

The correlation between crassulacean acid metabolism and water uptake in *Senecio medley-woodii*

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Abstract. The combination of a chamber for CO₂ gas exchange with a potometric measuring arrangement allowed concomitant investigations into CO₂ gas exchange, transpiration and water uptake by the roots of whole plants of *Senecio medley-woodii*, a species which exhibits Crassulacean acid metabolism. The water-uptake rate showed the same daily pattern as malate concentration and osmotic potential. The accumulation of organic acids resulting from nocturnal CO₂ fixation enhanced the water-uptake rate from dusk to dawn. During the day the water-uptake rates decreased with decreasing organic-acid concentration. With gradually increasing water stress, CO₂ dark fixation of *S. medley-woodii* was increased as long as water could be taken up by the roots. It was also shown that a reestablished water supply after drought caused a similar increase which in both cases ameliorated the water uptake in order to conserve a positive water balance for as long as possible. This water-uptake pattern shows that Crassulacean acid metabolism is not only a water-saving adaptation but also enhances water uptake and is directly correlated with the amelioration of the plant water status.

Key words: Crassulacean acid metabolism – Gas exchange (CO₂) – *Senecio* – Transpiration – Water uptake.

Introduction

Higher plants growing in arid climates have adapted their morphology and physiology to periodic water stress. Crassulacean acid metabolism (CAM) is generally considered to be an adaptation which enables a plant to avoid desiccation caused

by excessive water loss during periods of dwindling soil water resources (Kluge and Ting 1978; Schulze and Hall 1982).

During periods of sufficient soil water, CAM plants not only profit from nocturnal CO₂ fixation but also from CO₂ uptake during daytime. Therefore, a humid and relatively cool season favours carbon gain in CAM plants (Kluge and Ting 1978). In periods of moderate water stress the daytime CO₂ uptake is attenuated concomitant with the increasing soil water potential (Kluge and Fischer 1967; Batholomew 1973; Osmond et al. 1979). In addition, during severe drought dark fixation is progressively reduced and there is a correlation between nocturnal acidification and plant water potential (Szarek and Ting 1975; von Willert et al. 1983a). Recent investigations based on calculations at the cellular level, provided evidence that with a small change in water potential the amount of water that could be stored is comparable to the amount lost during the entire dark period in transpiration (Lüttge et al. 1982). Thus the increased osmotic potential of cells subsequent to the nocturnal acid accumulation (Lüttge and Ball 1977) could ameliorate the water economy of a CAM plant not only at the cellular level but also for the whole plant via an enhanced water uptake by the roots. This would explain the close connection between plant water status and the pattern of CAM as has been shown in investigations with plants in their natural habitat (Osmond 1975; Szarek and Ting 1975; Nobel 1976; Hartsock and Nobel 1976; von Willert et al. 1983a, b, 1984).

Investigations of CAM and plant water status should consist of measurements of water loss and water uptake which results in the water balance, and assess their implications for carbon gain. To study these interrelationships, simultaneous measurements of water uptake, transpiration and CO₂ gas exchange were made with the succulent *Senecio*

medley-woodii Hutchins. from South Africa. The experimental arrangement combined a gas-exchange chamber with a recording potometer.

Material and methods

Plant material. *Senecio medley-woodii*. Hutchins., a leaf succulent from Natal (Republic of South Africa) (Fig. 1) has leaves which are amphistomatic and uniformly covered with dead trichomes on both surfaces (Ruess 1983). Plants raised from cuttings were cultivated in a quartz sand substrate in the greenhouse until they were about 25 cm high. The apex with the youngest leaves was cut about one month before the plants were used for measurements, so that only six to seven mature leaves remained. At the start of each experiment the plant was transferred to a growth chamber (Z 1200E OJU-P; Weiss, GiesSEN, FRG). The growing medium was carefully washed from the rooting system and the plant then cultivated by hydroculture in the plant-growth chamber at a 12/12 h day/night period with an irradiation of 120 W m^{-2} (photon irradiance $510 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The air temperature was 17°C and the water-vapour pressure deficit 0.43 kPa (about 80% relative air humidity) day and night.

After precultivation, the plant was fixed into the measuring arrangement which was housed in a second plant-growth chamber. A fully climatized chamber for CO_2 gas exchange, after Koch et al. (1968) (GWK 8; Walz, Effeltrich, FRG) but with a modified cuvette for the plant (Ruess 1983), was used to measure CO_2 gas exchange with an infrared gas analyzer (UNOR4; Maihak, Hamburg, FRG), and also to determine transpiration. Dew points were measured with dew-point mirrors (TS3; Walz, Effeltrich, FRG) and air flow with electronic mass-flow meters (Model 5810; Brooks, Hatfield, UK).

