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The influence of leaf windows on the utilization and absorption of radiant energy in seven desert succulents

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Abstract

Four fluorescence parameters [F_v/F_m = the intrinsic efficiency of energy conversion *via* photosystem 2 (PS2); F_v'/F_m' = the efficiency of energy conversion *via* PS2 in the light; P = fraction of absorbed radiant energy utilized for photosynthesis; and D = fraction of absorbed radiant energy dissipated as heat] were measured on leaves of seven species of succulents having epidermal windows. While the function of leaf windows has reportedly been to increase absorption of radiant energy and, hence, the rate of photosynthesis in these species, recent evidence indicates that this translucent portion of epidermal tissue, lacking chlorophyll, may also result in photoinhibition in these species, especially for those with growth habits aboveground. Species with aboveground and belowground growth habits were compared with their leaf windows covered with reflective tape and with windows unobstructed. Results showed no increase in photoinhibition for these species resulting from the radiant energy penetrating the window tissue. Although the efficiency of the photosynthetic mechanism was not significantly influenced by the additional radiant energy provided by the window for individual species, there were significant differences in the efficiencies of radiant energy capture (F_v'/F_m') and utilization (P) between the two growth habits. Species with an aboveground growth habit were less efficient in radiant energy utilization compared with the species having a belowground growth habit.

Additional key words: chlorophyll fluorescence; *Haworthia truncata*; *Lithops olivacea*; *Opthalmophyllum longum*; *Peperomia dolabriformis*; *Peperomia graveolens*; *Peperomia pulchella*; photoinhibition; photosynthesis; *Senecio rowleyanus*.

Introduction

Leaves of certain succulents in five families (Asteraceae, Liliaceae, Mesembryanthemaceae, Piperaceae, and Urticaceae) have translucent portions of epidermal tissue known as "windows" (Jacobsen 1976). These windows occur in a wide range of sizes and shapes among species. This achlorophyllous tissue presumably allows incident radiant energy to penetrate into the succulent water-storage parenchyma (hydrenchyma) that fills the interior of the leaf (Krulik 1980, Moore *et al.* 1998, Egbert 2000). Theoretically, this allows radiant energy to strike both sides (inside and outside the leaf) of the thin, chlorophyllous layer that surrounds the hydrenchyma, thus increasing the surface area available for radiant energy absorption while minimizing the surface area exposed to water loss (Krulik 1980, Moore *et al.* 1998). This additional radiant energy could allow increased rates of photosynthesis in these species (Krulik 1980, Moore *et al.* 1998; but see Egbert and Martin 1999, 2000).

Radiant energy absorption and photosynthetic rates may also be enhanced in these species as a result of the light-cell interactions in the clear, succulent tissue that subtends the window (Terashima and Saeki 1983, Vogelmann 1993). The cellular structure and water content of this tissue may increase backscattering of radiant energy or cause focusing of radiant energy and thus increase the pathlength for radiant energy absorption by the chlorophyllous layer (Vogelmann 1989, Carter 1991, Richter and Fukshansky 1996).

Contrary to the above expectations, recent studies of window-leaved succulents indicated that photosynthetic rates did not significantly decline when radiant energy was prevented from entering the windows (Egbert and Martin 1999, 2000). Furthermore, some of the results obtained indicated that the additional radiant energy provided by the windows resulted in reduced photosynthetic activity.

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Given the unexpected results of the above studies and the general scarcity of information on the physiological role of epidermal windows, the goal of the current study was to use chlorophyll (Chl) fluorescence analysis to determine the fate of radiant energy absorbed through leaf windows in four species of window-leaved succulents. Radiant energy penetration into the leaves through the windows was excluded by covering the windows with reflective tape, and fluorescence parameters were compared to leaves with unobstructed windows.

In addition, fluorescence characteristics of seven species with windows were determined in the chlorophyllous tissue measured through the window and in the same tissue measured directly from the outside of the leaf in order to compare the utilization efficiency of internal *versus* external radiant energy for photosynthesis.

