shortened and Prolonged Dark Period. The characteristics of the MB are greatly influenced by the length of the previous dark period (see also 5). When the light period commences 2 h before its normal onset, *i.e.* at a point during the dark period when CO_2 dark fixation is still high, the duration of the MB and the maximum CO_2 uptake rate during the MB are increased (Fig. 4A). The initial period of CO_2 uptake at 30 µbar external CO_2, measured after onset of illumination, is prolonged by about 45 min compared to the corresponding period during a standard dark/light transient (Figs. 2C and 4C), *i.e.* CO_2 fixation via PEP carboxylase increasingly contributes to carbon gain during the early phase of the MB. Low O_2 concentration, given during the early part of the MB, causes little (about 15%) stimulation of CO_2 uptake (Fig. 4B). In

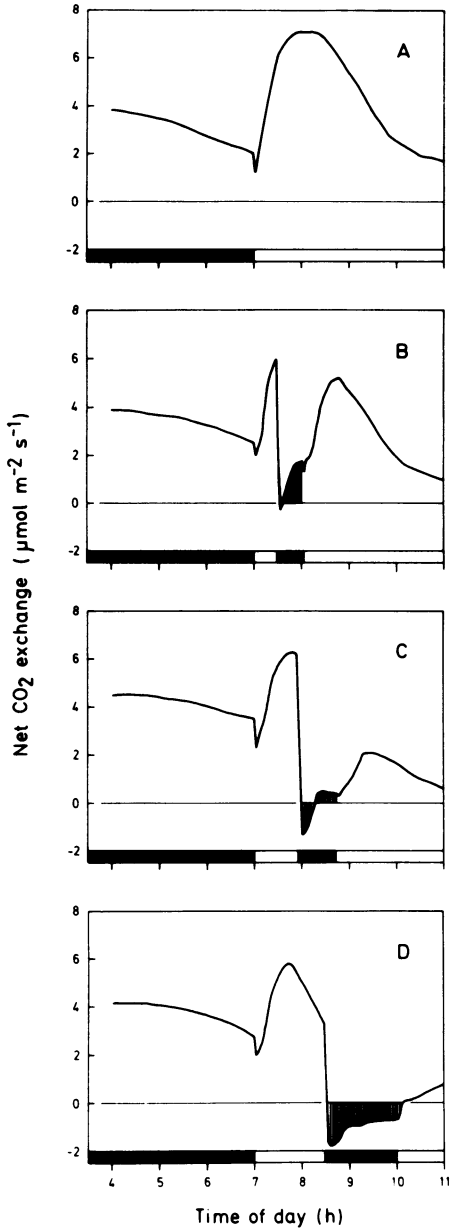

FIG. 3. Effects of dark interruptions (shaded areas) on net CO_2 exchange during the early 12-h light period following a 12-h dark period.

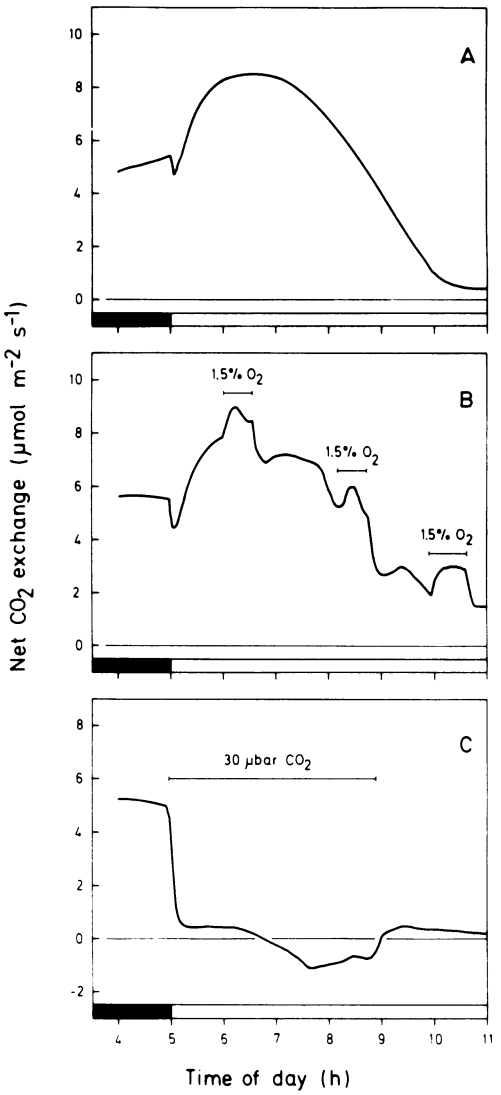

FIG. 4. Net CO_2 exchange during the early light period following a shortened (10-h) dark period (A), and the effects of decreased ambient O_2-concentration (B) and of decreased ambient CO_2 partial pressure (C). Plants were preadapted to 12-h light (07.00–19.00)/12-h dark cycles.