The plant was immobilized as a result of its fixation in the gas-exchange cuvette and the rooting system was bathed in the water container of a potometric system (Fig. 2). The control unit, which could be separated into two parts for inserting the plant, was fixed to the cuvette. An inner container attached to the control unit of the potometer (Fig. 2; 8) caught substrate and root particles to prevent their interfering with the weighing process. The hydroculture solution in the outer container on the balance (Fig. 2; 9, 12) also filled the inner container. A 7-mm-thick oil seal (Fig. 2; 10) prevented uncontrolled water loss to the ambient air. The weight loss of the outer container with the solution was measured as the water uptake of the plant. An air space existed in the inner container between control unit and solution surface. The control unit was temperature-controlled to prevent condensation of water vapour. A capillary tube allowed the pressure between air space in the potometer and the outside to be equalized. The temperature of the solution was measured (Fig. 2; 3) and controlled at 17°C by an external unit via the cooling coil (Fig. 2; 6).

It is known for numerous plants that a low O_2 partial pressure influences water-uptake rates (Kramer 1949; Slatyer 1967; Winter 1974). Thus, O_2 partial pressure was monitored by an electrode (Polling Oxi 722BSB; Camille Bauer, Basel, Switzerland) and oxygen-enriched air circulated through a silicon-rubber tube of 1 mm inner diameter. Oxygen diffusion through the tube wall into the solution allowed the O_2 partial pressure to be maintained at $6\text{--}8 \text{ g m}^{-3}$.

Irradiation was measured by a solarimeter (CM5; Kipp and Zonen, Delft, The Netherlands) and photon irradiance by a quantum sensor (LI 1905; Licor, Lincoln, USA). Data acquisition was made with a data-transfer unit (DTU 3240; Solartron, Farnborough, UK) and computation was performed at the computation center of the University of Zürich, using pro-

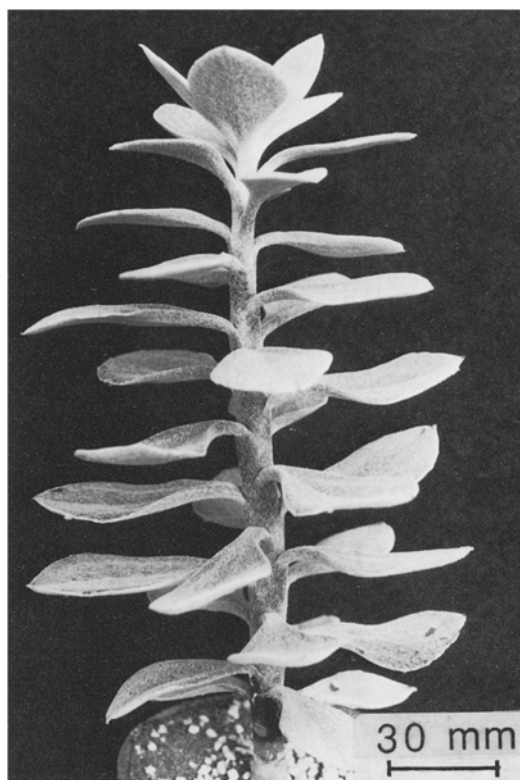


Fig. 1. Young unbranched plant of *Senecio medley-woodii*

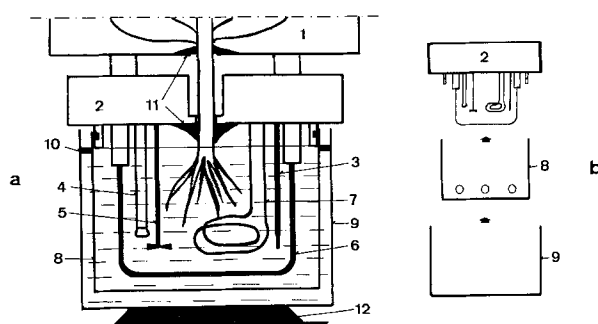


Fig. 2a, b. Recording potometer. a Assembled during experiments; b Explosion view. 1 Gas-exchange cuvette with plant; 2 control unit; 3 platinum resistance thermometer; 4 O_2 electrode with stirrer (5); 6 stainless-steel tube for cooling; 7 silicon-rubber tube for O_2 partial-pressure control; 8 inner container with holes; 9 outer container; 10 oil cover; 11 seal (Terostat IX, Teroson, Weinheim, FRG); 12 electronic balance (PL 1200 with converter GC 20; Mettler, Greifensee, Switzerland)

grams after Eller and Koch (1976). Malate was determined enzymatically (Bergmeyer 1970) and the osmolarity by an osmometer (Tp3B; Vogel, Giessen, FRG). Values for CO_2 gas exchange, transpiration and water uptake are given for the projected leaf area determined with a planimeter.

