Drought-induced changes in the nocturnal CO_2 fixation pattern of Senecio medley-woodii

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Abstract

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Senecio medley-woodii Hutchins., a succulent with crassulacean acid metabolism (CAM) was used for the investigations reported. The same diurnal environmental conditions were maintained throughout the experiments, with the exception that irrigation was stopped at day one of the investigations. CO_2 gas exchange and transpiration were monitored with a computer controlled gas exchange cuvette. With continued conditions of drought, the pattern of nocturnal CO_2 fixation changed and the maximum value of CO_2 fixation shifted from early to later in the night. A three-phase interrelationship between this shift of the peak value of CO_2 dark fixation and the changes in daily carbon gain and the transpirational water loss was observed. The hypothesis is advanced that the time between night fall and the occurrence of the maximum of nocturnal CO_2 fixation is an indicator for the plant water status of *Senecio medley-woodii*. In phase I the CO_2 fixation maximum occurs early in the night and coincides with a good water status of *S. medley-woodii*, whereas phase III with maximum values late in the night indicates severe drought stress. In phase II the shift from early to late takes place. In this phase the plant is subject to gradually increasing drought and the CAM-features are expressed most clearly (high CO_2 dark fixation and stomatal closure during the light period). Data from other authors support the notion that the hypothesis might also be valid for other CAM-plants.

Key words: Crassulacean acid metabolism, CAM, *Senecio medley-woodii*, CO_2 dark fixation, plant water status

Introduction

Plants with a well expressed crassulacean acid metabolism (CAM) are characterized by a net CO_2 uptake during the dark period. CO_2 fixation rates usually vary during this dark period. Numerous investigations have been concerned with CO_2 exchange and transpiration of CAM-plants and their modulation by environmental conditions during the light and the dark period (for reviews see e.g. Lüttge 1987, Griffiths 1988). However, only in a few investigations has the shape of the curve of CO_2 fixation (or the resulting

acidification) been correlated with environmental conditions (Szarek et al. 1973; Medina and Delgado, 1976; Hanscom and Ting, 1977, 1978; Osmond et al., 1979; Griffiths et al. 1986; Ong et al., 1986; Lüttge, 1987), and in only a few instances (e.g. Osmond et al., 1979; Lüttge, 1987) have the changes in the nocturnal CO_2 fixation pattern been explicitely correlated with plant water status, and not simply with environmental conditions or drought.

Over the past years we have completed numerous investigations on water relations and drought stress with the CAM plant *Senecio medley-woodii* (e.g. Ruess and Eller, 1985; Ruess et al., 1988; additional unpublished data). From these we gained the impression that there is a close link between the nocturnal variation of CO_2 uptake rates and water availability from the soil, and thus of changing plant water status. With regard to plant water and plant water status we clearly have to distinguish between water that is essential for the plant's biomass (e.g. water used as a solute in the cytoplasm) and the so called utilizable water (von Willert et al., 1990) which the plant can use for, e.g. growth, or which is lost by transpiration. If the plant is subject to drought, then the amount of utilizable water decreases, the plant water status changes which in turn induces changes in the plant's physiology. The first reaction of the plant is certainly to reduce transpirational water loss by stomatal regulation which in turn will affect CO_2 gas exchange.

Drought stress on plants has two sources. Firstly, it arises from high water vapour saturation deficits (VPD) of the ambient air, frequently combined with leaf temperatures above the ambient air temperature resulting from high solar energy input (=aerial drought). The result is a high value of ΔW (water vapour partial pressure difference leaf – ambient air) which is the driving force for the water loss by transpiration. High values of ΔW result in high transpirational water loss which is only avoided by closing the stomata. Second, decreasing water availability from the soil produces soil drought stress on a plant since the latter cannot replace its water loss through transpiration by water uptake via the roots. If the water budget (water loss minus water uptake) of a plant is negative this plant is subject to drought and its utilizable water reserves (von Willert et al. 1990, 1992) will decrease. There is no known method to properly quantify the amount of utilizable water (von Willert et al., 1990) and plant water status is only quantified by the percentage of water saturation (full turgor), a value not applicable to succulents as was outlined by von Willert et al. (1992). Nevertheless, we can attempt to find correlations or interrelationships without resorting to quantification. Such investigations may provide valuable information for further research.

