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Light-Stress and Crassulacean Acid Metabolism

By

ULRICH LÜTTGE[1)]

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Summary

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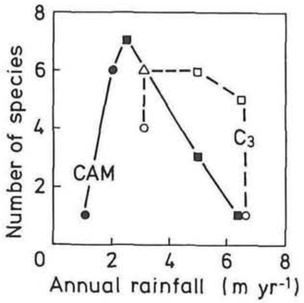
Environmental cues driving the evolution and diversification of plants with crassulacean acid metabolism (CAM) are widely accepted to have been primarily CO_2 (HCO_3^-) supply and subsequently H_2O supply. Light-stress is largely considered to act via amplification of water stress. Can light-stress per se affect CAM? CAM plants show various ways of acclimation to high light. In the field sun exposed CAM plants (e.g. rosettes of bromeliads, *Aloë*; *Kalanchoë* species) often respond with changes of pigmentation from dark green to strongly red or yellow. Changes in xanthophyll-cycle capacity serving thermal dissipation of excess photosynthetic excitation energy have been shown. Acclimation often seems to be strongly related to N-nutrition. CAM plants are known to be subject to acute and chronic photoinhibition. This was mostly related to phases when they perform C_3-photosynthesis, i.e. in the early morning (phase II) and especially in the afternoon (phase IV). High internal CO_2 concentrations generated during the day by malic-acid remobilization and decarboxylation (phase III) have been widely taken as a protective mechanism feeding photochemical energy dissipation, and thus, avoiding photoinhibition. However, high rates of nascent oxygen evolution occur simultaneously, and indeed, phase III-photoinhibition is in fact observed both in the field and in phytotrons even if water is sufficiently available. A considerable number of C_3/CAM-intermediate species are known. Especially in the genus *Clusia* an extraordinary plasticity of C_3-CAM-C_3 changes can be observed. It was shown, that this plasticity supports rapid responses to changing light intensities, where xanthophyll cycle and CAM-induction (phase III CO_2-concentrating mechanism) add to control of high-irradiance stress. CAM may be not so much an adaptation to very severe single-factor stress but by flexibility and plasticity it appears to be an ideal adaptation to variable and multi-factor stress, where CO_2, H_2O and light appear to be major control parameters but light may also act as decisive factor depending on parameter constellations.

[1)] Institute of Botany, Darmstadt University of Technology, Schnittpahnstrasse 3-5, D-64287 Darmstadt, Germany.

Evolution and Diversification - CO_2, H_2O and Light

In the phylogenetic tree of plants CAM is found as early as in the Isoëtaceae among the Lycopodiopsida and some Polypodiaceae among the Pteridopsida (LÜTTGE 1987). In the fresh water plants of some Isoëtaceae CAM serves CO_2 (HCO_3^-) acquisition where phosphoenolpyruvate carboxylase (PEPC) activity in the dark period avoids the competion for CO_2 with other photosynthesizing organisms pertaining during the light period (RAVEN & al. 1988, ROBE & GRIFFITHS 1990, KEELEY 1996). Hence, it is now postulated that the primary cue driving evolution of CAM was CO_2-supply requiring a CO_2-concentrating mechanism (GRIFFITHS 1989). However, the diversity of submerged fresh water CAM species - to which a few angiosperm species are also adding (KEELEY 1996) - is very limited. Hence, the major environmental force subsequently driving CAM diversification is always considered to have been and to be H_2O supply (RAVEN & SPICER 1996), since nocturnal CO_2 uptake and fixation increase water use efficiency of stomata bearing plants with stomatal opening during the dark period and stomatal closure during the dry and hot parts of the light period. The latter avoids the high evaporative demand of the atmosphere by refixing behind closed stomata CO_2 derived from CO_2 prefixed as malate during the previous night (LÜTTGE 1987). But how is high light as a stress factor entering the game? PITTENDRIGH 1948 has made a census of 40 epiphytic bromeliads in Trinidad and grouped them in 3 categories according to their light demand, i.e. I) an exposure group, II) a sun group and III) a shade tolerant group. CAM was dominant in the exposure and sun groups and never occurred in the shade tolerant group (Fig. 1). On the basis of these data the evolution of CAM and epiphytism was assessed in context, and it was concluded that the ancestral stock must have come from relatively moist but exposed habitats in the Andes, and that CAM can have evolved both before epiphytism and after epiphytism (GRIFFITHS & SMITH 1983, SMITH & al. 1986, SMITH 1989). This somewhat relates CAM evolution to high-irradiance stress which is amplified in the epiphytic habitat. However, the latter also amplifies water stress. In the distribution of Trinidadian epiphytic bromeliads CAM peaks at the lower end of the average annual rainfall scale (Fig. 1). Thus, it remains ambiguous whether high irradiance or restricted water availability were the primary cues determining the distribution of epiphytic bromeliads among Pittendrigh's three groups and evolution of CAM in the whole family. Indeed, in CAM-ecophysiology high-light stress is mostly interpreted as a factor amplifying water stress.



