The influence of leaf "windows" on Crassulacean acid metabolism in the South African succulent *Senecio rowleyanus* (Asteraceae)

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Abstract

Net CO_2 exchange rate (P_N) of shoots and diel fluctuations in titratable acidity of leaves of *Senecio rowleyanus* were measured to determine whether penetration of radiant energy through leaf "windows" (narrow, translucent strips on the leaf epidermis) resulted in increased CAM. Nocturnal P_N and nighttime increases in acidity were compared among plants with windows covered with reflective adhesive tape, transparent adhesive tape (to control for potential effects of the adhesive), and no tape. The windows did not significantly enhance the degree of CAM in *S. rowleyanus*.

Additional key words: CO_2 gas exchange; chlorenchyma; water-storage parenchyma.

Introduction

Plant adaptations to arid environments include anatomical, morphological, and physiological modifications. Some of these adaptations maximize water acquisition, increase water-use efficiency, and protect plant tissue from desiccation. One common example of a morphological adaptation that increases the efficiency of water use is a low surface area-to-volume ratio (Shields 1950, Fitter and Hay 1981). Whereas leaves of many succulents are thick, the leaves of *S. rowleyanus* form an almost perfect sphere that exposes a minimum amount of surface area per volume of leaf. Although apparently advantageous in minimizing water loss, this shape may be detrimental by decreasing the leaf surface area available for radiant energy absorption and, as a result, limiting CO_2 exchange. The amount of irradiated leaf surface area in *S. rowleyanus* is further reduced by its growth habit in southwestern Africa. Shoots grow prostrate along the surface of the ground, reducing radiant energy interception by the undersides of the leaves. A morphological adaptation that

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may help compensate for this reduction in radiation interception is a translucent "window" on the leaf surface (Kaul 1977).
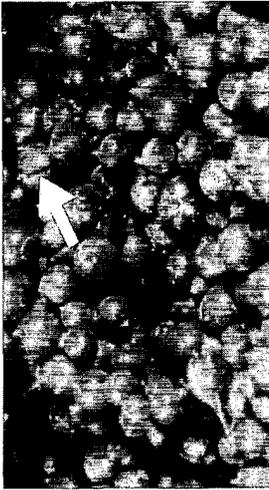

Fig. 1. The crescent-shaped window on the leaf surface of *S. rowleyanus*. Leaf shown is approximately 0.8 cm in diameter.

The leaf window in *S. rowleyanus* is a narrow, translucent, crescent-shaped band of tissue on the epidermis of the adaxial side of the lamina (Fig. 1). The window lacks chlorophyll and stomata and is developmentally an extension of the water-storage parenchyma that fills the interior of the leaf (Hillson 1979). Except at the window, a thin band of chlorophyllous tissue occurs just underneath the epidermis of the pea-shaped leaf. Radiation entering the window is presumably transmitted through the water-storage parenchyma, thereby irradiating the chlorenchyma tissue from the inside of the leaf, in effect increasing the surface area of the leaf irradiated for photosynthesis (Krulik 1980). Radiation penetration into leaves through epidermal windows is not limited to this plant. Species in the southern African genus *Lithops* grow underground with only their leaf tips, containing one or more epidermal windows, exposed for radiation interception. Results from several studies indicate that radiation is transmitted through the window and internal water-storage parenchyma to the photosynthetically active tissue located along the sides of the underground leaves (Eller and Ruess 1982, Eller and Nipkow 1983, Eller and Grobbelaar 1986). Although several studies include speculation regarding potential increases in photosynthesis due to photosynthetic photon flux density (PPFD) penetration through the leaf windows in *S. rowleyanus* (Rauh and Hutchinson 1973, Moore and Langenkamp 1991, Christensen-Dean and Moore 1993), there are no values to support or reject such speculation. Therefore, the objective of this study was to assess whether PPFD penetration through the windows of the leaves of *S. rowleyanus* results in enhanced metabolic activity due to the increased surface area available for PPFD interception. This objective was evaluated by measuring (*1*) gas exchange of attached shoots and detached leaves, (*2*) nocturnal increases in titratable acidity, and (*3*) window orientation relative to source radiation.