Results

To ascertain if the transfer from cultivation in a sandy substrate to hydroculture might affect the

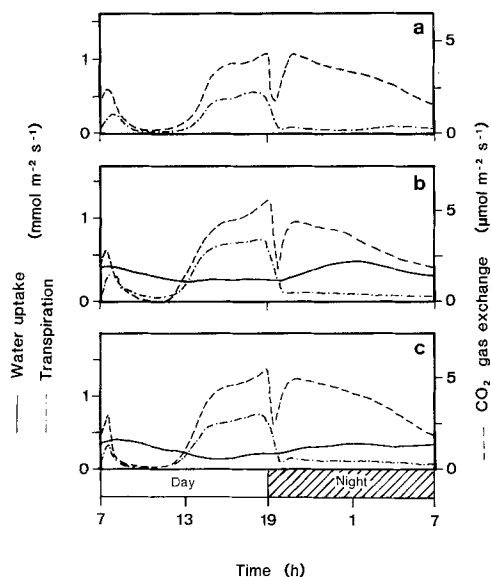


Fig. 3a-c. CO₂ Gas exchange, transpiration and water uptake of *S. medley woodii* **a** cultivated in the quartz sand substrate and at **b** the first and **c** fourth day after the transfer of the plant to hydroculture in the potometric arrangement. Air temperature 17° C, relative air humidity 80%, photon irradiance 480 μmol m⁻² s⁻¹

physiology of a plant, measurements were made before and after the transfer. Figure 3 shows CO₂ gas exchange, transpiration and water uptake of a plant prior to its transfer from the quartz substrate to hydroculture in the potometer (Fig. 3a) and at the first and the fourth day after transfer (Fig. 3b,c). The pattern of CO₂ gas exchange and transpiration was that of a typical CAM plant with a good water supply (Kluge and Ting 1978; Osmond 1978). During the day the transpiration pattern was the same as for CO₂ gas exchange but nocturnal transpiration was very low. The CO₂ gas exchange and transpiration did not alter after the transfer as could be observed from day to day in experiments with unchanged cultivation conditions. The slight increase after transfer to the potometer resulted from the improved water supply in hydroculture. Additional experiments showed that after 4–6 d the daily variations in the gas-exchange pattern were small and no major changes were observed in experiments which ran for up to two weeks. The water uptake varied from the first to the second or third day after the transfer to hydroculture but then remained at the same level for consecutive days. To avoid these initial variations, all plants used for the water-uptake experiments were precultivated in hydroculture for at least 5 d.

In plants performing the C₃ and C₄ pathway of photosynthesis, water uptake follows the daily

Table 1. Leaf dimensions of young and adult leaves of *S. medley-woodii* with abundant (A) or limited (L) water availability

	Young leaves		Adult leaves	
	L	A	L	A
Leaf thickness (mm)	1.6	5.3	1.7	4.5
Δ leaf thickness (A-L; mm)	—	3.7	—	2.8
Leaf surface (cm ²)	16.32	12.98	23.24	23.90
Fresh weight (g)	1.05	2.02	1.60	3.11
Surface expansion (cm ² g ⁻¹)	15.5	6.4	14.5	7.7

course of transpiration rates but with a certain delay (Kramer 1949; Mingeau 1969; Ehrlér et al. 1966; Brunner and Eller 1974, 1975). *Senecio medley-woodii* showed a diurnal course of water uptake (Fig. 3c) which was very different from such a pattern. Water-uptake rates increased during the night, reached a maximum in the early morning and decreased again. Most remarkable was the increase in the water-uptake rate during the night, although transpiration was almost nil. Such an uptake pattern was observed in all similar measurements and accounted for a positive water balance over the 24-h period. In the experiment shown in Fig. 3 the daily net water gain was 6.7 mol m⁻² d⁻¹ at the fourth day. In a similar experiment the value was 6.67 mol m⁻² d⁻¹ at the fourth day and was still 5.06 mol m⁻² d⁻¹ at the tenth day.

A positive water balance during the course of several days raises the question of water-storage capacity. In the latter experiments mentioned above, a calculation of the surplus water uptake (in cm⁻³) over the 10-d period divided by the total leaf area of the plant gave a value of 1.3 mm for the amount by which the leaf thickness should increase to meet storage requirements. A comparison of leaf dimensions (Table 1) shows that the leaves of *S. medley-woodii* can vary their surface expansion and thus leaf thickness according to water availability (or better, surplus of water uptake over water loss). The observed differences in leaf thickness (Table 1) are much larger than the required 1.3 mm in our experiment.

At the end of the 10-d experiment the daily variation in malate content was 114 μequivalents (g⁻¹ fresh weight) or 60% of the evening value of malate. During the same time the osmolarity of the cell sap increased by 95 mosmol, which is equal to an increase in osmotic potential of 2.28 bar. Such an increase in the osmotic potential could be the driving force for water gain during the night.

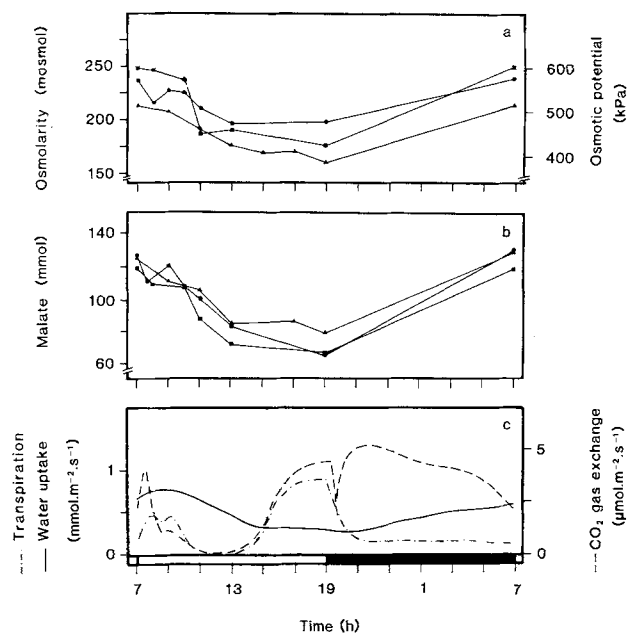


Fig. 4a-c. Daily course of **a** osmolarity and **b** malate content of *S. medley-woodii* leaves, and **c** CO₂ gas exchange, transpiration and water uptake of the whole plant. Air temperature 17°C, relative air humidity 80%, photon irradiance 480 μmol m⁻² s⁻¹