	C_3	CAM
Ex	○	●
Su	□	■
Sh	△	

Fig. 1. Distribution of 40 epiphytic Bromeliaceae species of the exposure group (Ex), the sun group (Su) and the shade group (Sh) of PITTENDRIGH 1948 with C_3 photosynthesis (open symbols, dashed line) and CAM (closed symbols, solid line) in Trinidad related to annual rainfall. (After GRIFFITHS & SMITH 1983.)

Acclimation

Can we thus perhaps come closer to our quest for unique roles of light-stress if we change the time scale and consider actual ecophysiological behaviour, such as light acclimation within species, within clones or in individual plants? Are there specific light regulated responses? Among tropical rosette plants, e.g. the bromeliads *Ananas* and *Bromelia*, or *Aloë*, there are many species which can grow similarly well in deep shade, in semi shade and under full sun exposure. Often propagating via ramets individuals occurring under such a variety of conditions may belong to the same genetic clone. They may show strong acclimation of pigmentation ranging from dark to pale green and bright yellow or red with increasing sun exposure. Sun protectants have been found in CAM plants, e.g. epidermal wax providing reflection of light (ROBINSON & OSMOND 1994, BACHEREAU & al. 1998), phenolics in general (BACHEREAU & al. 1998), and anthocyanins (BARKER & al. 1997). In *Aloë vera* the red pigment has been identified as the xanthophyll rhodoxanthin (Fig. 2, DIAZ & al. 1990).

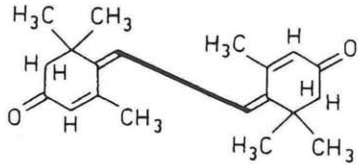


Fig. 2. Rhodoxanthin, a xanthophyll with its two ionon rings. The carbon chain with the other 20 C-atoms is given by the solid line linking the terminal ionon rings.

Rhodoxanthin may only acts as a simple sun protectant. It is not clear if it is also involved in dissipation of excessive excitation energy like other xanthophylls, such as the components of the xanthophyll cycle zeaxanthin, antheraxanthin and violaxanthin serving control of photoinhibition by preventing or limiting over-reduction of photosynthetic electron-transport-chain components and by thermal energy dissipation (DEMMIG-ADAMS & ADAMS 1992, HORTON & al. 1994, PFÜNDEL & BILGER 1994, SCHINDLER & LICHTENTHALER 1996). The xanthophyll cycle with interconversions of zeaxanthin (no-epoxide), antheraxanthin (one epoxide on one of the ionon-rings) and violaxanthin (two epoxides, i.e. one on each of the two ionon-rings) is clearly operative in CAM-performing green tissues (D'AMBROSIO & al. 1994, ADAMS & DEMMIG-ADAMS 1996). Increased levels of zeaxanthin have been recorded in exposed rosettes of *Aloë vera* (DIAZ & al. 1990) and *Bromelia humilis* (FETENE & al. 1990), but a more detailed study of acclimation has been performed with the Crassulaceae *Crassula argentea* (ADAMS & DEMMIG-ADAMS 1996). High light acclimated plants of *C. argentea* display two important adaptations as compared to low light acclimated plants: they have a greater capacity to use absorbed light energy through photosynthesis and a greater capacity for energy dissipation via the xanthophyll zeaxanthin cycle. The correlations between these parameters at full sunlight were remarkable with the ratios of leaves acclimated to high light to low light leaves given as follows: photosynthetic capacity, 2.4; zeaxanthin levels, 3.7; zeaxanthin plus antheraxanthin levels, 3.3; thermal energy dissipation (non-photochemical fluorescence quenching), 2.3. Differences have even been observed between exposed upper and shaded lower faces of individual leaves of *C. argentea*, and also in other CAM plants (*Kalanchoë*, WINTER & AWENDER 1989, *Cotyledon*, ROBINSON & OSMOND 1994) gradients within the thick succulent leaves related to light gradients have been documented. However, both adaptations - photosynthetic capacity and xanthophyll cycle, respectively - may be very different responses to environmental cues. In *Kalanchoë pinnata* (LÜTTGE & al. 1991b) and in *B. humilis* (FETENE & al.

