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Photosynthesis but not CAM responded flexibly to changes in irradiance in *Plectranthus marrubioides* (*Lamiaceae*)

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Abstract

Well-watered plants of *Plectranthus marrubioides* Benth., a crassulacean acid metabolism (CAM) species naturally inhabiting sun exposed succulent places, were grown at photosynthetically active photon flux densities (PPFD) of either 150 (LL) or 300 (HL) μmol m^{-2} s^{-1} in a controlled environment. Photosynthesis of LL plants was saturated by irradiance of *ca.* 500 μmol m^{-2} s^{-1} while in HL plants saturation was not reached up to 1200 μmol m^{-2} s^{-1} and photosynthetic capacity was nearly 50 % higher than in the LL plants. However, maximum photon yield was 55 % lower and compensation irradiance was 25 % higher in LL plants. The former also had larger, more succulent leaves, *i.e.,* they were morphologically more sun adapted. On the other hand, nocturnal accumulation of malic and citric acid, nighttime CO_2 gain, and the low relative carbon recycling were independent of the prevailing PPFD. Furthermore, photosynthetic performance was flexibly and reversibly adjusted in HL plants after transfer to 600 or 150 μmol m^{-2} s^{-1} while nocturnal CO_2 uptake was not influenced. Photosynthesis showed a high acclimation potential to high PPFD and patterns of gas exchange became more C_3-like the higher the irradiance was, without a direct effect on CAM in *P. marrubioides.*

Additional key words: Crassulacean acid metabolism; gas exchange; irradiance response; malic acid accumulation.

Introduction

Early research on crassulacean acid metabolism (CAM) was done on relatively few, selected species mainly native to extreme desert habitats (Kluge and Ting 1978,

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Osmond 1978). So, the opinion elicited that CAM was a rather static metabolic adaptation of very specialized plants to long-term drought periods. Meanwhile, it has become clear that CAM is much more widespread among plants from very different habitats, including submerged water plants (Keeley 1996). The phenomenon of CAM can dynamically cover large diversity of expression and combinations of gas exchange patterns (Griffiths 1988b) in many species from many families (Smith and Winter 1996). These plants optimize their carbon metabolism and gas exchange very flexibly in response to changes in environment. Thus, the mode of photosynthesis can reversibly and quickly be changed from approximately that of a C_3 plant to an exclusive nighttime CO_2 uptake within very few days with or without greatly affecting nocturnal accumulation of malic acid (Winter and Smith 1996).

One of the species showing a high plasticity of CAM is *P. marrubioides* (Herppich 1989, Herppich and Herppich 1996). *Plectranthus* is a genus in the large family *Lamiaceae*, widespread in the Old World tropics and subtropics but also occurring in Asia, Australia, New Zealand and even Hawaii (Jacobsen 1981). It includes most of the relatively few succulent species in the *Lamiaceae* (Jacobsen 1981) and the only five CAM taxa described up to now in this family (Kluge and Ting 1978, Herppich and Herppich 1996).

In *P. marrubioides*, which is native to the East African Highlands and the western Higher Escarpment in Yemen (Deil 1988), patterns of CAM and diurnal gas exchange respond very flexibly to a variety of environmental stimuli (Herppich *et al.*, unpublished). This taxon is preferentially found sun exposed or semi-shaded on shallow soils in rocky grassland and dry secondary succulent highland places up to an altitude of more than 2200 m (Deil 1988). Its natural habitats are characterized by high mean annual temperatures (≈20 °C), frequent mist and dew fall in the early morning hours and the evening and high, periodic rainfalls (annual mean > 600 mm, Deil 1988). Therefore, besides episodic droughts and high temperatures, pronounced short and long-term changes in irradiance ranging from very high incident irradiation to low PPFD during prevailing mist may be the major environmental constraints affecting CAM and photosynthesis.

Only very limited information about photosynthetic performance and CAM in the genus *Plectranthus* is available (Herppich and Herppich 1996). Therefore, controlled environment experiments with the high mountain CAM species *P. marrubioides* were done to elucidate the functional interrelationships between irradiance and photosynthesis, CAM and gas exchange patterns.

Material and methods

Plants of *Plectranthus marrubioides* Benth., collected in Yemen (Deil 1988), were propagated from cuttings in plastic pots containing sandy soil. During greenhouse growth in a semi-controlled environment [day: temperature (T_D) = 27±5 °C, relative humidity (RH_D) = 50±15 %; night: T_N = 15±5 °C, RH_N = 85±10 %), plants were abundantly watered every third day. Mean integrated daily irradiance (400-700 nm) was 12±3 mol(photon) d^{-1}, as derived from measurements of PPFD with a *LI-190 S* quantum sensor (*LI-COR*, Lincoln, U.S.A.). Several plants were transferred to a growth chamber (*Ecophyt* Model *VEPHQ 5/1350*, *Heraeus-Voetsch*, Balingen, Germany) and, if not other stated, acclimated to PPFDs of about 150 (LL) or 300 (HL) μmol m^{-2} s^{-1}. Radiation was provided by high-pressure mercury vapour lamps (*HQI-TS 250 Watt/D*, *Osram*, München, Germany). If not other specified, temperature and relative humidity were set to 30 °C and 50 % during the day and 20 °C and 70 % at night. Duration of photoperiod was 12 h in all experiments.