A detailed analysis of the diurnal course of CO₂ gas exchange, transpiration and water uptake of the plant and also the osmotic potential and the malate content of the leaves is presented in Fig. 4. During the 6-h period, from 7 h to 13 h, malate was consumed and the osmotic potential decreased. A second period, from 13 h to 19 h, started with an increase in the CO₂-uptake rate after the midday depression (Fig. 4c) and was characterized by a constant low level of malate and a low osmotic potential. The third phase, from 19 h to 7 h, is that of nocturnal malate accumulation, which was paralleled by an increase in the osmotic potential.

Water uptake showed the same daily pattern as malate and osmotic potential. Evidently, water uptake is correlated with the osmolarity of the cell sap as the consequence of nocturnal acid accumulation (mainly malate) and its consumption during daytime and is not correlated with water loss by transpiration.

To investigate the reactions of *S. medley-woodii* to reduced water availability, a water shortage was induced by lowering the O₂ partial pressure in the water container of the potometer to a value of 1 g(O₂) m⁻³, and maintaining this value throughout the experiment. It is well known that such a low O₂ partial pressure reduces water uptake

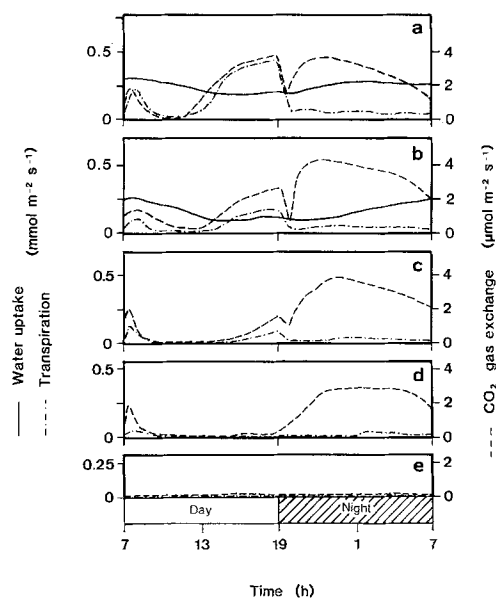


Fig. 5a-e. CO₂ Gas exchange, transpiration and water uptake of *S. medley-woodii* during increasing water stress induced by O₂ deficiency in the hydroculture solution followed by cutting the roots. **a** Third and **b** tenth day of stress in hydroculture; **c** 2, **d** 5 and **e** 30 d after cutting the roots. Air temperature 17°C, relative air humidity 80%, photon irradiance 480 μmol m⁻² s⁻¹

(Kramer 1949; Slatyer 1967) and can even cause a water-stress-induced shift from C₃ to CAM metabolism (Winter 1974). We gave preference to this method since osmotically active substances (inorganic or organic) added to the solution might be absorbed by the roots (Slatyer 1961; Lagerwerff et al. 1961; Lawlor 1970).

The results are shown in Figs. 5 and 6. Daytime CO₂ uptake decreased from day to day, while nocturnal CO₂ fixation increased from an initial value of 105 mmol m⁻² d⁻¹ to 149 mmol m⁻² d⁻¹ (+41.9%) on the tenth day (Figs. 5a, b, 6a). Within the same period, transpiration was attenuated (Figs. 5a, b, 6b) but the water balance (Fig. 6c) remained positive day and night. On the tenth day a net gain in water of 5.57 mol m⁻² d⁻¹ was measured. The greater part of the daily water gain was caused by a marked increase in the uptake rate between the beginning and the end of the night (Fig. 5b). This increased water uptake during the dark period coincides with the well-known accumulation of osmotically active products of CO₂ fixation in the same period.

On the tenth day of the experiment, further water uptake was prevented by cutting the roots off the plant. In this way, a severe water stress was applied to the plant which reacted to the stress with an instantaneous decrease in CO₂ fixation,