1990) zeaxanthin levels were clearly determined by irradiance during growth in a phytotron. They were very low in shade grown plants. In light grown plants they were rather independent of N nutrition (N starvation versus NO_3^- supply; Tab. 1). Conversely, in both species maximum rates of photosynthetic electron transport, as detected via measurements of O_2 evolution, were strongly modulated by N nutrition, where in fact N supply appeared more important than high irradiance during growth for expression of high rates of O_2 evolution (Fig. 3). For *K. pinnata* the conclusion was that external factors elicit flexible responses of the photosynthetic mechanism in this constitutive CAM plant; high-light grown plants were not shade tolerant; low-light grown plants were shade tolerant but not shade demanding (LÜTTGE & al. 1991a). Based on studies with plants of *Kalanchoë daigremontiana* and *Hoya carnosa* grown under a range of different light intensities ADAMS & al. 1987 concluded that CAM plants can adjust to shaded conditions but are susceptible to photoinhibition (see next two sections for photoinhibition in different phases of CAM) when exposed to higher light intensities than experienced during growth.

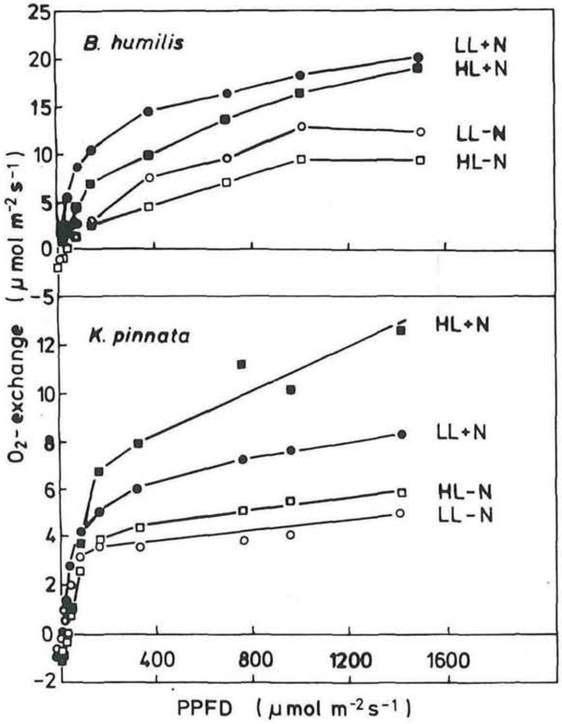


Fig. 3. Light-dependence of O_2-evolution of leaf samples of *Bromelia humilis* (after FETENE & al. 1990) and *Kalanchoë pinnata* (after LÜTTGE & al. 1991b) grown at high light (HL) and low light (LL) with (+N) and without (-N) nitrogen supply.

Table 1. Zeaxanthin levels ($\mu g\ g^{-1}$ fresh weight) in plants of *Bromelia humilis* and *Kalanchoë daigremontiana* grown under high light with (+N) and without (-N) supply of nitrogen as nitrate. Zeaxanthin levels in low light plants plus or minus N were undectable. (After FETENE & al. 1990 and LÜTTGE & al. 1991a).