contrast, when the dark period is extended until net CO_2 dark fixation has ceased, only a small MB occurs (Fig. 5A). Reduction in the ambient O_2 concentration to 1.5% during maximum CO_2 uptake of the MB leads to a relatively high (60%) stimulation of CO_2 fixation (Fig. 5B), and with leaves exposed to 30 μbar CO_2, net CO_2 loss is observed continuously after the beginning of the light period (Fig. 5C). Deacidification starts immediately after onset of illumination (Fig. 6C).

At the end of a shortened dark period, CO_2 fixation via PEP carboxylase appears to continue with high rates into the light period, thereby increasing the magnitude of the MB (Fig. 4A). Under these conditions, substrate (PEP) availability for dark CO_2 fixation may still be high and/or the cytoplasmic malic acid concentration may not yet have reached inhibitory levels at the onset of illumination. Following illumination, there is however only minor net synthesis of malic acid (Fig. 6A), probably due to the simultaneous operation of β-carboxylation and decarboxylation processes (9).

A time-dependent component is more important in determining the characteristics of the MB than the total amount of carbon fixed in the previous dark period. For example, even though net carbon gain during 12 h darkness was at 30 μbar external CO_2, 20% of that at 330 μbar and 15% of that at 1,000 μbar CO_2, the MB, measured at 330 μbar CO_2, was not greatly affected (data not shown). Increasing leaf temperatures from 15 to 30°C at night greatly reduced nocturnal net carbon gain (from 100–16%) but also had little effect on the magnitude of the MB which was determined at 20°C (data not shown).

Leaf Temperature. The magnitude and duration of the MB markedly decreases when leaf temperature during the MB is increased from 15 to 30°C (Fig. 7). At 15 and 20°C, maximum rates of CO_2 uptake occur about 1 h after onset of illumination, whereas at 30°C a brief 20-min increase in CO_2 uptake is followed by a sharp decline in net CO_2 uptake, and approximately 1 h after onset of illumination slight net CO_2 loss is observed. When exposed to CO_2-free air during the period of the MB, leaves kept at 30°C show net CO_2 loss almost instantaneously after the beginning of the light period, while at 15°C about 1 h elapses until net CO_2 loss commences (Fig. 8). High temperatures not only