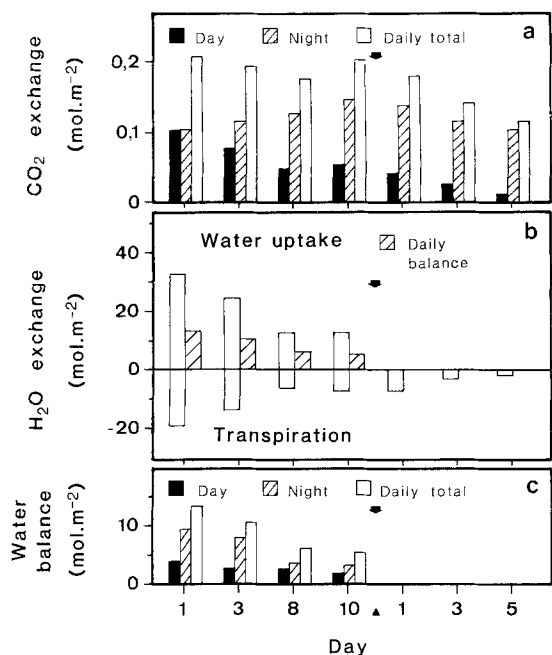


Fig. 6a-c. CO₂ Gas exchange, transpiration and water uptake of *S. medley-woodii* during increasing water stress induced by O₂ deficiency in the hydroculture solution followed by cutting off the roots (arrow). The values shown are summations for the light and the dark period and also for the day. Air temperature 17° C, relative air humidity 80%, photon irradiance 480 $\mu\text{mol m}^{-2} \text{s}^{-1}$

not only during the day but also during the night. Hence, the imposition of moderate water stress (from the first to the tenth day) enhanced nocturnal CO₂ fixation gradually but, by contrast, the first day of severe water stress (roots cut off) induced an immediate reduction in CO₂ fixation, i.e., CO₂ dark fixation increased despite a water shortage as long as water uptake was feasible for the plant. Thirty days after cutting off the roots no measurable CO₂ gas exchange could be observed (Fig. 5e).

The above experiment, involving a change from a sufficient water supply to water-stress conditions, was reversed in the experiment shown in Figs. 7 and 8. A potted plant which had not been irrigated for 20 d was then transferred to the potometer (hydroculture solution supplied with sufficient O₂). On the 20th day (Fig. 7a), some CO₂ uptake could still be measured indicating that the plant had not yet reached the extreme water-stress situation shown in Fig. 5e. After transfer of the plant to the potometer, a 2-h period for thermal equilibration of the experimental arrangement preceded the restart of measurements.

The first-measured water-uptake rate of 2.13 $\text{mmol m}^{-2} \text{s}^{-1}$ (Fig. 7b) exceeded any previously measured values proving that the root system of

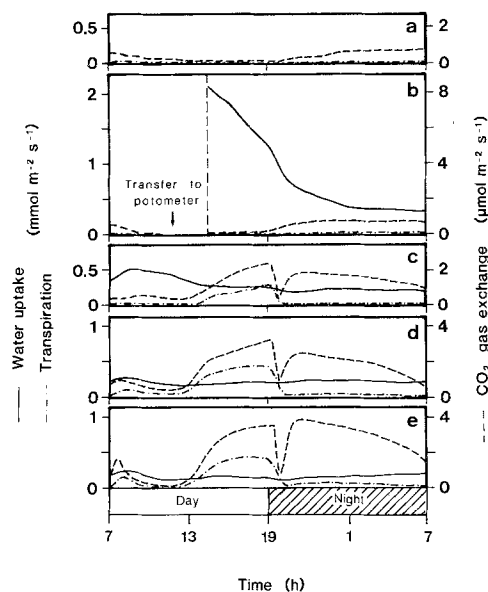


Fig. 7a-e. CO₂ Gas exchange, transpiration and water uptake of *S. medley-woodii* after a drought period **a** before the transfer into the potometer, **b** the first, **c** second, **d** third and **e** sixth day after a good water supply was reestablished by hydroculture. Air temperature 17° C, relative air humidity 80%, photon irradiance 480 $\mu\text{mol m}^{-2} \text{s}^{-1}$

the plant was in a suitable condition to react promptly to the improved water supply. It is probable that the uptake rates were even higher prior to the restart of monitoring. There was a water gain of 34.53 mol m^{-2} from 14 h to 19 h but, in addition to this high initial gain, the uptake during the 2-h unmonitored thermal-equilibration period should also be considered. A lower but still considerable uptake rate was maintained during the following 24-h period (Fig. 7b, c) until the same level as that of a similar plant with a constant good water supply (Fig. 5a) was established.

The water gain in the first 5 h monitored equalled six daily mean water gains measured in another experiment for a plant with a constant good water supply (5.5 mol m^{-2}). During the first night the water gain was 22.68 mol m^{-2} which is sevenfold the value of the nocturnal water gain of a plant with good water supply. From the third day (Figs. 7d, 8c) the water gain was reduced, evidence that a satisfactory plant water status had been reestablished.