	+ N	- N
B. humilis	9.4	13.2
K. pinnata	6.7	5.4

CAM Flexibility: C_3 Photosynthesis in Phases II and IV

After considering the aeons of evolution and the months and weeks of acclimation we can reduce or refine time scale once again and study flexibility within days and hours. We recognise that with its four phases *sensu* OSMOND 1978 CAM is very flexible in itself because expression of phases can be readily adjusted to environmental conditions. Nocturnal CO_2 fixation via PEPC in phase I at low atmospheric evaporation demand leads to high water use efficiency, but stomata may also close partially or totally during the night, when water stress is severe (LÜTTGE 1987, GRIFFITHS 1988). The most sensitive to water stress is stomatal opening during phases II and IV (SMITH & LÜTTGE 1985). These are dark-light and light-dark transition phases in the early morning and in the afternoon, respectively, where stomata are open and atmospheric CO_2 is fixed partially via PEPC and partially via ribulosebisphosphate carboxylase/oxygenase (Rubisco; see LÜTTGE 1987). Phase II is normally rather short except in the genus *Clusia* (BALL & al. 1991, BORLAND & al. 1993, ROBERTS & al. 1996, 1997, BORLAND & GRIFFITHS 1997), but phase IV often is extensive. Conditions during phase IV are much like those in C_3 plants performing C_3 photosynthesis. Water stress rapidly abolishes stomatal opening during times of phases II and IV (SMITH & LÜTTGE 1985). However, phase IV is also highly modulated by light. ADAMS & al. 1989 and ADAMS & DEMMIG-ADAMS 1996 have observed that cladode faces of cacti exposed in different directions of the compass show different photosynthetic performance due to the irradiance received during the day. That nocturnally accumulated malate is more rapidly remobilized, decarboxylated and exhausted as an internal CO_2 source at high light intensity during the day is known already for a long time (Fig. 4; KLUGE 1968, NOSE & al. 1977). This may shift stomatal opening for phase IV C_3 photosynthesis to times earlier in the afternoon as compared to situations with lower light intensity, provided, of course, water availability is not limiting. In the experiment of Fig. 4 at low light a minimal level of malate - which still remained much higher than at high light - was only reached towards the end of an extended light period, and only then stomata opened for phase IV CO_2 uptake, while at the 2.4-fold higher light intensity this occurred 6 hours earlier. With phases II and IV in the morning and afternoon CAM plants perform C_3 photosynthesis with open stomata at times of the day when light intensities are not extreme. Therefore, one might have thought that photoinhibition is not a big problem. Photoinhibition is readily measured recording chlorophyll fluorescence and determining potential quantum yield of photosystem II (PS II), "F_v/F_m", after various periods of dark adaptation. Values of $F_v/F_m < 0.83$ indicate photoinhibition, which may be due to energy dissipation via built-up of an electrochemical proton gradient across the thylakoid membranes and via generation of heat in the xanthophyll cycle if reversible within a short time in the dark (minutes up to an hour), to damage and repair of light-harvesting complex (LHC) proteins if reversible over night (D1-protein of LHC II), and to photodestruction if not reversible over night (BJÖRKMAN & DEMMIG 1987, THIELE & al. 1998). Such measurements of F_v/F_m now show that photoinhibition is almost always observed. It may be considered a special attribute of phase IV because the high internal CO_2-concentrations during phase III are taken as a protective mechanism (see next section). The experiment of Fig. 5 with the C_3 photosynthesis/CAM-intermediate species *Clusia minor* shows that, in fact, acute photoinhibition was most pronounced in phase IV of CAM and much lower in phases II and III, and even in the C_3-state F_v/F_m-ratios as low as in phase IV were only reached at higher light intensities. In the cacti discussed by ADAMS & DEMMIG-ADAMS 1996 photoinhibition was largely due to thermal energy dissipation via the xanthophyll cycle. However, photoinhibition can become quite severe, when malic acid is rapidly exhausted as an internal CO_2-source at high irradiance, and when water stress, salinity stress or other kinds of osmotic stress prevent stomatal opening and CO_2-uptake at a time when otherwise phase IV would have occurred. Then CO_2 is not available, neither from internal sources nor from the atmosphere, and overenergization of the photosynthetic apparatus can become a considerable problem. This has been found, for example, in the Aizoaceae *Mesembryanthemum crystallinum* when CAM had been induced in this facultative halophyte and facultative CAM plant by salinity stress. Overenergization in the afternoon with the malate pool exhausted and stomata closed leads to strong oxidative stress and an antioxidative defence system needs to build up. MISZALSKI & al. 1998 have discovered that expression of chloroplastic Fe-superoxide dismutase is induced together with CAM in *M. crystallinum*. The role of internal CO_2 remobilised from nocturnally accumulated malate for control of photoinhibition has also been elucidated in experiments with sun and shade leaves of *Kalanchoë pinnata* where nocturnal fixation of CO_2, and hence, malate accumulation were prevented by applying CO_2-free air. With this treatment as compared to controls sun leaves experienced greater photoinhibition when exposed to high light; shade leaves experienced a high degree of photoinhibition regardless of whether malic acid had been allowed to accumulate in the previous dark period or not (ADAMS & OSMOND 1988).

High Internal CO_2 – Concentrations and Light – stress: Phase III

After direct measurements of internal CO_2-concentrations in CAM plants during organic acid remobilization (phase III) had shown that these were as high as a few per cent (COCKBURN & al. 1979, SPALDING & al. 1979, KLUGE & al. 1981) and thus photosynthetic CO_2 reduction must work under condition of substrate saturation, it became widely accepted that CAM would not be prone to photoinhibition in phase III. In the field phase III of CAM coincides with the daily time of most strong insolation, but with saturating CO_2 levels excitation energy would be effectively consumed by photochemical work (OSMOND & al. 1979, 1982, WINTER & DEMMIG 1987, ADAMS & OSMOND 1988, GRIFFITHS & al. 1989). However, almost at the same time and partially even in the work of the same authors who suggested limited photoinhibition in phase III doubts also rose their heads (ADAMS 1988, ADAMS & al. 1987, 1988, KEILLER & al. 1994). In fact, acute photoinhibition is often observed at midday, i.e. during phase III of CAM, both in the laboratory (or phytotron) and in the field (Fig. 5 and 6).