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Materials and methods

Plants: Six individuals of *S. rowleyanus* Jacobs (Asteraceae) were purchased from a commercial supplier and divided into 59 pots (5×5 cm). Potting medium was a 1 : 2 ratio of sand and standard greenhouse soil mixture (6 : 2 : 1 : 1, respectively, of soil, peat, vermiculite, and *Perlite*). Plants were grown in a greenhouse from 26 May through 16 June 1995, watered every other day (allowing for drying between waterings), and fertilized weekly with a 0.02 % solution of *Technigro* (*Fisons*, Warwick, NY, USA) fertilizer (20 : 18 : 18; N : P : K). Average environmental conditions in the greenhouse during growth were: 1100 μmol m^{-2} s^{-1} maximum PPFD, although PPFD on most days was lower due to clouds; 28/18 °C day/night air temperatures; and 2.5/0.6 kPa day/night vapor pressure deficit (vpd). To replicate natural growth patterns, shoots extending from the pot were placed on a horizontal, green plastic surface at the same height as the soil level.

Once active growth was observed, some plants were transferred to a growth chamber. Half the plants were placed under an average PPFD of 100 μmol m^{-2} s^{-1} (range: 50 to 150 μmol m^{-2} s^{-1}; designated LL) and the other half under an average PPFD of 455 μmol m^{-2} s^{-1} (range: 400 to 510 μmol m^{-2} s^{-1}; designated HL), both with a 12 h photoperiod. Growth chamber conditions were: 25/19 °C day/night temperatures, and 2.2/0.7 kPa day/night vpd. Plants were watered as described above. Fertilizer was applied monthly with a dilute solution of 18 % of each of total N, P_2O_5, and K_2O (trace elements also included). All plants were growing vigorously when used in the experiments; only plants from the HL treatment were used in the gas exchange and acidity experiments. Plants from the greenhouse were used for the gas exchange experiments using detached leaves.

To prevent radiant energy entry through the windows, silver-colored, reflective adhesive tape (*3M Scotch Silver*, St. Paul, MN, USA) was cut to exactly match each window on each leaf (range: 12 to 30 leaves) of a shoot one week prior to gas exchange. The reflective tape had 0 % transmittance and 94 % reflectance of PPFD measured with an integrating sphere. The one week period was intended to allow depletion of stored saccharides in the leaves that may have resulted from the additional PPFD provided by the windows. As a control for possible effects of the presence of the reflective tape on the leaf epidermis, gas exchange of shoots with leaf windows covered by transparent, adhesive tape (*United Tape Co.*, Cumming, GA, USA; 97 % transmittance) was measured in a second group of plants. A third group of plants was measured with no tape on the windows of their leaves. To examine whether the reflective tape might have increased internal radiation scattering in the leaf, black plastic tape (*3M Scotch Plastic*, St. Paul, MN, USA) was attached to the underside of the reflective tape, and then both were cut to cover the windows on leaves of a fourth group of plants.

Leaf diameters and maximum widths of the windows were measured on leaves used in the gas exchange experiments. Because the leaves of *S. rowleyanus* are nearly perfectly spherical, leaf and window surface areas were calculated using a method similar to that of Krulik (1980).

To mark shoots for determination of growth rates, a fine thread was tied loosely around the base of selected stems in the LL and HL treatments. Shoot measurements, including leaf and window diameters, number of leaves per shoot and shoot length, were recorded after 5 weeks of growth. Window orientation was observed and recorded for 15 new shoots (range 8 to 16 leaves per shoot) grown under the LL conditions in the growth chamber.

Gas exchange: The open gas exchange system, differential infrared gas analyzer, cuvettes, *etc.*, are described in Harris and Martin (1991) and Gravatt and Martin (1992). Gas exchange rates were measured on attached shoots of plants grown in the growth chamber and detached leaves of plants grown in the greenhouse. Six shoots, with lengths ranging from 16 to 23 cm and having 12 to 30 leaves each, were sealed in the gas exchange cuvettes for 6 d. To minimize plant-to-plant variability, gas exchange of shoots with leaf windows covered with reflective tape was measured for 3 d, followed by measurements during the remaining 3- d using the same shoots but with the reflective tape replaced by clear tape. Shoots remained attached to the plant throughout the experiment. Gas exchange of six plants with no tape on the leaf windows was measured for 3 d. In all experiments, cuvette environmental conditions were the same as those in the growth chamber (see above). Ambient CO_2 concentrations ranged from 370 to 400 cm^3 m^{-3}.