The massive water gain during the first light period by the plant in the potometer led to increased CO₂ dark fixation from the first hour of the following night (Figs. 7b, 8a). By the second day, transpiration was marked, evidence of stomatal opening during the light period, and CO₂ uptake in the light was feasible for the plant as a consequence of the improved water status. From

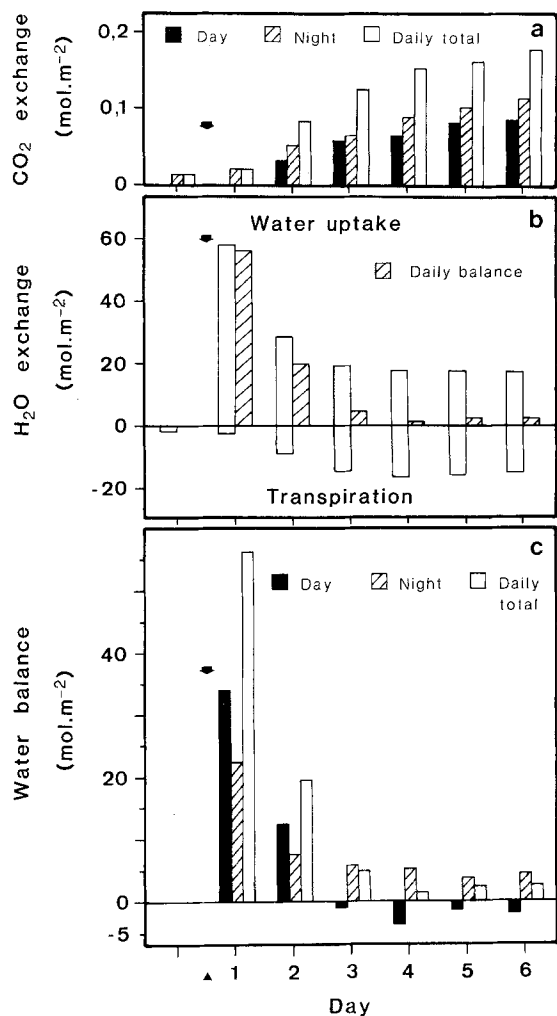


Fig. 8a-c. CO₂ Gas exchange, transpiration and water uptake of *S. medley-woodii* after a drought period and during the first 6 d with a water supply reestablished by hydroculture (arrow, transfer to potometer). The values shown are summations for the light and dark period and also for the day. Air temperature 17°C, relative air humidity 80%, photon irradiance 480 $\mu\text{mol m}^{-2} \text{s}^{-1}$

the first to the second night, CO₂ dark fixation (Fig. 8a) more than doubled (first night 0.021 mol m⁻², second night 0.053 mol m⁻²). During the subsequent days there was an increase not only in CO₂ dark fixation but also in daytime CO₂ uptake and transpiration (during day and night) (Figs. 7, 8). After 6 d (Fig. 7e) the plant showed the typical CO₂-exchange and transpiration pattern of a CAM plant with a good water supply.

Discussion and conclusions

In our experiments the reaction of *S. medley-woodii* to increasing and decreasing short-term water stress was investigated. After a rainfall in arid re-

gions, plants are confronted with a sudden transition from drought to humid soil conditions. In these habitats, high evaporation rates limit the time available for plants to collect water to refill their water reserves in order to survive the following drought. In such cases, root growth of succulents is quick (Kausch 1965) and the ability to take up water is immediate, as can be seen from Figs. 7 and 8.

In contrast to mesic plants, full turgescence of succulents is only reached after a certain period of abundant water supply. For *S. medley-woodii* at least a 20-d period in hydroculture is needed to gain full turgor which then is paralleled by a daily transpiration water loss equal to the water uptake during the same time (daily water balance=0). Prior to this maximal plant water status the daily water balance remains positive if water can be taken up (Fig. 3).

In C₃ and C₄ plants, water-uptake rates increase during the light period with an increasing amount of water loss by transpiration, and water uptake decrease steadily during the night, i.e. the driving force is primarily a consequence of loss of turgescence during daytime due to transpiration (Brunner 1975; Brunner und Eller 1974). In *S. medley-woodii* the daily course of the water-uptake rate is proportional to the daily variations of both the osmotic potential of the cell and the concentration of malate, or more fundamentally, the generation of the CO₂ acceptor phosphoenolpyruvate from osmotically inactive starch or glucan (Kluge and Ting 1978). Therefore, in CAM plants the driving force for the rate of water uptake is the basic CAM feature, CO₂ dark fixation, which is modulated by environmental conditions and the physiological status of the plant, and is not primarily transpiration as it is in C₃ and C₄ plants.

Assuming a constant amount of osmotically active substances per cell, the water collected by the plant roots raises cell water potential and thus water uptake should decrease if the uptake rate is higher than the concomitant transpirational loss. Consequently, the water-uptake rate tends to decrease with improving plant water status. However, in a CAM plant the concentration of osmotically active substances rises during night as the consequence of CO₂ fixation (Lüttge and Ball 1977) provoking a gradual increase in the water-uptake rate (Figs. 3, 4, 5a, b, 7e). Therefore, the higher the nocturnal CO₂ fixation, the greater is the increase of the water-uptake rate (Δ water uptake) from dusk to dawn, as the close correlation established in Fig. 9 clearly demonstrates. In this figure the results of seven experiments with well-

watered and water-stressed plants are compiled. This finding, resulting from water-uptake measurements concomitant with CO₂ gas-exchange measurements, explains the correlation between nocturnal acidification and plant water potential as shown by Szarek and Ting (1975) and von Willert et al. (1983a). In addition, the high level of dark-fixation products causes high water-uptake rates during the early morning until the products are consumed (Fig. 4); consequently, nocturnal acidification and plant water potential are causally connected during both the light and the dark period.