The investigation of drought impact on plants requires a clear distinction of the different parameters of drought which must be monitored or held at defined and constant values. In the results presented below, plants were cultivated and monitored in plant growth chambers. In these chambers the environmental conditions for the plant investigated can be held identical for every day of the experiment. The aim of this work was to analyze whether there is a quantifiable relationship between the nocturnal CO_2 fixation pattern and drought stress induced by decreasing water availability from the soil. Therefore, the daily pattern of ambient air temperature, VPD, irradiation and air circulation around the plant was held constant during the entire period of the experiments.

Material and methods

The CAM plant used in this investigation is a succulent Asteraceae, *S. medley-woodii* Hutchins, from the province of Natal (Republic of South Africa). Cuttings were propagated in the greenhouse

and, after rerooting, were transferred to a plant growth chamber for further cultivation under the same environmental conditions as during the measurements (see Fig. 1 and Fig. 4, respectively). Plants were well watered during this period.

Photon irradiance was measured by a quantum sensor (LI1905, LICOR, Lincoln, USA). Gas exchange was measured with a fully climatized cuvette system as described by Ruess and Eller (1985). Automatic control of the cuvette system was made via an online computer system (VME-bus system, ELTEC, Mainz, BRD) combined with a data transfer unit (Orion 3530 A, Schlumberger, Farnborough, GB) which was also used for data acquisition. Values for CO_2 gas exchange and transpiration are based on the projected (single) leaf area. Fit of curves in Fig. 2 and 4 was made with the computer software GRAPHER (Golden Software Inc., Golden, USA).

To quantify the impact of drought from the water vapour saturation deficit of the air (VPD), the value of VPD, integrated for the 24 h period (IVPD), was calculated according the formula

$$IVPD = \int_{24h} VPD \cdot dt.$$

Results and discussion

Results from a 60 day experiment on changes of the diurnal CO_2 gas exchange pattern caused by soil drought are shown in Fig. 1. The plant was well watered and precultivated under environmental conditions given in Fig. 1 until the experiment started with the plant being fixed in the gas exchange monitoring equipment. The environmental conditions during the experiments remained the same as before but watering was stopped at day one. The daily pattern of the CO_2 gas exchange (Fig. 1) at the second day is typical for a well watered CAM-plant. The highest dark fixation value was monitored at about two hours after the onset of the dark period. At day 22 the CO_2 fixation pattern (Fig. 1) had markedly changed. The morning peak still existed but during time of the light period a respiratory CO_2 output occurred. Despite 22 days of drought, a high dark fixation persisted but with a pattern different from that at day two. The peak value of CO_2 uptake was reached with a time lag of about 7 h after the onset of the dark period. If the time lag (hours after the night fall) for the peak value of CO_2 dark fixation is correlated with the duration of the drought (days after the last irrigation) three phases are obtained (Fig. 2). For about the first 6 days (phase I) the time lag was fairly constant and around 2 h. In phase II the shape of the CO_2 fixation curve during the night changed. The time lag for the peak value increased steadily but after about 20 days of drought the lag was constant and about 7 h (phase III). The pattern of nocturnal CO_2 fixation did not change for the rest of the experiment. With further continuing drought conditions the curve of the nocturnal CO_2 uptake flattened and it was increasingly difficult to define a peak value which after about 40 days reached its maximum between 6 and 8 h after onset of darkness.