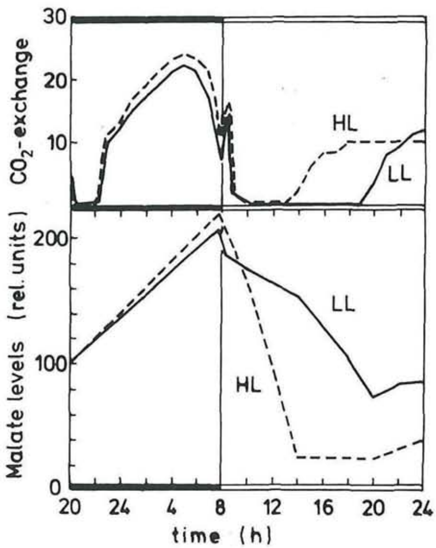


Fig. 4. CO_2-exchange and malate levels in phyllodia of plants of *Kalanchoë tubiflora* grown at low light and measured in the light period (open bar on abscissa) following the dark period (dark bar on abscissa) shown at the same low light intensity as experienced during growth (LL) and at a much higher (2.4-fold) light intensity (HL), respectively. (After KLUGE 1968.)

Fig. 6 shows that there are no distinct tendencies along a C_3-, C_3/CAM- and CAM-distribution of *Clusia* species with respect to both acute phase-III-time and chronic predawn photoinhibition, and if anything, acute photoinhibition at midday even appears to be more pronounced in CAM states than in C_3 states. Perhaps without the high CO_2 levels saturating CO_2 reduction and photochemical work, photoinhibition in phase III would be even larger than actually observed. The other side of the medal of high CO_2 assimilation rates in photosynthesis behind closed stomata are stoichiometrically high rates of evolution of nascent oxygen in the water splitting reactions at PS II. As a consequence internal built-up of high oxygen levels can occur, with up to twice the atmospheric partial pressure observed in a species of *Kalanchoë*. This has been known just as long as the high internal CO_2 levels are known (SPALDING & al. 1979) but has long been overlooked by workers in the context (see MAXWELL & al. 1998, i.e. 20 years later).

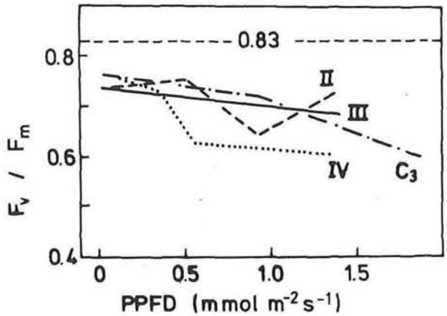


Fig. 5. Light-response characteristics of acute photoinhibition given by measurements of potential quantum yield of photosystem II (F_v/F_m) in plants of the C_3/CAM intermediate species *Clusia minor* in the C_3-state (C_3) and in the CAM-state during phases II, III and IV of CAM. Plants were kept at the photosynthetic photon flux densities (PPFD) indicated and darkened for 10 min before F_v/F_m was determined. (After HAAG-KERWER 1994.)

Plasticity of C_3/CAM Intermediate Species as Compared to C_3-Plants: How to Deal with Variable and Suddenlight – Stress?

Plasticity is exhibited by perennial C_3/CAM-intermediate species which can repeatedly perform reversible transitions between C_3 photosynthesis and CAM. The C_3/CAM-bromeliad *Guzmania monostachia* is subject to extreme variation in light intensity in its epiphytic habitat especially in relation to rainy season/dry season transitions and uses a number of photoprotective mechanisms to regulate photosynthesis and prevent long term damage, *viz.* both the shift to CAM and xanthophyll pool size and the extent of zeaxanthin-antheraxanthin-violaxanthin interconversion (MAXWELL & al. 1994, 1995). The C_3 species *Clusia multiflora* and the C_3/CAM intermediate species *Clusia minor* occur sympatrically in the field in northern Venezuela (GRAMS & al. 1997, LÜTTGE 1999). Somewhat surprisingly the C_3 species dominates open fully sun exposed sites while the C_3/CAM intermediate species appears to prefer semi-shaded sites. With CAM as a mechanism for drought- and light-stress tolerance one might have expected the opposite pattern. Both species have been studied extensively both in the field (GRAMS & al. 1997) and in phytotrons (HERZOG & al. 1999, see LÜTTGE 1999 for further references).