Gas exchange measurements: CO_2 exchange and transpiration were measured with an IRGA (*Binos 1*, *Leybold-Heraeus*, Hanau, Germany) in an open circuit system (*Walz*, Effeltrich, Germany) described by Herppich (1989). Dew point mirrors (*TS3CI*, *Walz*, Effeltrich, Germany) were used to record dew points of the airstream entering and leaving the climatized leaf chamber (*GK 2* or *GMK 3*, *Walz*, Effeltrich, Germany); leaf temperature was measured with copper-constantan thermocouples. Temperature and humidity in the leaf chamber corresponded to that in the growth chamber. Rates of CO_2 exchange and transpiration, and leaf conductance for water vapour (g_{H2O}), calculated after Caemmerer and Farquhar (1981), were related to the leaf surface because leaves were amphistomatous (Willert *et al.* 1995). Temperature and humidity during gas exchange measurements were always adjusted to that in the growth chamber.

Radiant energy use characteristics of photosynthesis were obtained from normal CO_2/H_2O gas exchange measurements during phase IV (Osmond 1978). In these cases irradiation was provided by a high-pressure mercury vapour lamp (*Planta HRI-T 2000 W*, *Radium*, Wipperfürth, Germany) and PPFD was varied by different layers of an optical neutral grey plastic foil.

Leaf malic acid content and water status analysis: Fully expanded mature leaves, *ca.* 1 and 9 h after irradiation, were immediately copied onto paper, their fresh mass was determined, they were dried to constant mass at 90 °C, and dry mass was determined after cooling in a desiccator. Projected area of the paper traces was measured with an area meter (*Delta T Devices*, Cambridge, U.K.), and leaf surface was estimated as twice the projected leaf area. Leaf water content (WC) was calculated from the difference between fresh and dry masses and related to leaf surface area (WC_{LA}). Using hot water extracts of the dried leaves, malic and citric acid contents were determined enzymatically (Möllering and Gruber 1966, Möllering 1974).

Results

Studying photosynthetic radiant energy-use characteristics of CO_2/H_2O gas exchange under natural ambient CO_2 concentrations (C_a) is difficult in CAM plants because malic acid is decarboxylated at changing rates and leaf internal CO_2 concentration (C_i) is largely varied during phase III, comprising a large part of the photoperiod (Osmond 1978). Therefore, the duration of phase III was investigated by repeatedly turning off the light at different times during the day (Fig. 1). Decarboxylation started slowly in the morning, as indicated by the low rates of carbon loss, *i.e.*, the negative values of CO_2 exchange rates (Fig. 1*A*,*B*). It was maximal *ca.* 4.5 h after irradiation during an undisturbed photoperiod (Fig. 1*C*). Decarboxylation of malic acid seemed to be completed or, at least, became negligible after *ca.* 8 h (Fig. 1*B*,*C*). Thereafter, both constant rates of dark respiration, R_D (Fig. 1*B*) and, after reirradiation, of photosynthetic CO_2 uptake (Fig. 1*A*,*C*) were obtained. Net dark, *i.e.*, CAM CO_2 fixation was not observed during the rest of the light period (Fig. 1*B*). Thus, the last 4 h of the light period could be used for determination of photosynthetic response to irradiance in *P. marrubioides*.