The integrated values of CO_2 gas exchange and transpiration for the light and the dark period for this 60 day experiment are presented in Fig. 3. Also included in Fig. 3 are the values of water use efficiency (CO_2 fixed/H_2O transpired; WUE) for the 24 h period. Changes in CO_2 exchange, transpiration and WUE coincided with the observed three-phase pattern of time lag of the CO_2 dark fixation maximum. Phase I with an almost constant time lag of 2 h was paralleled by a nearly constant and high CO_2 fixation and an extremely high transpirational water loss during the light period. Evidently, in this period the plant water status was still very good, the plant did not show signs of drought stress (e.g. by reducing substantially transpiration in the light period) and the WUE was low. During this phase nocturnal transpiration was very high and at a constant
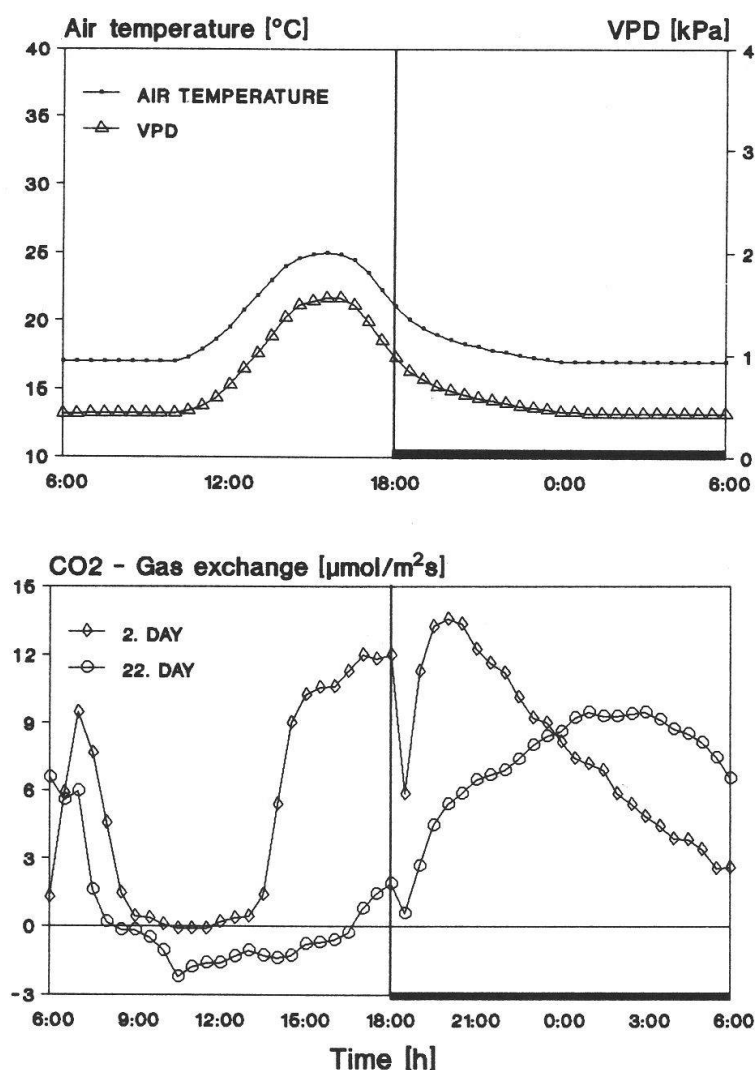
Fig. 1. CO_2 gas exchange of *Senecio medley-woodii* at days 2 and 22 after irrigation was stopped. Diurnal changes of ambient air temperature and water vapour deficit similar to conditions in the natural habitat. Photon irradiance 550 μmol m^{-2} s^{-1}.

level. CO_2 dark fixation increased in this time period. This for us is an indication that the plant already sensed somehow that water availability from the soil decreased. Such a reaction of *S. medley-woodii* with increasing values of CO_2 dark fixation at the onset of drought is well known to us and has been reported before (Ruess et al., 1988). We believe this increase of CO_2 dark fixation to be a feature common for CAM-plants as we have observed it also with other CAM-plants (e.g. *Cotyledon orbiculata*).