Materials and methods

Species selected represent aboveground (*Peperomia dolabriformis* R. Knuth, *Peperomia graveolens* Rauh & Barth., *Peperomia pulchella,* and *Senecio rowleyanus* Jacobs) and belowground [*Haworthia truncata* Schönland, *Lithops olivacea* L. Bol., and *Opthalmophyllum longum* (N.E.Br.) Tisch.] growth habits. Plants were purchased from commercial suppliers (*Burks' Nursery*, Benton, AR, USA; *Highland Succulents*, Gallipolis, OH, USA; *Living Stones Nursery*, Tucson, AZ, USA; *Sunrise Nursery*, Lawrence, KS, USA) and potted in a medium consisting of 1 part sand to 2 parts standard greenhouse soil mixture (6 : 2 : 1 : 1 of soil, peat, vermiculite, and *Perlite*). For species with a belowground growth habit, soil was added to cover the roots, stems, and leaves, except for the leaf tips where the windows were located. The window leaf tips were thus at the same level as the soil surface. The radiation source only irradiated these plants through the windows, and photosynthetic photon flux density (PPFD) was measured incident on the window surface. Plants were grown for a minimum of two weeks prior to experimentation in a growth chamber with a 14 h photoperiod, 30/20 °C, and 2.5/0.9 kPa day/night air temperature and vapor pressure deficit, and 340 μmol m^{-2} s^{-1} average PPFD (range: 315 to 500 μmol m^{-2} s^{-1}). Plants were watered twice a week and fertilized monthly with a dilute solution of 18 % of each of total N, P_2O_5, and K_2O (trace elements also included).

For leaf temperature measurements, windows were covered with reflective tape (see below) one day prior to measurements. A shaded temperature sensor was held to the non-window leaf side for one minute prior to recording the temperature. Temperatures were measured on leaves without the tape treatment and those with the tape treatment at mid-day (14:00 to 15:00), the end of the day (21:00 to 22:00), and the end of the night (07:30 to 08:30).

The PPFD penetrating the leaf tissue of *L. olivacea* was measured with a *LI-190SB* quantum sensor and *LI-COR LI-185B* light meter (Lincoln, NE, USA). A detached leaf with only the apical window exposed to the radiation source was placed on top of the light sensor. Half-centimeter cross-sectional slices were then removed starting from the basal end of the leaf. All leaves and leaf slices completely covered the light-sensitive portion of the sensor. The last slice of tissue included only the apical portion of the leaf constituting the window. Because the window is surrounded by chlorophyllous leaf tissue, a small amount of green tissue was included with the window tissue. Chl content of the cross-sectional slices of leaf tissue was also measured (Šesták *et al.* 1971). Leaf water content was determined by weighing leaf slices before and after drying for 3 d at 65 °C.

Chl fluorescence was measured with a *PAM-2000* portable fluorometer (*Walz*, Effeltrich, Germany) using procedures described by Demmig-Adams *et al.* (1996). An attached leaf at the end of the night was used to measure F_0 and F_m. F_v was then derived from $F_m - F_0$. Plants were returned to the growth chamber, and then the same leaves were used at mid-day to measure F, F_m', and F_0' under the same PPFD as in the growth chamber. The equations of Demmig-Adams *et al.* (1996), $P = (F_m' - F)/F_m'$ and $D = 1 - (F_v' - F_m')$, were used. The fluorescence sensor was typically positioned so that fluorescence was measured from above the leaf through the clear window. In addition, fluorescence was measured directly from the green tissue on the sides of the leaves. For the latter measurements on species with a belowground growth habit, a hole was cut in the side of the pot and the soil removed to expose the side of the leaf to the fluorescence sensor.

To prevent penetration of radiant energy into the leaves through the windows, silver, reflective adhesive tape (*3M Scotch Silver*, St. Paul, MN, USA) was cut to exactly match each window on each leaf and affixed one week prior to testing. The reflective tape had 0 % transmittance and 94 % reflectance of PPFD measured with an integrating sphere.

Because the sample sizes were small, a re-sampling ("bootstrapping") statistical method (Simon 1992), which lacks assumptions about the nature of the data (Potvin and Roff 1993), was used to compare means. In all cases, significant differences among means were inferred when $p < 0.05$.

Results and discussion

Because covering the windows with tape reduces the amount of radiant energy entering the leaf (Egbert 2000) it was predicted that leaves with unobstructed windows would be warmer than those with their windows covered. Obstructing the windows, however, did not cause a significant change in leaf temperatures in any of the species examined (values not shown).