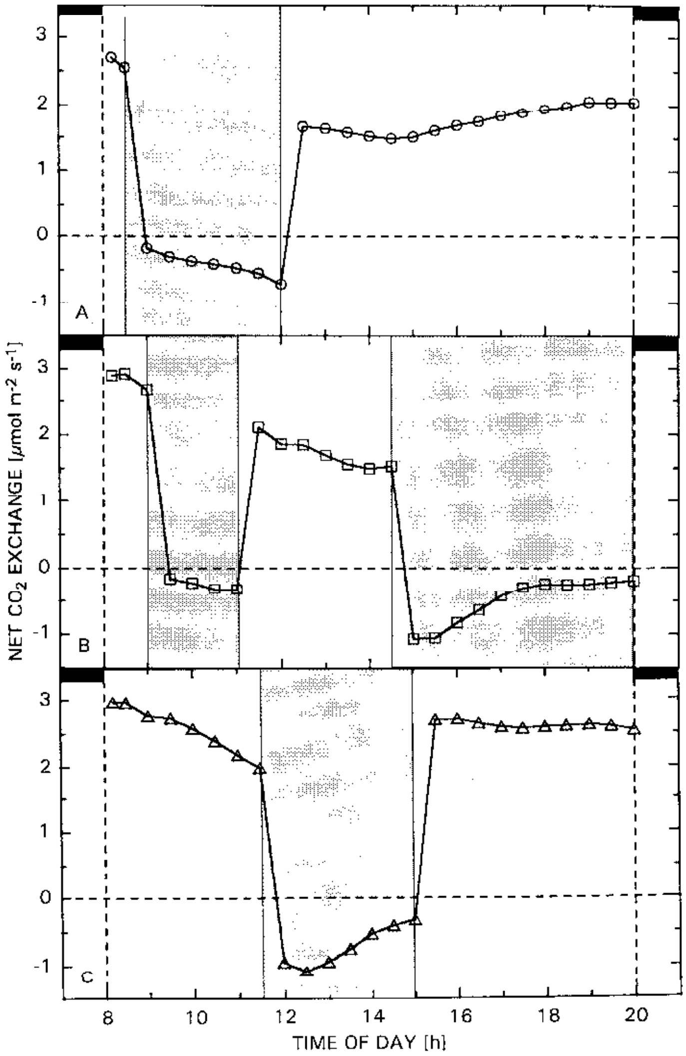

Fig. 1. Influence of the timing of dark interruption of the normal irradiation period on the course of net CO_2 exchange rate during the different phases of CAM in *P. marrubioides*. The duration of darkening is indicated by grey areas, normal night periods by black bars. Shown are three independent experiments (*A*-*C*). Climatic conditions were 25 °C and 60 % RH at a PPFD of *ca.* 600 μmol m^{-2} s^{-1} during the day, and 15 °C and 90 % RH at night.

The HL and LL plants of *P. marrubioides* differed in their photosynthetic response to irradiance (Fig. 2). HL plants showed a 25 % lower compensation irradiance but 15 % higher R_D, 45 % higher apparent photon yields and 30 % higher net CO_2 exchange rate near or at radiant energy saturation (Table 1). The latter was reached at about 500 μmol m^{-2} s^{-1} in the LL plants, while in the HL plants it was not attained at 1200 μmol m^{-2} s^{-1}, the highest PPFD tested. Using the 'Kok-method' as described by Villar *et al.* (1994), which utilizes the 'Kok-effect', it was estimated that about 70 % of R_D might have been maintained in the light.

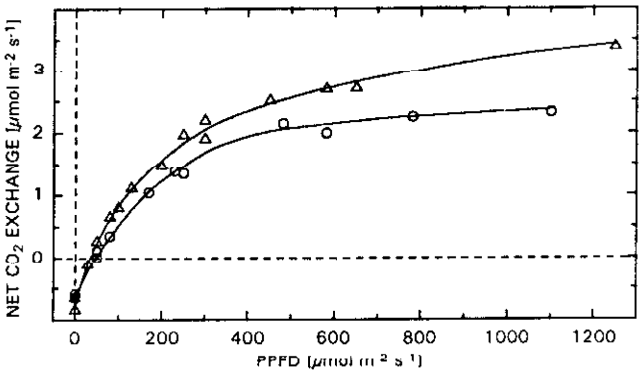

Fig. 2. Representative examples of irradiance response curves of net CO_2 exchange rate of plants adapted to PPFDs of about 150 (○) or 300 μmol m^{-2} s^{-1} (Δ). One curve comprises two irradiance response measurements on two consecutive days at a temperature of 25 °C and 60 % RH. The irradiances were changed randomly and care was taken that a true steady state was attained to minimize hysteresis effects.

Table 1. Irradiance responses of net CO_2 exchange and dark respiration (R_D) rates of mature leaves of well-watered plants adapted to different PPFD [μmol m^{-2} s^{-1}]. Means (±SD) of 3 observations. Day/night temperature and relative humidity were 30/20 °C and 50/70 %, respectively.

	PPFD	
	150	300
R_D [μmol m^{-2} s^{-1}]	0.62±0.04	0.72±0.14
Maximum photon yield [mol(CO_2) mol^{-1}(photon)]	0.027±0.001	0.042±0.008
PPFD at compensation irradiance [μmol m^{-2} s^{-1}]	42±2	35±1
Saturation irradiance for P_N [μmol m^{-2} s^{-1}]	>500	>1200
Net CO_2 exchange at or near saturation irradiance [μmol m^{-2} s^{-1}]	2.54±0.31	3.20±0.33

While photosynthetic performance was largely influenced by irradiance during growth, neither nocturnal carbon gain nor malic or citric acid accumulation differed significantly in plants adapted to either HL or LL (Table 2), although total malic acid pools might have been a little more depleted at HL. Relative carbon recycling (Griffiths 1988b), determined as [(Δmalate - NCG_N)/Δmalate], seemed to be not influenced by different irradiances. Nevertheless, leaves of HL plants had a 20 %

higher water content, calculated on a leaf surface basis (WC_{LA}), and had a slightly but not significantly higher specific leaf mass (SLM, Table 2). In another experiment increasing PPFD from 240 to 330 μmol m^{-2} s^{-1} and increasing daytime temperature from 25 to 28 °C resulted in a slightly larger (9 %) Δmalate, mostly due to a little more effective decarboxylation of malic acid (Fig. 3). However, neither rates of decarboxylation nor timing of start or end of decarboxylation differed markedly. Under both irradiances the malic acid content continued to increase for *ca.* 3 h after irradiation and reached its lowest level *ca.* 5 to 6 h later. Net accumulation of malic acid did not begin before darkness (Fig. 3).