Phase II (from about day 6 to about day 20) with CO_2 dark fixation maxima continuously shifting towards the end of the dark period coincided with a change from net CO_2 uptake to CO_2 release during the light period, combined with a drastic reduction of transpiration during the same period (most pronounced between day 6 and 9). CO_2 fixation and transpiration in the dark decreased much slower and the WUE was high. In this phase the plant water status deteriorated, the plant attempted to reduce water loss by closing the stomata during the light period (Fig. 3) but could still maintain a fairly high CO_2 gain through dark fixation. Evidently, in this period, the plant benefited mostly
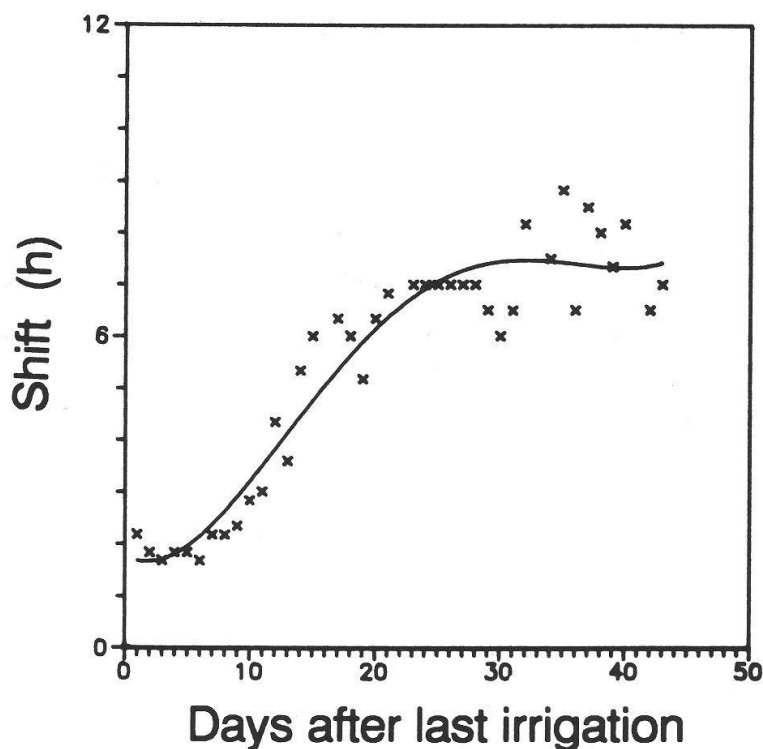
Fig. 2. Time shift of the maximum of CO_2 dark fixation from early to late in the night in a drought-stressed plant of *Senecio medley-woodii*. Curve fitting with the computer software GRAPHER.

from the CAM-feature CO_2 dark fixation combined with stomatal closure during the light period to avoid excessive water loss.

In phase III (from about day 20) CO_2 gain for the 24 hour period was first attenuated (Fig. 3), then decreased steeply and eventually turned into a CO_2 output (day 60). Since transpiration was only a little attenuated, the WUE was markedly reduced. In this period the plant started to shed leaves since *S. medley-woodii* is drought deciduous (von Willert et al., 1992). In this phase the transition from a physiologically active CAM-plant (full CAM and super CAM, after Kluge and Ting, 1978) to a plant in a drought induced resting phase with a minimal CO_2 exchange and transpirational water loss occurred.