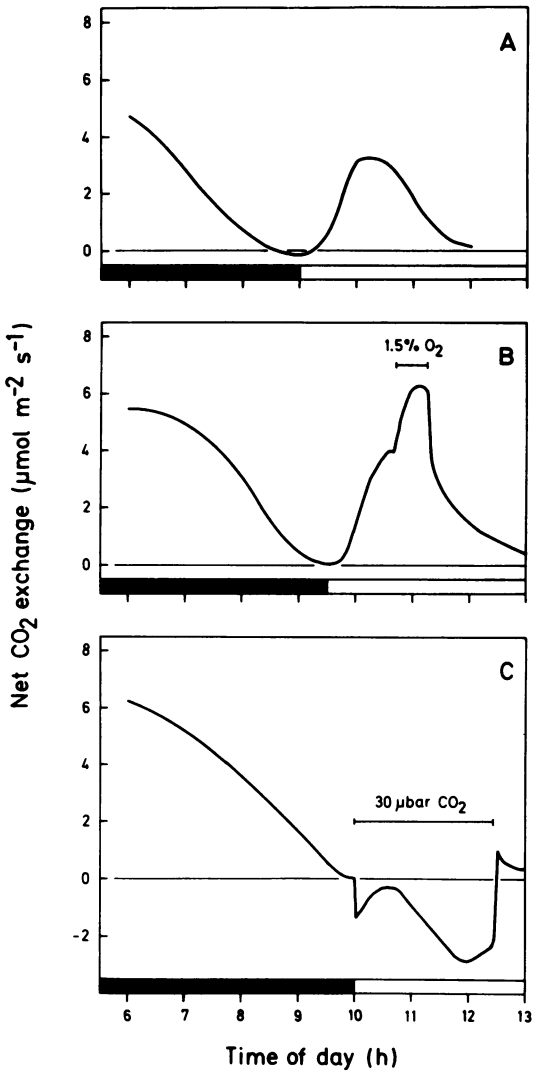

FIG. 5. Net CO_2 exchange during the early light period following a prolonged (14- to 15-h) dark period (A), and the effects of decreased ambient O_2 concentration (B) and of decreased ambient CO_2 partial pressure (C). The standard 12-h dark period was prolonged in each experiment until net CO_2 dark fixation had reached zero. Plants were preadapted to 12-h light (07.00–19.00)/12-h dark cycles.

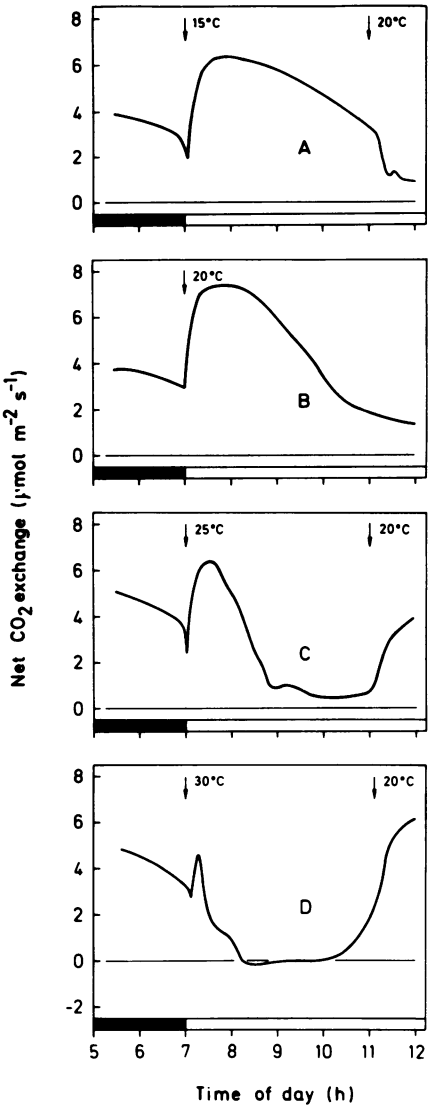

FIG. 7. Effect of leaf temperature on net CO_2 exchange during the first 4 h of the light period following a standard 12-h dark period at 20°C. The leaf-air vapor pressure difference ranged from 10 to 14 mbar at the four temperature regimes.

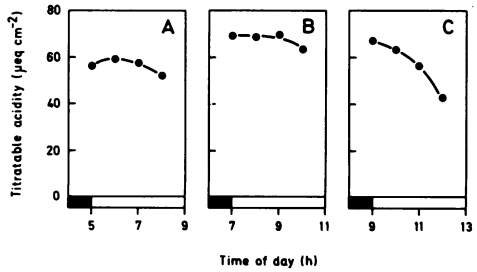

FIG. 6. Titratable acidity during the first 3 h of illumination following a shortened dark period (A), following a standard 12-h dark period (B), and following a prolonged dark period (C). Plants were preadapted to a 12-h light (07.00–19.00)/12-h dark cycle. The experiment was performed in a growth cabinet. PAR = 1 mmol photons m^{-2} s^{-1}; leaf temperature = 20–22°C.

seem to increase the rate of malic acid decarboxylation but also effect earlier onset of malic acid decarboxylation.

CONCLUSIONS

Data on CO_2 exchange presented here are consistent with the operation of both PEP carboxylase and RuBP carboxylase during the MB of CO_2 uptake. CO_2 fixation via PEP carboxylase occurs primarily during the initial phase of the MB, whereas C_3 photosynthetic CO_2 fixation via RuBP carboxylase becomes predominant during the later part of the MB. The time point when CO_2 fixation has reached its maximum value in the course of the MB indicates the onset of malic acid decarboxylation which is presumably initiated by release of malic acid from the vacuoles. These processes are favored by high temperatures. The subsequent decline in net uptake of external CO_2 is probably due to increased intercellular CO_2 partial pressure resulting in stomatal closure (2) and due to inhibition of PEP carboxylase by an increased cytoplasmic concentration of malic acid (13).