Table 2. Influence of PPFD [μmol m^{-2} s^{-1}] on nocturnal accumulation of malic and citric acids (Δmalate, Δcitrate) and the malate content obtained after completed decarboxylation (basic level). Furthermore, the integrated nighttime CO_2 gain (NCG_N) and the relative CO_2 recycling [(Δmalate - NCG_N)/Δmalate] as well as specific leaf mass (SLM) and leaf water content related to total leaf area (WC_{LA}) of HL and LL plants are shown. Means ± SD (for gas exchange measurements *n* = 5, otherwise *n* = 45). Values of SLM and WC_{LA} are given in dimensions easily allowing comparison with values published in older literature (*e.g.*, WC_{LA} ≈ degree of succulence). Day/night temperature and relative humidity were 30/20 °C and 50/70 %, respectively.

	PPFD	
	150	300
Δmalate [mmol m^{-2}]	18.0 ± 5.3	18.7 ± 2.8
Basic level of malic acid [mmol m^{-2}]	4.2 ± 2.9	3.3 ± 1.3
Δcitrate [mmol m^{-2}]	1.4 ± 0.5	1.5 ± 0.8
NCG_N [mmol m^{-2}]	17.3 ±1.3	16.6 ± 2.3
Relative recycling [%]	5	11
WC_{LA} [g_{H2O} dm^{-2}]	7.61 ± 0.52	9.00 ± 0.69
SLM [g_{DM} m^{-2}]	34.46 ± 2.32	36.46 ± 2.90

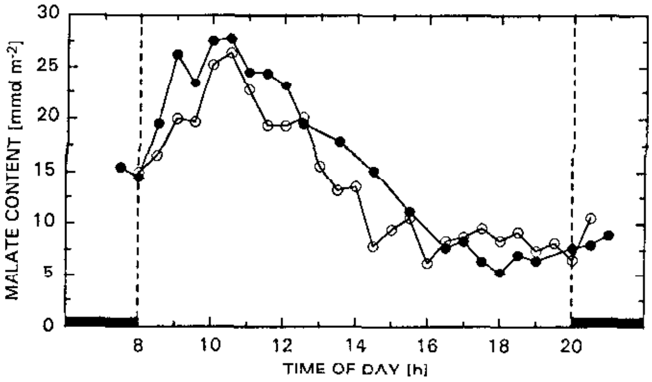

Fig. 3. *Changes in malate content of mature leaves of P. marrubioides plants during a normal 12 h* light period under a PPFD of 240 (○) and 330 μmol m^{-2} s^{-1} (●). Climatic conditions were either 25±3 or 27±3 °C and 50±5 % RH during the day, and 13±2 or 15±2 °C and 90±5 % RH at night.

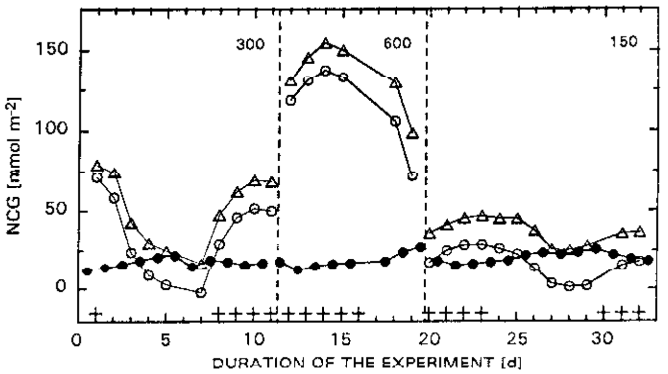

Fig. 4. Changes of net carbon gain (NCG) during the day (O), the night (●) and in 24 h (Δ) in *P. marrubioides* growing under different PPFDs, succeedingly varied from 300 to 600 and back to 150 μmol m^{-2} s^{-1}, under well-watered (indicated by +) and drought stress conditions. Changes in PPFD are indicated by dotted lines. Temperature and relative humidity were 30 °C and 50 % during the day, and 20 °C and 70 % during the night.

Table 3. Mean water use efficiencies [mmol(CO_2 gained) mol^{-1}(H_2O lost)] during the day (WUE_D) and at night (WUE_N) of well-watered ($+H_2O$) and droughted ($-H_2O$, third day after withholding watering) plants adapted to PPFDs of 150, 300 or 600 μmol m^{-2} s^{-1}. Climatic conditions were $T_{D/N}$ = 30/20 °C and $RH_{D/N}$ = 50/70 %.