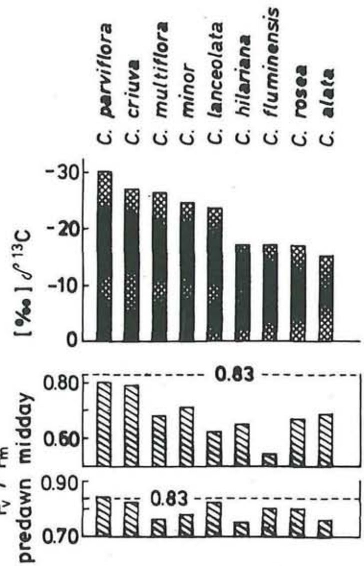


Fig. 6. Acute and chronic photoinhibition given by measurements of potential quantum yield of photosystem II (F_v/F_m) for several species of *Clusia* at midday after short periods of dark adaptation (acute photoinhibition at times of the day when CAM plants are in phase III) and before dawn after the nocturnal dark period. Data are averages from the literature on measurements in the field as well as in the laboratory. The species are arranged from left to right according to increasing (decreasingly negative) carbon isotope ratios ($\delta^{13}C$) which indicate dominant C_3-photosynthesis on the left hand and dominant CAM on the right hand of the distribution and C_3/CAM intermediate behaviour in between. (After LÜTTGE 1999)

A detailed comparison of the two species has been run in the exposition chambers of the GSF-Research Center for Environment and Health (Neuherberg/Munich, Germany), where photosynthetic photon flux densities (PPFD) of 1200 μmol m^{-2} s^{-1} can be reached and the typical bell shaped daily courses of PPFD can be simulated (HERZOG & al. 1999). Both species were grown at low light intensities (PPFD of 4 mol m^{-2} d^{-1}) and well watered and showed C_3-type CO_2 exchange under these conditions (day zero in Fig. 7). Subsequently they were transferred to high light intensity without or with drought as additional stress, and the behaviour after 5 days is shown in Fig. 7. At 24.5 mol m^{-2} d^{-1} the C_3 species was able to use the increased PPFD for increased CO_2 uptake, drought reduced CO_2 uptake at 24.5 mol m^{-2} d^{-1} only a little on day 5. However, 33.5 mol m^{-2} d^{-1} (without drought) strongly inhibited photosynthesis. The C_3/CAM intermediate species switched from C_3-photosynthesis to CAM after transfer to high light intensity and performed rather similarly under all three conditions, i. e. at 24.5 mol m^{-2} d^{-1} with and without water and at 33.5 mol m^{-2} d^{-1} with water, although drought reduced the expression of phase II. (The curves for CO_2 exchange in Fig. 7 show the day time phases II to IV of CAM, and with the first and last points of the daily time courses presented they give an idea of dark period CO_2 uptake. Day/night oscillations of organic acid levels were also induced but are not shown. Switching to CAM clearly allowed the C_3/CAM intermediate species to perform much better than the C_3 species after transfer to the higher light intensity of 33.5 mol m^{-2} d^{-1}. Acute and some chronic photoinhibition, with $F_v/F_m < 0.83$ during the day and at the end of the dark period, respectively, occurred after transfer of the plants to the stress conditions (Fig. 7), and both species behaved in the same way in this respect. Which mechanism(s) did they use for dissipation of excitation energy, as only the C_3/CAM intermediate species had the option of the phase III CO_2-concentrating mechanism? The daily course of zeaxanthin levels in both species follows the bell shaped curve of PPFD during the day and is closely correlated to thermal energy dissipation (non-photochemical fluorescence quenching, NPQ; Fig. 8; PPFD not shown). Diurnal changes of zeaxanthin and antheraxanthin levels have been demonstrated in CAM plants before, with highest levels at midday for the times of stronger insolation, i. e. during phase III (MAXWELL & al. 1995, ADAMS & DEMMIG-ADAMS 1996). PPFD-dependence and time course of zeaxanthin accumulation at 1000 μmol m^{-2} s^{-1} are also shown for the two *Clusia* species in Fig. 8. Drought stress in addition to the light stress of 22.5 mol m^{-2} d^{-1} to which these plants were subjected somewhat increased zeaxanthin levels in both species. Most noteworthy, however, is the observation that the C_3 species at midday in the diurnal cycle (upper panels in Fig. 8) operated close to the maximum levels shown to be attainable in the short term experiments (two lower panels in Fig. 8) while the C_3/CAM intermediate species, which was in the CAM state in these experiments, at midday only used about a third to half of its maximum zeaxanthin-accumulation capacity. It may be also noted that in plants of the C_3/CAM intermediate species submitted to 1000 μmol m^{-2} s^{-1} PPFD zeaxanthin peaked after a few minutes and then declined again to some extent. Thus, evidently the C_3 species which only has the zeaxanthin cycle for acute dissipation of excitation energy as heat operates close to the limits of this mechanism. Conversely, the C_3/CAM intermediate species is far from exhausting this option having the additional option of the phase-III CO_2-concentration mechanism to dissipate energy via photochemical work. This also explains another observation made in these experiments. After several days at high light intensities in these plants grown at low light intensities, i. e. from day 4 onwards, the fully developed laves of the C_3-species became necrotic and rapidly died, but this never happened in the C_3/CAM intermediate species. In long-term exposure, however, the plants of the C_3 species are not destroyed at all; they can grow new leaves having a photosynthetic apparatus well adapted to the higher light intensities. This now explains the natural distribution of the two species. The C_3 species must adapt to the open sites, which it dominates, right from the state of seedling emergence. It may not experience much variability in light conditions. The C_3/CAM intermediate species at the semi-shaded sites, which it dominates, may utilize variable light conditions by reversible C_3/CAM transitions. It is not observed that the C_3 species much intrudes the sites of the C_3/CAM intermediate species. However, the reverse does occur and the latter can grow side by side with the former in fully exposed sites. Its CAM option may help it to intrude the exposed habitat of the C_3 species without being subject to intermediate photodestruction of leaves as seen in the exposition chamber study described above. With respect to our theme of light-stress and CAM it appears that the CAM option is not so much an advantage at strong continuous stress but allows adaptations by flexible strategies.