In our experiment with the water-stressed plant, the insertion of the plant into the potometric arrangement leads to an increase of CO₂ dark fixation (Figs. 7, 8). After the initial high level of water uptake due to the very low water potential of the water-stressed plant (Figs. 7b, 8c), the daily water balance (water uptake – transpiration) remains positive (Fig. 8c). This clearly indicates that the plant has not yet reached its maximal water status (maximal turgor) or, from a different viewpoint, the water-storage capacity of the plant at this time is not fully utilized.

However, by the second day the improved water status allows *S. medley-woodii* to reestablish daytime CO₂ uptake (Fig. 8a) since the transpirational water loss (6.83 mol m⁻²), caused by the daytime opening of the stomata, is overcompensated by the daily water uptake (29.3 mol m⁻²). This water-uptake surplus explains the early reaction of a CAM plant to improved water supply by stomatal opening during daytime, as shown by Szarek and Ting (1974; 1975) and Osmond et al. (1979).

It is essential for a succulent plant to store as much water as can be harvested since a sufficient water reserve is crucial for its survival. Therefore, the plant has to make use of any physiological and-or physical process which may enable it to increase water reserves as long as water is available. Hence, a negative water balance during the light period, resulting from transpirational water loss exceeding water uptake, should at least be compensated by nocturnal water gain. In our experiment this is always the case (Fig. 8c, third to sixth day). We have shown that the accumulation of CO₂ dark-fixation products enhances the water-uptake rate from dusk to dawn (Fig. 9), and it can be seen from Fig. 8c how a CAM plant modulates transpiration and daily water-uptake rates in order to obtain a positive daily water balance.

However, we must emphasize that the daily variation of water-uptake rates in CAM plants results from two driving forces. Firstly, there is the

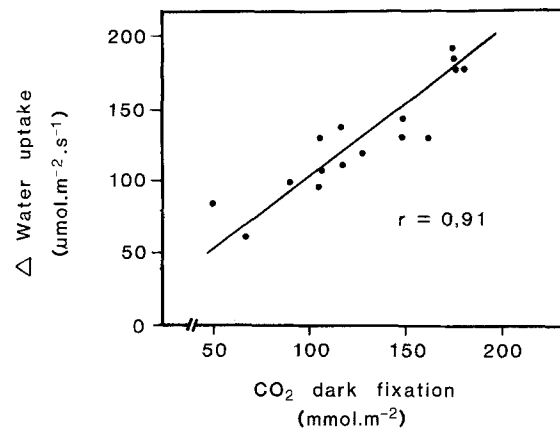


Fig. 9. Correlation between nocturnal carbon gain and the increase of the water-uptake rate from dusk to dawn (= Δ water uptake). Results compiled from seven experiments with well-watered and water-stressed plants

cell potential varying slowly according the plant water status and a slowly changing cell osmolarity. Secondly, there is the diurnal oscillation of cell osmolarity caused by the diurnal formation and consumption of CO₂ dark-fixation products (Fig. 4), and sometimes there is a small additional variation caused by transpirational water loss (e.g. detectable between 16 h and 21 h in Fig. 5a, b). From the second to the sixth day the total water uptake during the night decreases as a consequence of the improved plant water status (Fig. 10a, I). However the increase of the water uptake concomitant with the increased nocturnal CO₂ fixation (Fig. 10b, I) prevents an even more pronounced decline of the total water uptake. Therefore, CO₂ dark fixation, which shows a linear relationship with acid accumulation (Medina and Osmond 1981; Medina 1982) improves the daily water uptake and enables the plant to conserve a positive daily water balance until the maximal plant water status (maximal turgor) is reached. Since CO₂ dark fixation brings about an improvement of the plant water status, we suggest that the increase of CO₂ dark fixation in this case (Fig. 8a) is primarily a plant reaction for collecting as much water as is necessary to attain the maximal plant water status. That the increase in nocturnal CO₂ fixation also contributes to the daily carbon gain is evident.

If our suggestion is correct, then a CAM plant has to increase nocturnal CO₂ dark fixation when a period of good water supply changes gradually to water-stress conditions; this lowers the cell water potential in order to maintain a positive water balance as long as water is still available at a reasonably low soil water potential. Our results from the water-stress experiment (Figs. 5, 6)