	PPFD					
	150		300		600	
	$+H_2O$	$-H_2O$	$+H_2O$	$-H_2O$	$+H_2O$	$-H_2O$
WUE_D	3.2	3.9	2.6	2.5	4.3	4.5
WUE_N	8.1	11.3	8.6	11.1	5.7	13.7

Varying the PPFD from 300 to 600 and back to 150 μmol m^{-2} s^{-1} did not influence integrated total nighttime carbon gain (Fig. 4). However, relative to the values observed at 300 μmol m^{-2} s^{-1}, daytime CO_2 uptake and total daily carbon gain were enhanced by more than 170 % at the higher PPFD and reduced by nearly 50 % at the lower PPFD in well-watered plants (Fig. 4). Therefore, the contribution of CAM to total daily CO_2 gain declined from 38 and 26 % to 13 % with raising the PPFD and the patterns of gas exchange became much more C_3-like the higher the PPFD (Fig. 5). Midday depression of gas exchange was greatly reduced and stomata were widely open throughout the light period at the highest PPFD (Fig. 5*C*, *circles*). Short-term drought led to only a slow reduction of integrated carbon uptake at day and seemed to reversibly and slightly stimulate that at night at all irradiances tested (Fig. 4). Molar water use efficiencies were a little improved at the highest PPFD and were similar in well-watered plants and at the third day after withholding watering (Table 3). The nighttime WUE seemed to be reduced at a PPFD of 600 μmol m^{-2} s^{-1}, when the plants were sufficiently watered but amended at the beginning of drought.

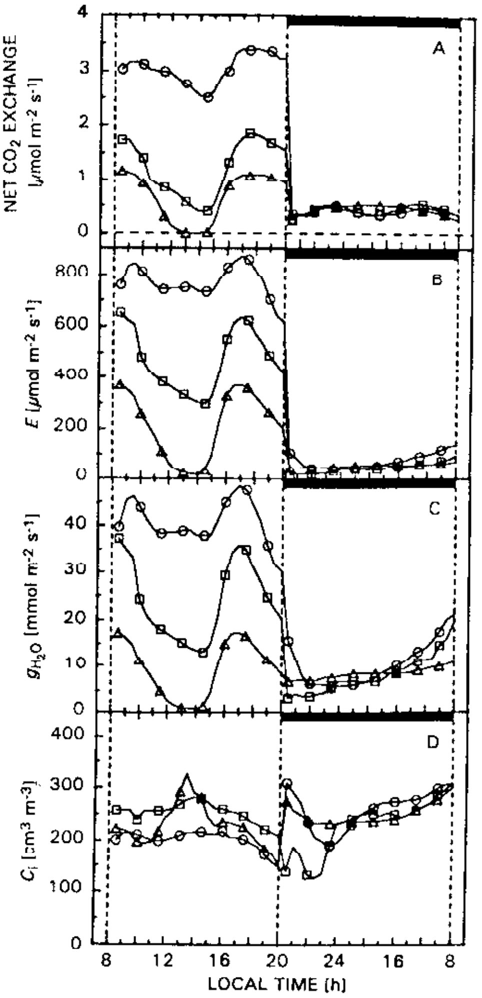

Fig. 5. Representative examples of diurnal changes in (*A*) net CO_2 exchange rate, (*B*) transpiration rate (*E*), (*C*) leaf conductance for water vapour (g_{H2O}), and (*D*) leaf internal CO_2 concentration (C_i) of *P. marrubioides*, determined under PPFD of 150 (Δ), 300 (☐) and 600 (○) µmol m^{-2} s^{-1} under abundant water supply. The number of symbols does not represent the number of data points. Nighttime is indicated by black bars. Climatic conditions were as in Fig. 4.

Discussion

In the classical view on the functional interrelationship between the response of CAM to radiation and photosynthetic performance of CAM plants, mostly worked out for desert plants (*e.g.*, Nobel 1982, 1988), it is accepted that both nocturnal acid accumulation and nighttime CO_2 uptake increase curvilinearly with the irradiance of the previous day. This may result from an improved supply of storage saccharides necessary as precursors of PEP for nocturnal CO_2 fixation and a more complete consumption of the malic acid pool and, thus, increased storage capacity of the vacuole (*e.g.*, Kluge and Ting 1978, and references therein). Nevertheless, CAM may be 90 % saturated at about 22 mol m^{-2} d^{-1} ($\approx$ 520 µmol m^{-2} s^{-1} for 12 h, Nobel 1982).