CAM and Variable Stress

Can we generalize this conclusion from the behaviour of two *Clusia* species? The hackneyed view of a CAM plant is a succulent plant thriving under the merciless sun of deserts, i. e. under strong single-parameter stress. However, LANGE & al. 1975 already noted that in the Negev, i. e. one of the harshest deserts, CAM plants are extremely rare. The only stem-succulent types in the Negev are a few species of *Caralluma*, where *C. negevensis* is a CAM species but does not occur in the open desert habitat and uses shady sites between rocks as an "ecological niche"(LANGE & al. 1975, WINTER & TROUGHTON 1978b). Arido-active dwarf shrubs with C_3 photosynthesis are much more successful at the exposed sites in the Negev. The only other CAM type occurring in the Negev appears to be the winter annual C_3/CAM-intermediate therophytes *Mesembryanthemum nodiflorum* and *Mesembryanthemum forskahlii* (WINTER & TROUGHTON 1978a,b). A census of CAM species is not available, but we can make some guesses. The typical desert CAM plants are the leaf succulent Agavaceae (c. 300 species) and the stem succulent Cactaceae (c. 1,500 species). Conversely, there are c. 19,000 species of Orchidaceae and 2,500 species of Bromeliaceae which are more typical inhabitants of shaded, semi-shaded and semi-exposed habitats of rain forests and of canopies, because many of them are epiphytes, i. e. c. 14,000 among the Orchidaceae and c. 1,150 among the Bromeliaceae. (For these numbers see KRESS 1989.) Very many Orchidaceae and Bromeliaceae are CAM plants. Assuming about 50 % (WINTER & SMITH 1996), this limited evaluation would count more than 5times as many CAM species of typical forest sites than of deserts, and even among the Cactaceae 150 species are epiphytic (KRESS 1989). Thus, it appears that more CAM diversification is observed under variable multifactor stress as typical for the forest sites and to a much lesser extent under strong single factor stress as given in deserts. Hence, indeed, the pair of *Clusia* species described above, the C_3 species *C. multiflora* and the C_3/CAM species *C. minor*, may have a general message for us; that is, flexibility and plasticity being a major ecophysiological advantage of CAM, where CO_2, H_2O and light appear to be the major control parameters, but light may become a decisive factor depending on the parameter constellations. A special form of very rapidly variable "light stress" is given by the dynamics of light flecks on the forest floor (KÜPPERS & al. 1997, LOGAN & al. 1997, WATLING & al. 1997, ADAMS & al. 1999).