Our hypothesis maintains that the CAM-plant *S. medley-woodii* in a good plant water status with a sufficient water supply from the soil has its maximum of CO_2 dark fixation in the first two hours of the night whereas a plant subject to drought shifts this CO_2 fixation maximum with increasing stress (= deteriorating plant water status) towards a time later in the night. We suppose that the magnitude of this time lag is an indicator of the actual plant water status but we cannot yet quantify this interrelationship. We have presented only data of one 60 day experiment but *S. medley-woodii*, in further experiments on water translocation (13 plants, 30 to 90 days of drought, publication of data in preparation), displayed the same 3-phase pattern of CO_2 dark fixation as outlined above. We obtained similar results with the CAM-plant *Cotyledon orbiculata* (Crassulaceae) and we presume this shift of the maximum values of nocturnal CO_2 fixation to represent an indicator for the actual plant water status of all CAM-plants. This hypothesis is supported by results from field investigations by Osmond et al. (1979) and Griffiths et al. (1986). There, drought-stressed *Opuntia inermis* were watered which not only led to an increase of the water content of the cladodes but to a shift of the nocturnal
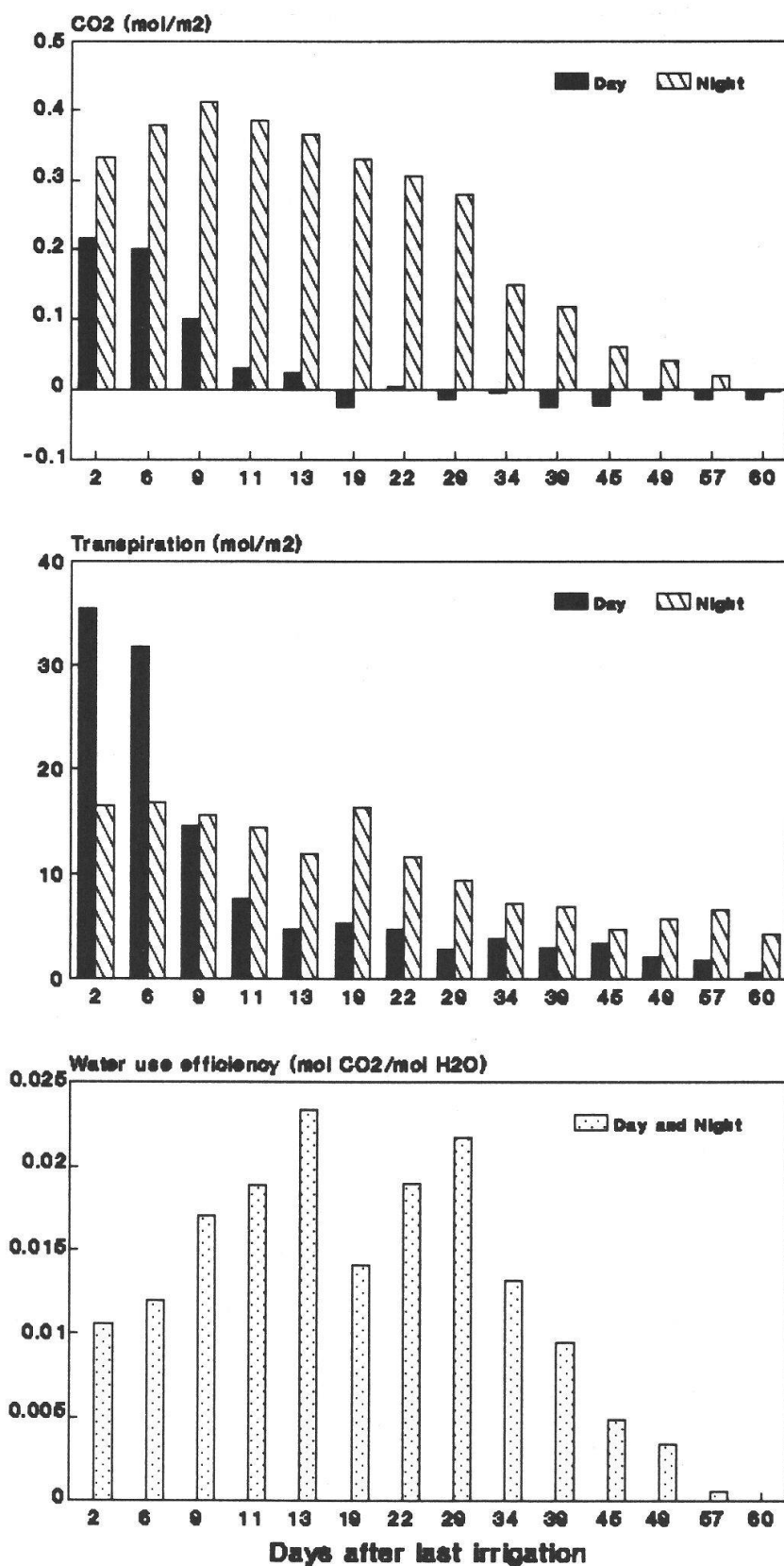
Fig. 3. Modulation of the CO_2 gas exchange, transpiration and values of water use efficiency for a plant of *Senecio medley-woodii* after irrigation was stopped at day 1. Environmental conditions as given in Fig. 1.
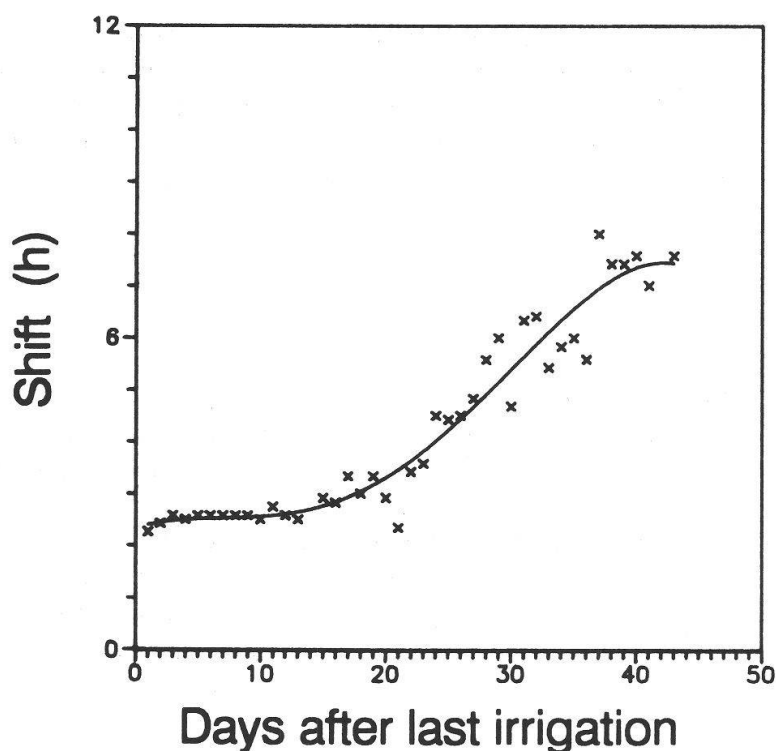
Fig. 4. Time shift of the maximum of CO_2 dark fixation from early in the night to late in the night (data after Ruess et al., 1988). Environmental conditions: Air temperature 17 °C, VPD 390 Pa, photon irradiance 440 µmol m^{-2} s^{-1}, 12/12 h light/dark-period. Curve fitting with the computer software GRAPHER.