The finding that in *P. marrubioides* varying the photon fluence rates within a broad range had no influence on CAM seemingly contrasted with reports on several CAM species from very different habitats (*e.g.*, Ong *et al.* 1986, Nobel 1988, Medina *et al.* 1993, Zotz and Winter 1993). However, both nocturnal processes may be either insensitive to PPFD (Martin *et al.* 1986, Griffiths 1988a, Fetene and Lüttge 1991) or even decrease at higher photon fluence rates (Ong *et al.* 1986, Schmitt *et al.* 1988, Borland and Griffiths 1989). Both the nocturnal carbon gain and Δmalate or the depletion of the total malic acid pool might have been irradiance saturated at LL in *P. marrubioides*. Such a behaviour is common in obligate shade-demanding CAM plants (Adams 1988). However, *P. marrubioides* mainly occupies sun-exposed places at a high altitude and may thus be a sun plant (Deil 1988). In the hemiepiphytic *Tillandsia usneoides*, successfully growing from deep shade to full sun, nocturnal CO_2 uptake and acid accumulation are insensitive to changes in PPFD from 200 to more than 1000 µmol m^{-2} s^{-1} (Martin *et al.* 1986), only declining at very low PPFD. Therefore, low irradiance saturation of CAM may not necessarily be linked to shade requirement (Adams 1988), although the responsibility may be influenced by growth conditions (Fetene *et al.* 1990, Lüttge *et al.* 1991).

In *P. marrubioides* the relative amount of CO_2 recycling (Griffiths 1988b) was low and nearly constant because nighttime CO_2 uptake and Δmalate were not influenced by changes in PPFD. Similar behaviour has been reported for well-watered and well-fertilized *Bromelia humilis* (Fetene and Lüttge 1991). In the investigations on *P. marrubioides* nighttime temperatures were moderate and, thus, R_D was only minor (Herppich 1989, Fetene and Lüttge 1991). The low relative recycling may also indicate a negligible nighttime diffusion resistance in these well-watered plants as the rates of recycling increased with decreasing leaf conductance (Herppich 1989).

The small amount of nocturnally accumulated citric acid, accompanying Δmalate, was also not influenced by PPFD in *P. marrubioides*. Similar is the finding for some CAM species of the genus *Clusia* (Franco *et al.* 1992). On the other hand, *Ananas comosus* (Borland and Griffiths 1989, Medina *et al.* 1993) and *B. humilis* (Fetene *et al.* 1990) show a higher Δcitrate at higher PPFD during the preceding day. In low citrate CAM plants like *P. marrubioides* the degree of nocturnal accumulation of citric acid may be merely a consequence of the capability to synthesize malate (Medina *et al.* 1993, Herppich and Herppich, unpublished), *i.e.*, of the phospho*enol*pyruvate carboxylase activity. If this applies, nocturnal accumulation of citric acid ought to be irradiance saturated in same the range of PPFD which was found for Δmalate in *P. marrubioides*.

In contrast to CAM, P_N and total daily carbon gain largely depended on the photon fluence rates in well-watered plants of *P. marrubioides*. Photosynthetic performance rapidly acclimated to both higher and lower irradiances than experienced during growth. Apparent photon yield, compensation irradiance and R_D indicate that *P. marrubioides* is a sun plant (Lüttge *et al.* 1986). These cardinal points of irradiance-saturating curves also show that the acclimation of the photosynthetic apparatus to low irradiances may be limited, as both photon yield and compensation irradiance allow more effective work in plants grown at the higher PPFD. Although higher photon yields and low compensation irradiance are normally linked to shade adaptation (Lüttge *et al.* 1986) and, *e.g.*, found in CAM plants grown under low irradiance (Schäfer and Lüttge 1988, Borland and Griffiths 1989), Adams *et al.* (1987) report that both parameters may be unaffected by a wide range of PPFD in both typical sun and shade CAM plants (Griffiths *et al.* 1989).

Additionally, maximum P_N at or near saturation irradiance and the fact that in HL plants saturation by irradiance was not achieved at the highest PPFD tested, pointed out the pronounced acclimation potential of *P. marrubioides* to high irradiances. This seemed to be more prominent in the *Lamiaceae* than in other CAM plants, whether shade demanding like *Hoya carnosa* or *Kalanchoë uniflora* (Adams *et al.* 1987, Schäfer and Lüttge 1988) or well adapted to high irradiances like *K. daigremontiana* or *B. humilis* (Adams *et al.* 1987, Fetene *et al.* 1990) or at least high irradiance tolerant (*T. usneoides*, Martin *et al.* 1989).