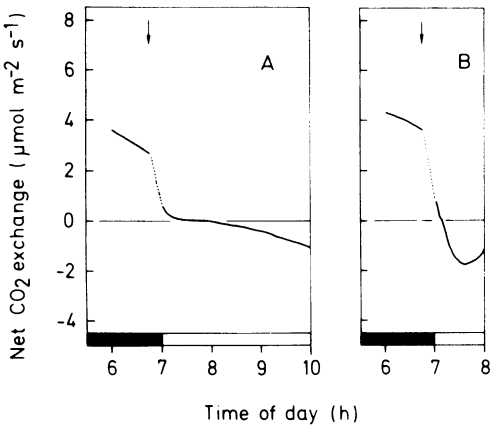

FIG. 8. Effect of CO_2-free air (arrow indicates time point of application) on net CO_2 exchange at 15°C (A) and 30°C (B) during the early light period following a 12-h dark period at 20°C and 330 μbar CO_2. CO_2 exchange during the decrease in CO_2 partial pressure is arbitrarily indicated by a dotted line, because accurate measurement of CO_2 exchange during this period was not possible.

Acknowledgments—We are grateful to Otto Ludwig Lange for supporting these studies, and to Doris Faltenbacher for competent technical assistance. Calculations were made at the Rechenzentrum der Universität Würzburg.

LITERATURE CITED

1. ALLAWAY WG, B AUSTIN, RO SLATYER 1974 Carbon dioxide and water vapour exchange parameters of photosynthesis in a crassulacean plant, *Kalanchoë daigremontiana*. Aust J Plant Physiol 1: 397–405
2. COCKBURN W, IP TING, LO STERNBERG 1979 Relationship between stomatal behavior and internal carbon dioxide concentration in CAM plants. Plant Physiol 63: 1029–1032
3. LANGE OL, ED SCHULZE, L KAPPEN, M EVENARI, U BUSCHBOM 1975 CO_2 exchange pattern under natural conditions of *Caralluma negevensis*, a CAM plant of the Negev desert. Photosynthetica 9: 318–326
4. LANGE OL, J TENHUNEN, M BRAUN 1982 Midday stomatal closure in mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber. I. Comparison of the behavior of various European mediterranean species. Flora. 172: 563–579
5. MARCELLE R 1975 Effect of photoperiod on the CO_2 and O_2 exchanges in leaves of *Bryophyllum daigremontianum* (Berger). *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. Dr W Junk, The Hague, pp 349–356
6. MEYER CP 1979 Regulation of gas exchange in *Kalanchoë daigremontiana* Hamet et Perr. PhD thesis. University of Melbourne
7. OSMOND CB, WG ALLAWAY 1974 Pathways of CO_2 fixation in the CAM plant *Kalanchoë daigremontiana*. I. Patterns of $^{14}CO_2$ fixation in the light. Aust J Plant Physiol 1: 503–511
8. OSMOND CB, O BJÖRKMAN 1975 Pathways of CO_2 fixation in the CAM plant *Kalanchoë daigremontiana*. II. Effects of O_2 and CO_2 concentration on light and dark CO_2 fixation. Aust J Plant Physiol 2: 155–162
9. OSMOND CB, JAM HOLTUM 1981 Crassulacean acid metabolism. *In* MD Hatch, NK Boardman, eds. The Biochemistry of Plants. Academic Press, New York, pp 283–327
10. OSMOND CB, DL NOTT, PM FIRTH 1979 Carbon assimilation patterns and growth of the introduced CAM plant *Opuntia inermis* in Eastern Australia. Oecologia 40: 331–350
11. TENHUNEN JD, OL LANGE, M BRAUN 1981 Midday stomatal closure in mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber. Oecologia 50: 5–11
12. WINTER K 1980 Carbon dioxide and water vapor exchange in the crassulacean acid metabolism plant *Kalanchoë pinnata* during a prolonged light period. Plant Physiol 66: 917–921
13. WINTER K 1982 Properties of phosphoenolpyruvate carboxylase in rapidly prepared, desalted leaf extracts of the crassulacean acid metabolism plant *Mesembryanthemum crystallinum*. Planta 154: 298–309
14. WONG SC 1979 Elevated atmospheric partial pressure of CO_2 and plant growth. Oecologia 44: 68–74