To increase the total amount of plant tissue available for CO_2 uptake, gas exchange was also measured using detached leaves (about 102 leaves; range 95 to 119) having windows covered with reflective tape for one week and using leaves with no tape. All leaves were selected randomly from plants in the greenhouse. Leaves were placed in the gas exchange cuvettes with their windows oriented directly toward the light source, and P_N was measured for 3 d. This was repeated three times. Cuvette environmental conditions were the averages of the conditions in the greenhouse during the seven days prior to gas exchange measurements: 32/17 °C day/night temperatures, 2.6/0.8 kPa day/night vpd, 455 μmol m^{-2} s^{-1} PPFD. The CO_2 concentrations in the gas exchange cuvettes ranged from 352 to 386 cm^3 m^{-3}.

In all experiments, gas exchange parameters for attached shoots and detached leaves were calculated according to equations in Šesták *et al.* (1971) and Farquhar and Sharkey (1982) using values from the second day and night of each experiment.

Titratable acidity: Reflective and clear tape was affixed to the leaf windows of different plants in the growth chamber for one week prior to collection as described above. Fifteen leaves for each of the three treatments (reflective, clear, and no tape) were collected at lights-on (09:00 h) and lights-out (21:00 h). Tissue was weighed and then immediately frozen at -15 °C for 24· h. Upon thawing, each leaf was pulverized in deionized water with a mortar and pestle. Acidity of the resultant slurry was determined by titration with 0.001 M NaOH to a pH of 7.0. Dry masses were obtained after evaporation of the slurry and drying at 65 °C for at least 3 d. Acidity is expressed as mmol kg^{-1}(dry mass).

Statistical analysis: Because most sample sizes were small, it was difficult to determine whether or not the data met the conditions necessary for use of parametric statistics. Thus, a resampling (or "bootstrapping") method (Simon 1992), which lacks

assumptions about the nature of the data (Potvin and Roff 1993), was used. In all cases, pairs of means were tested for significant differences ($p \leq 0.05$).

Results and discussion

The presence of nighttime CO_2 uptake and a significant difference between daytime and nighttime tissue acidity (reflective tape, $p = 0.003$; clear tape, $p = 0.015$; and no tape, $p = 0.040$) indicate that *S. rowleyanus* is a CAM plant (Figs. 2 and 3). This
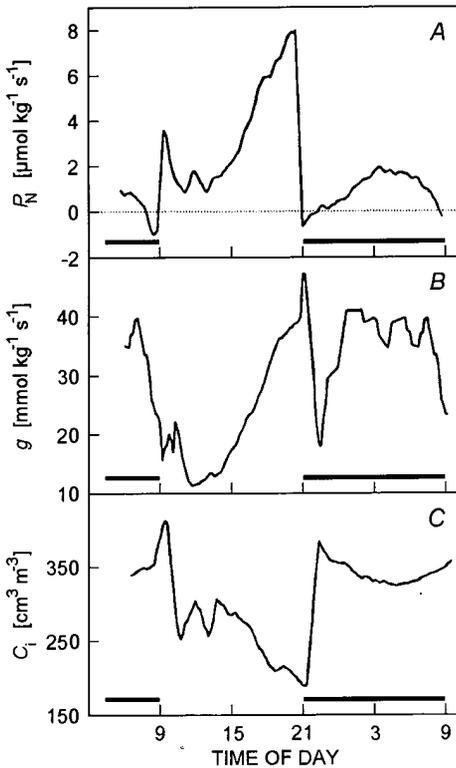

Fig. 2. Net CO_2 exchange, P_N (*A*), stomatal conductance, g (*B*), and leaf internal CO_2 concentration, C_i (*C*) over a 24 h period for an attached shoot of *S. rowleyanus*. Leaf windows on the plant were not covered with tape. Plants were grown for five weeks in a growth chamber (see Materials and methods for environmental conditions during growth and during gas exchange measurements). Values are calculated on a dry mass basis. The horizontal black bars indicate night.

study constitutes the first report of CAM in *S. rowleyanus*, although CAM has been reported previously in other species of this genus (Szarek and Ting 1977, Szarek 1979, Fioretto and Alfani 1988). Although leaf-to-leaf variability was high, there were no significant differences in the change of acidity among the three tape treatments, $p = 0.42$ (Fig. 3). Integrated nighttime and daytime CO_2 exchange of shoots having leaf windows covered with reflective tape did not differ significantly from those of shoots with leaves having no tape or transparent tape (Fig. 4). Because the transparent tape did not alter gas exchange of the shoots, controls in the experiments using detached leaves omitted the transparent tape treatment.