CO_2 fixation maximum towards a time earlier in the night. Griffiths et al. (1986) found in field investigations on the CAM-bromeliads *Aechmea aquilegia* and *A. fendleri* that these bromeliads, growing on drier sites, tended to have their CO_2 dark fixation maxima later in the dark period. Smith and Lüttge (1985), in experiments on *Kalanchoe daigremontiana* in plant growth cabinets with controlled environmental conditions also observed that drought-stressed plants had their CO_2 dark fixation maximum later during the night than well watered plants.

One could argue that the shift of the maximum of nocturnal CO_2 fixation towards a later time of night is caused by the high air temperatures (and also the VPD) prevailing until about midnight (Fig. 1). This is definitely not the case since we have already shown this timeshift with ambient air temperature held constant (Ruess et al., 1988). That experiment was also made with *S. medley-woodii* and covered a 77 day drought period, but with different environmental conditions and a large branched plant in a larger plant container. Air temperature was 17 °C and was held constant during the entire 24 h period and over the entire 77 days. VPD was also constant (about 390 Pa) and, if we make the same correlation as in Fig. 2, we obtain a similar 3-phase pattern (Fig. 4) as in Fig. 2. There are differences regarding onset and duration of the three phases resulting from parameters different from the experimental conditions in the experiment above (Fig. 1, 2): phase I lasted longer (until about day 15) since aerial drought was lower. For this experiment IVPD amounted to 33.6 MPa · s wereas IVPD was 62.7 MPa · s for the experiment of Fig. 1. Moreover, the water reserves in the soil were much larger because the container had a soil volume about 15 times that used in the experiment of Fig. 1.

Phase II (from about day 15 to about day 38) lasted 1.6 times longer than in Fig. 2 and this is most probably also a consequence of the lower aerial drought-stress of this investigation. In phase III the time lag is the same as shown in Fig. 2. If data on the 24 h CO_2 gain for this experiment (Ruess et al., 1988, Fig. 5) are compared with the 3-phase pattern of Fig. 4, as was done above (Fig. 2 and 3), we get the same correlation as outlined above.

Conclusions

It is evident from the results presented above and published earlier by different researchers (Osmond et al., 1979; Ruess et al., 1988; Smith and Lüttge, 1986; Griffiths et al., 1986) that the maximum of nocturnal CO_2 fixation in various CAM-plants (*Aechmea aquilegia, A. fendleri, Cotyledon orbiculata, Kalanchoe daigremontiana, Opuntia inermis, Senecio medley-woodii*) tends to shift to a time later in the night if the plants are drought stressed. This shift is not a direct consequence of higher temperatures or higher values of the VPD early into the night (Ruess et al., 1985; Smith and Lüttge, 1985; Fig. 4 above) but a higher IVPD enhances transpirational water loss, and the water status of the plant will deteriorate earlier after the onset of drought compared with lower values of the IVPD (Fig. 2 and 4).

We present the hypothesis that the actual position of the maximal value of CO_2 dark fixation relative to the onset of the dark period is an indicator for the actual plant water status of a CAM plant. We consider a peak value of CO_2 fixation early in the night, at least for *S. medley-woodii*, to be an indicator for a good water status of that plant, or even that this plant is at its best plant water status if the peak value occurs within the first one and a half h of the night. Consequently, a peak value occurring later during the night signals a deteriorated plant water status but we cannot yet quantify the amount of decrease in utilizable water. To this aim, more data based on different environmental conditions are needed.

For the first time we have shown that for *S. medley-woodii* the modulation of transpiration and the changes in the CO_2 fixation pattern, especially during the night, and also the sums of CO_2 fixation during the light, the dark and the 24 h period are linked with the shift of the CO_2 dark fixation peak. We suppose that these correlations also exist with other CAM-plant since the shift of the CO_2 fixation peak also was observed with species of the Bormeliaceae (*Aechmea*), the Crassulaceae (*Cotyledon, Kalanchoe*) and the Cactaceae (*Opuntia*) as outlined above.

The change of the CO_2 dark fixation pattern and the shift of its maximum to a time earlier or later in the night occurs in three phases (Fig. 2 and 4) which correlate with three phases in the changes of CO_2 fixation and transpirational water loss (Fig. 3; see also Ruess et al., 1988). We, therefore, conclude that the time lag from the onset of dark at least for *S. medley-woodii*, is a measure for its water status. Such an indicator could in the future be very helpful for a nondestructive supervision of plant water status.

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