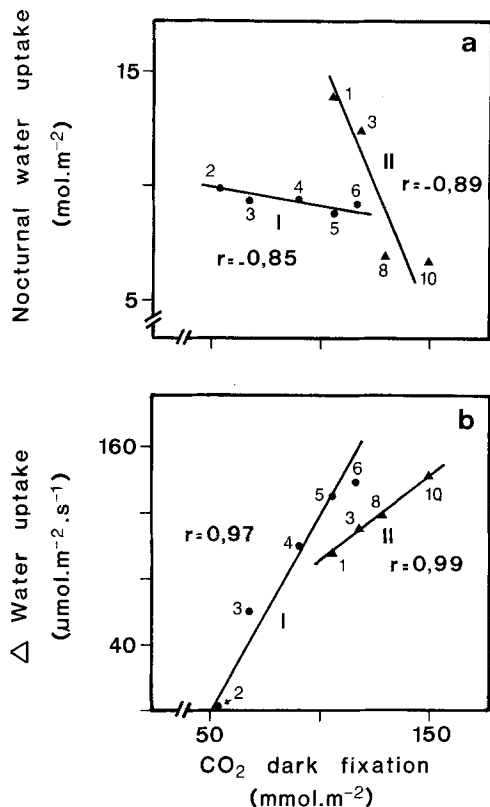


Fig. 10a, b. Correlation between nocturnal carbon gain, nocturnal water uptake (a), nocturnal carbon gain and the increase of the water uptake rate from dusk to dawn (Δ water uptake) (b). I, Water supply reestablished after drought (results from the experiment shown in Figs. 7, 8). II, Increasing water stress (Results from the experiment shown in Figs. 5, 6). Numbers give the day of the experiment

support this opinion. Dark fixation of CO_2 increases after water stress has been applied (Fig. 6a) indicating that the capacity for CO_2 dark fixation was not fully utilized by the plant with a good water supply, a fact that cannot be easily understood if CO_2 dark fixation is considered to be only a tool to maximize carbon gain. One could, however, argue that the CO_2 -fixation capacity was a limiting factor during the day and that at the start of water stress, daytime CO_2 uptake is attenuated to reduce water loss, causing a consequent compensating increase in CO_2 dark fixation. However, this does not explain why the daytime CO_2 uptake is reduced when the plant still has a positive daily water balance (Fig. 6b).

It is known that plants react to decreasing air humidity with a feed-forward reaction resulting in stomatal closure (Farquhar 1978), and that this occurs before there is a significant decline in leaf water status. Similarly, *S. medley-woodii* reduces daytime CO_2 uptake and thus transpirational water loss (Fig. 6b, first to eighth day) enabling

the economic long-term use of plant water reserves (Cowan 1982). The perception of declining water availability to the roots could result in a similar feed-forward reaction by the plant: the plant perceives the lowered water-uptake rates and conserves a positive water balance by enhancing CO_2 dark fixation in order to lower cell potential. Water uptake is thereby maintained despite decreasing soil water potential (Fig. 6b). The mode of perception of such changes could be through turgor changes which are involved in controlling the day-night rhythm of CAM as shown in the model proposed by Lüttge et al. (1975). The plant cannot avoid a steady decline in water uptake, as can be seen from the total nocturnal water gain (Fig. 10a, II) but the increase of Δ water uptake (Fig. 10b, II) resulting from enhanced CO_2 dark fixation (Fig. 6a) enables *S. medley-woodii* to maintain a positive water balance (Fig. 6c). If the roots are cut off, then water uptake is prevented and the plant immediately reduces CO_2 dark fixation.

Summarizing, we recognise in *S. medley-woodii* three different ways of modulating CAM according to the water supply. Firstly, the reestablished water supply to a water-stressed *S. medley-woodii* results in enhanced CO_2 dark fixation and reestablished daytime CO_2 uptake and transpiration (Figs. 7, 8). A similar reaction by other CAM succulent given an improved water supply was observed by von Willert et al. (1983b, 1984); Szarek et al. (1973); Szarek and Ting (1975); Hanscom and Ting (1977); Osmond et al. (1979). Secondly, in the case of the transition from a good water supply to gradually increasing water stress, *S. medley-woodii* increases CO_2 dark fixation whereas daytime CO_2 uptake (and transpiration) decreases (Figs. 5, 6), as has been shown by Benzing and Renfrow (1971); Kluge et al. (1973); Medina and Delgado (1976); Medina et al. (1977) for other CAM plants. Thirdly, in the case of severe drought, *S. medley-woodii* ceases to take up CO_2 day and night (Fig. 5e), a fact that was also observed in the field (von Willert et al. 1984; Szarek and Ting 1974; Nobel 1977; Ting and Rayder 1982) for other CAM succulents.

In the first two cases, water is available and *S. medley-woodii* increased CO_2 dark fixation to ameliorate water uptake in order to conserve a positive water balance as long as possible (Figs. 6, 8). This could be another explanation for the auxiliary role attributed to CAM in *Sedum acre* L. by Kluge (1977). During the hot and dry period the CAM of *S. acre* could ameliorate the water economy of the plant rather than contribute substantially to carbon gain.

In the third case, as soon as water is no longer available, CO₂ uptake decreases gradually to nil because only a maximized water saving by total stomatal closure day and night can prevent the plant from desiccation. The rapidity of the nighttime stomatal closure depends, however, on the water-storage capacity of the plant and can be delayed 40 d in the case of *Ferocactus acanthodes* (Nobel 1977).

If we accept that for a CAM plant in an arid habitat, water harvesting is as essential as water saving, then the control of CO₂ dark fixation according to the availability of water from the soil, and perhaps also from the air (von Willert et al. 1984), has to overrule the aim to maximize carbon gain. Therefore, the substantial contribution of CO₂ dark fixation to carbon gain during periods of increasing water stress is directly correlated with the conservation of a high plant water status primarily by efficient water harvesting and only secondarily by water saving.

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