As the nocturnal carbon gain of *P. marrubioides* was not affected at all by such treatment, this high acclimation potential led to much more C_3-like overall gas exchange patterns the higher the PPFD. This not only drastically reduced the relative contribution of CAM to total daily carbon gain but, using the descriptive terms proposed by Neales (1975, *cf.* Kluge and Ting 1978), the 'full CAM plant' seemingly became a 'weak CAM plant' just by increasing the PPFD without any true effect on crassulacean acid metabolism. In other words, it was photosynthetic performance that very flexibly responded to that environmental signal, while CAM remained rather conservative in *P. marrubioides*. Although similar results have been reported for several other CAM species from different habitats and families (Kluge *et al.* 1973, Wagner and Larcher 1981, Winter and Awender 1989), nocturnal CO_2 gain has always been slightly enhanced in these studies. Hence such behaviour could have been typical for plants with high CAM plasticity, increasing their capability to quickly respond to dynamic changes in a highly variable environment. However, in *Clusia uvitana* integrated nocturnal CO_2 uptake became negative at low irradiance during the preceding day where phase III was greatly reduced (Zotz and Winter 1993). At very high irradiance (1200 μmol m^{-2} s^{-1}) photosynthetic performance was substantially enhanced and nighttime carbon gain ceased in *C. minor* (Schmitt *et al.* 1988). However, in contrast to the facultative epiphytes *C. uvitana* and *C. minor* which may partially grow shade adapted (Zotz and Winter 1993), *P. marrubioides* preferentially inhabits exposed places in regions where clouds and mist could frequently reduce incident PPFD (Al-Hubaishi and Müller-Hohenstein 1984). It may, therefore, be of great ecological advantage for optimal biomass production and reproductive success to maximize responsiveness to irradiance of the photosynthetic performance as much as possible while additionally maintaining a high degree of CAM to ensure survival and reduce photoinhibition (Griffiths *et al.* 1989) in a periodically drought prone habitat (Deil 1988, Herppich and Herppich 1996).

References

Adams, W.W., III: Photosynthetic acclimation and photoinhibition of terrestrial and epiphytic CAM tissues growing in full sunlight and deep shade. - Aust. J. Plant Physiol. **15**: 123-134, 1988.

Adams, W.W., III, Osmond, C.B., Sharkey, T.D.: Responses of two CAM species to different irradiances during growth and susceptibility to photoinhibition by high light. - Plant Physiol. **83**: 213-218, 1987.

Al-Hubaishi, A., Müller-Hohenstein, K.: An introduction to the vegetation of Yemen. Ecological basis, floristic composition, human influence. - Gesellschaft für technische Zusammenarbeit (GTZ), Eschborn 1984.

Borland, A.M., Griffiths, H.: The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. - J. exp. Bot. **40**: 53-60, 1989.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Deil, U.: Primäre und sekundäre Standorte sukkulentenreicher Pflanzengesellschaften in Südwest-Arabien. - Flora **180**: 41-57, 1988.

Fetene, M., Lee, H.J.S., Lüttge, U.: Photosynthetic acclimation in a terrestrial CAM bromeliad, *Bromelia humilis* Jacq. - New Phytol. **114**: 399-406, 1990.

Fetene, M., Lüttge, U.: Environmental influences on carbon recycling in a terrestrial CAM bromeliad, *Bromelia humilis* Jacq. - J. exp. Bot. **42**: 25-31, 1991.

Franco, A.C., Ball, E., Lüttge, U.: Differential effects of drought and light levels on accumulation of citric and malic acids during CAM in *Clusia*. - Plant Cell Environ. **15**: 821-829, 1992.

Griffiths, H.: Carbon balance during CAM: an assessment of respiratory CO_2 recycling in the epiphytic bromeliads *Aechmea nudicaulis* and *Aechmea fendleri*. - Plant Cell Environ. **11**: 603-611, 1988a.

Griffiths, H.: Crassulacean acid metabolism: A re-appraisal of physiological plasticity in form and function. - Adv. bot. Res. **15**: 43-92, 1988b.

Griffiths, H., Ong, B.L., Avadhani, P.N., Goh, C.J.: Recycling of respiratory CO_2 during crassulacean acid metabolism: alleviation of photoinhibition in *Pyrrosia piloselloides*. - Planta **179**: 115-122, 1989.

Herppich, W.B.: CAM-Ausprägung in *Plectranthus marrubioides* Benth. (Fam. *Lamiaceae*). Einfluß der Faktoren Licht, Blattemperatur, Luftfeuchtigkeit, Bodenwasserverfügbarkeit und Blattwasserzustand. - PhD Thesis. Westfälische Wilhelms-Universität, Münster 1989.

Herppich, W.B., Herppich, M.: Ecophysiological investigations on plants of the genus *Plectranthus* (Fam. *Lamiaceae*) native to Yemen and southern Africa. - Flora **191**: 401-408, 1996.

Jacobsen, H.: Das Sukkulentenlexikon. - G. Fischer Verlag, Stuttgart - New York 1981.

Keeley, J.E.: Aquatic CAM photosynthesis. - In: Winter, K., Smith, J.A.C. (ed.): Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. Pp. 281-295. Springer-Verlag, Berlin - Heidelberg - New York 1996.

Kluge, M., Lange, O.L., Eichmann, M.v., Schmid, R.: Diurnaler Säurerhythmus bei *Tillandsia usneoides*: Untersuchungen über den Weg des Kohlenstoffs sowie die Abhängigkeit des CO_2-Gaswechsels von Lichtintensität, Temperatur und Wassergehalt der Pflanze. - Planta **112**: 357-372, 1973.