The second set of gas exchange results using detached leaves also indicated that blocking radiation from entering the windows did not result in a decrease in CO_2 exchange (Fig. 5). In fact, the detached leaves with the reflective tape exhibited a statistically significant increase in CO_2 uptake relative to results of the detached
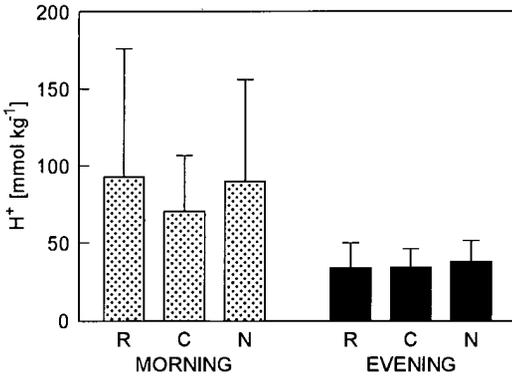

Fig. 3. Titratable acidity of *S. rowleyanus* leaves ($n = 15$) collected in the morning and evening for three treatments: windows covered with reflective tape (R), windows covered with clear tape (C), and windows with no tape (N). Plants were grown for five weeks in a growth chamber (see Materials and methods for environmental conditions during growth). No significant differences ($p > 0.05$) were found among the three morning treatments or the three afternoon treatments. However, significant differences between daytime and nighttime tissue acidities indicate CAM (reflective tape, $p = 0.003$; clear tape, $p = 0.015$; and no tape, $p = 0.040$). Vertical lines extending from the bars represent SD. Values are calculated on a dry mass basis.

leaves without tape. The gas exchange results using the detached leaves with reflective tape on top of black tape indicated no difference from the results obtained
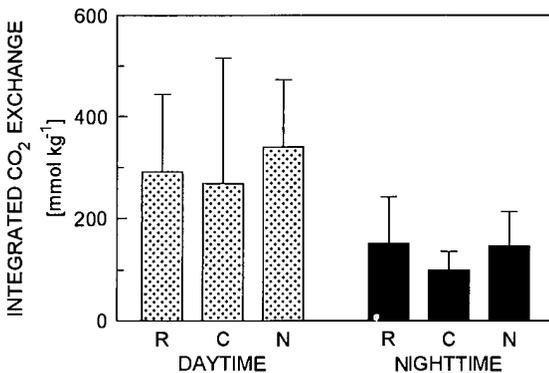

Fig. 4. Mean day and night integrated CO_2 exchange for six shoots of *S. rowleyanus* under three treatments: windows covered with reflective tape (R), windows covered with clear tape (C), and windows with no tape (N). Plants were grown for 5 weeks in a growth chamber (see Materials and methods for environmental conditions during growth and during gas exchange measurements). No significant differences ($p > 0.05$) were found among treatments for either daytime or nighttime values. Vertical lines extending from the bars represent SD. Values are calculated on a dry mass basis.

Table 1. Leaf and window diameters, shoot length, and number of leaves per shoot after 5 weeks of growth of *S. rowleyanus* under low irradiance, LL (PPFD 100 μmol m^{-2} s^{-1}) or high irradiance, HL (PPFD 455 μmol m^{-2} s^{-1}) in a growth chamber. Means of 47 (leaves) or 11 (shoots) samples are shown with SD in parentheses. In all HL to LL comparisons, no means were significantly different ($p > 0.05$), except the number of leaves per node.