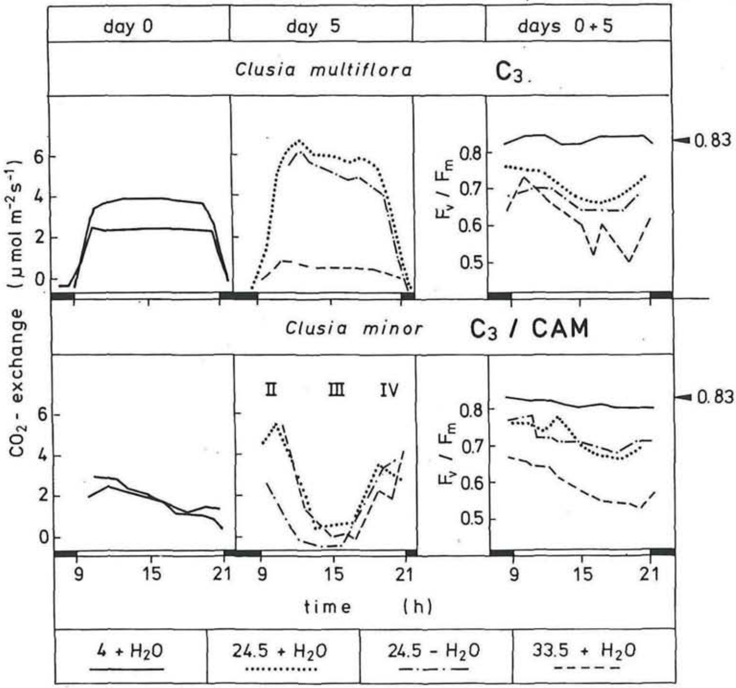


Fig. 7. CO_2-exchange and photoinhibition given by measurements of potential quantum yield of photosystem II (F_v/F_m) in the C_3-species *Clusia multiflora* and the C_3/CAM-intermediate species *Clusia minor*. Plants were grown at low light and after day 0, the last day at low light, they were transferred to two different high daily light doses applied in a bell-shaped time course (not shown) with additional stress due to withholding water in one case. The behaviour on day 0 (solid lines) and 5 days after the transfer (other lines) is shown; numbers at the bottom of the graph explaining the lines are daily doses of photosynthetic photon flux density (mol m^{-2} d^{-1}); roman numbers for gas exchange of *C. minor* on day 5 indicate CAM phases. (After HERZOG & al. 1999)

SKILLMAN & WINTER 1997 have studied this for the highly shade tolerant CAM bromeliad *Aechmea magdalenae* in a rainforest in Panama. These plants have a particularly high capacity of using light flecks during phase III of CAM and show a very high thermal energy dissipation (NPQ up to 5.5) in high light. This light-fleck use in phase III would enhance malate remobilisation and open the gates for phase IV CO_2 uptake possibly increasing overall carbon gain. Unfortunately no more work seems to be available on this phenomenon, which is not only important on the forest floor but also in canopy habitats (Uwe Rascher, Darmstadt, personal communication; MAXWELL & al. 1994, 1995) with their many epiphytic CAM plants. Even more intriguing still is the question of how C_3/CAM-intermediate plants, such as the bromeliad *Guzmania monostachia* and species of *Clusia*, might respond to light-flecks.

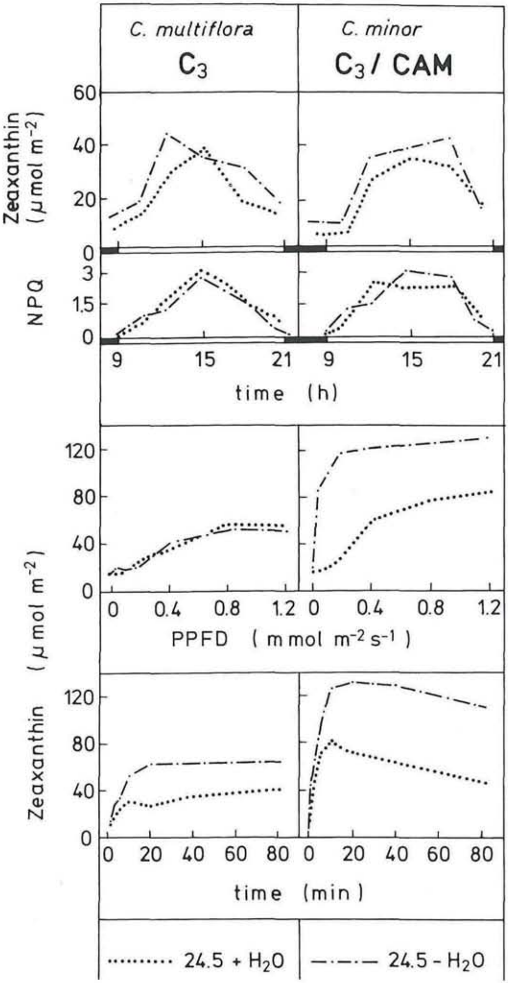


Fig. 8. Daily courses of zeaxanthin levels and thermal energy-dissipation (non-photochemical fluorescence quenching, NPQ) in the plants of *C. multiflora* and *C. minor* shown also in Fig. 7 on day 5 after transfer to high daily light doses (24.5 mol m^{-2} d^{-1}) with and without drought stress as indicated (2 upper panels). Lower panels show short term experiments with *C. multiflora* and with *C. minor* in the CAM-state where photosynthetic photon flux density (PPFD) was presented for 20 min, as indicated, or a PPFD of 1000 µmol m^{-2} s^{-1} was given for various times up to 80 min, as indicated, before analysis of zeaxanthin levels. (After HERZOG & al. 1999).

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