Kluge, M., Ting, I.P.: Crassulacean Acid Metabolism. Analysis of an Ecological Adaptation. - Springer-Verlag, Berlin - Heidelberg - New York 1978.

Lüttge, U., Ball, E., Fetene, M., Medina, E.: Flexibility of crassulacean acid metabolism in *Kalanchoë pinnata* (Lam.) Pers. I. Response to irradiance and supply of nitrogen and water. - J. Plant Physiol. **157**: 259-267, 1991.

Lüttge, U., Ball, E., Kluge, M., Ong, B.L.: Photosynthetic light requirement of various tropical vascular epiphytes. - Physiol. vég. **24**: 315-331, 1986.

Martin, C.E., Eades, C.A., Pitner, R.A.: Effects of irradiance on Crassulacean acid metabolism in the epiphyte *Tillandsia usneoides* L. (*Bromeliaceae*). - Plant Physiol. **80**: 23-26, 1986.

Martin, C.E., McKee, J.M., Schmitt, A.K.: Response of photosynthetic O_2 evolution to PPFD in the CAM epiphyte *Tillandsia usneoides* L. (*Bromeliaceae*). - Photosynth. Res. **21**: 145-150, 1989.

Medina, E., Popp, M., Olivares, E., Janett, H.P., Lüttge, U.: Daily fluctuations of titratable acidity, content of organic acids (malate and citrate) and soluble sugars of varieties and wild relatives of *Ananas comosus* L. growing under natural tropical conditions. - Plant Cell Environ. **16**: 55-63, 1993.

Möllering, H.: Malatbestimmung. - In: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse. Vol. 2. Pp. 1636-1639. Verlag Chemie, Weinheim 1974.

Möllering, H., Gruber, W.: Determination of citrate with lyase. - Anal. Biochem. **17**: 369-379, 1966.

Nobel, P.S.: Interaction between morphology, PAR interception, and nocturnal acid accumulation in cacti. - In: Ting, I.P., Kluge, M. (ed.): Crassulacean Acid Metabolism. Pp. 260-277. Amer. Soc. Plant Physiol., Rockville 1982.

Nobel, P.S.: Environmental Biology of Agaves and Cacti. - Cambridge University Press, Cambridge - New York - New Rochelle - Melbourne - Sydney 1988.

Ong, B.L., Kluge, M., Friemert, V.: Crassulacean acid metabolism in the epiphytic ferns *Drymoglossum piloselloides* and *Pyrrosia longifolia*: studies on responses to environmental signals. - Plant Cell Environ. **9**: 547-557, 1986.

Osmond, C.B.: Crassulacean acid metabolism: A curiosity in context. - Annu. Rev. Plant Physiol. **29**: 379-414, 1978.

Schäfer, C., Lüttge, U.: Effects of high irradiance on photosynthesis, growth and crassulacean acid metabolism in the epiphyte *Kalanchoë uniflora*. - Oecologia **75**: 567-574, 1988.

Schmitt, A.K., Lee, H.J.S., Lüttge, U.: The response of the C_3-CAM tree, *Clusia rosea*, to light and water stress. I. Gas exchange characteristics. - J. exp. Bot. **39**: 1581-1590, 1988.

Smith, J.A.C., Winter, K.: Taxonomic distribution of crassulacean acid metabolism. - In: Winter, K., Smith, J.A.C. (ed.): Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. Pp. 427-436. Springer-Verlag, Berlin - Heidelberg - New York 1996.

Villar, R., Held, A.A., Merino, J.: Comparison of methods to estimate dark respiration in the light in leaves of two woody species. - Plant Physiol. **105**: 167-172, 1994.

Wagner, J., Larcher, W.: Dependence of CO_2 gas exchange and acid metabolism of the alpine CAM plant *Sempervivum montanum* on temperature and light. - Oecologia **50**: 88-93, 1981.

Willert, D.J. von, Matyssek, R., Herppich, W.B.: Experimentelle Pflanzenökologie, Grundlagen und Anwendungen. - G. Thieme Verlag, Stuttgart 1995.

Winter, K., Awender, G.: Crassulacean acid metabolism and photochemical efficiency of photosystem II in the adaxial and abaxial parts of the succulent leaves of *Kalanchoë daigremontiana* grown at four photon flux densities. - Plant Physiol. **90**: 948-954, 1989.

Winter, K., Smith, J.A.C.: An introduction to crassulacean acid metabolism. Biochemical principles and ecological diversity. - In: Winter, K., Smith, J.A.C. (ed.): Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. Pp. 1-13. Springer-Verlag, Berlin - Heidelberg - New York 1996.

Zotz, G., Winter, K.: Short-term regulation of crassulacean acid metabolism activity in a tropical hemiepiphyt, *Clusia uvitana*. - Plant Physiol. **102**: 835-841, 1993.