PPFD	Leaf diameter [mm]	Window diameter [mm]	Leaves per node	Shoot length [cm]	Leaves per shoot
LL, initial	7.68 (0.68)	0.85 (0.16)	1.00 (0)	33.95 (12.50)	50.31 (23.73)
final	7.64 (0.59)	0.86 (0.21)	0.57 (1.14)	39.30 (12.42)	87.88 (45.60)
HL, initial	7.58 (0.63)	0.87 (0.22)	1.00 (0)	30.59 (16.41)	51.81 (28.70)
final	7.58 (0.57)	0.85 (0.16)	3.04 (1.65)	34.75 (17.10)	96.25 (55.08)

with detached leaves with reflective tape attached (values not shown). Therefore, it appears that the silver reflective tape was not altering the backscattering of radiation within the leaf tissue. Lack of a difference among the treatments substantiates the titratable acidity results, further indicating that radiation penetration through the leaf windows in *S. rowleyanus* does not result in increased CO_2 uptake.
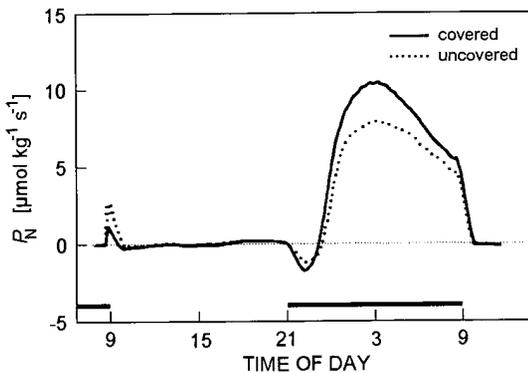

Fig. 5. Mean rates of net CO_2 exchange (P_N) over a 24 h period of three sets of detached leaves (approximately 100 leaves per set) of *S. rowleyanus*. Leaf windows were covered with reflective tape (*solid line*) or had no covering (*dashed line*). (See Materials and methods for environmental conditions during growth and during gas exchange measurements.) A significant difference, $p=0.05$, was found between the reflective tape and no tape treatment. The mean net P_N integrated over the 24 h period for the reflective tape treatments, 285 mmol kg^{-1}, was significantly different ($p = 0.05$) from the mean net integrated P_N, 188 mmol kg^{-1}, of leaves lacking tape. Values are calculated on a dry mass basis. The horizontal black bars indicate night.

In order to determine whether or not leaves orient their windows such that they maximize photon interception, as proposed by Kaul (1977) and Hillson (1979), window orientation relative to the direction of the source of irradiance was measured using LL plants. The apiculate tip of all leaves grew towards the overhead lamps such that the plane of the epidermis bearing the windows oriented vertically, rather than horizontally. This orientation reduced the amount of radiation intercepted. In

addition, our results (Table 1) do not substantiate prior claims that the width of the window increases, resulting in greater irradiation of the leaf interior, when leaves are grown under low irradiance (Rauh and Barthlott 1975, Kaul 1977).

Windows of *S. rowleyanus* constitute only 6.9 % of the total leaf surface area ($n = 24$, range 5.6-8.7 %, SD = 0.55). Thus, the minor influence of the windows on the physiology of the leaves reported here may not be surprising. On the other hand, despite their small size, their relative size is similar to *Lithops olivacea* (7.5 %; $n = 4$, range 6.3-8.4%, SD=0.89), which grows mostly underground. Such windows are presumably important for acquisition of photons in these species (Moore *et al.* 1998).

In summary, this is the first study to assess whether the windows of *S. rowleyanus* increase the amount of radiant energy intercepted and thus increase the activity of CAM in the leaf. Contrary to previous suggestions, *S. rowleyanus* does not orient its leaves such that their windows intercept the greatest amount of available radiation. In addition, the results of this study indicate that CAM was not enhanced by photon penetration through the epidermal windows of *S. rowleyanus*. Furthermore, in one instance, the level of CAM increased following exclusion of photon penetration through the leaf windows. This result is suggestive of possible photoinhibition in leaves with unobstructed windows. Evidence of photoinhibition in CAM plants growing in fully exposed locations has been reported previously (Adams *et al.* 1987a,b, Adams and Osmond 1988, Demmig-Adams and Adams 1992). Further study is necessary to determine if the entry of additional photons into the leaves of *S. rowleyanus* mediated by epidermal windows actually reduces photosynthetic activity.

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