PPFD decreased with increasing depth inside leaves of *L. olivacea* (Fig. 1*A*). One third of the incident radiant energy penetrated immediately below the unobstructed

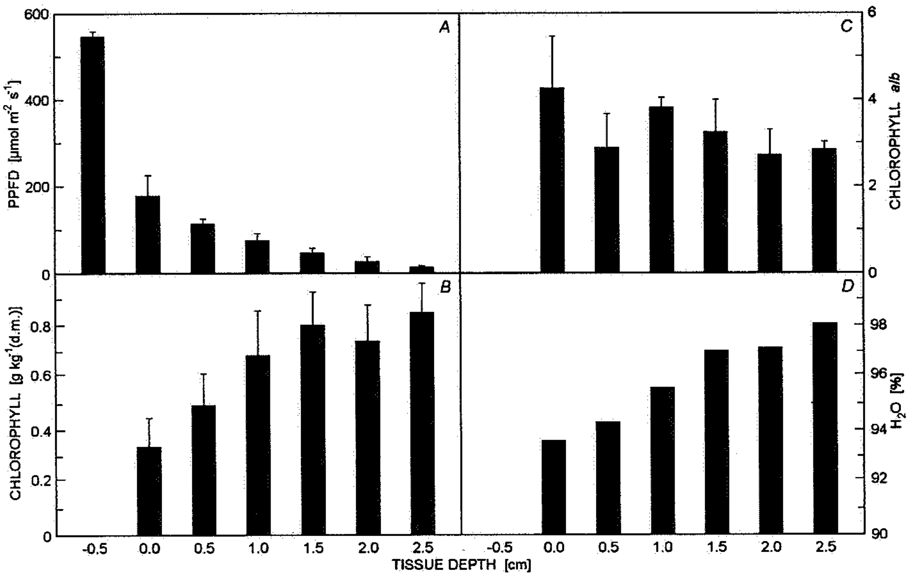

Fig. 1. PPFD (*A*), chlorophyll content (*B*) and *a*/*b* ratio (*C*), and leaf water content (*D*) in cross-sectional slices of *Lithops olivacea* from the top of the leaf (0), to the base (2.5 cm). *n* = 3 plants. *Vertical lines extending from bars* represent standard deviations. Standard deviations in panel D are < 0.01.

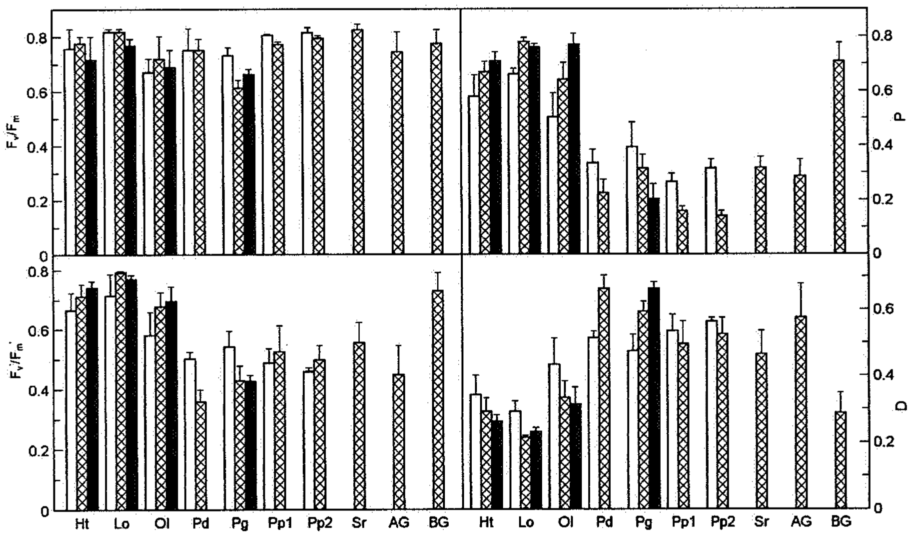

Fig. 2. Mean chlorophyll fluorescence values for seven plant species with leaf windows. F_v/F_m = intrinsic photosystem 2 (PS2) efficiency measured at the end of the night; F_v'/F_m' = actual PS2 efficiency measured mid-day; P = $(F_m' - F)/F_m'$ the fraction of radiant energy in PS2 utilized by photosynthesis; and D = $1 - (F_v' - F_m')$ the fraction of radiant energy in PS2 thermally dissipated. When measuring irradiance-adapted leaves, PPFD was the same as used in the growth conditions. *Haworthia truncata* (Ht), *Lithops olivacea* (Lo), *Opthalmophyllum longum* (Ol), *Peperomia dolabriformis* (Pd), *Peperomia graveolens* (Pg), *Peperomia pulchella*, older leaves (Pp1), *Peperomia pulchella*, younger leaves (Pp2), and *Senecio rowleyanus* (Sr). *Open bars* are measurements made through the windows (from above), *hatched bars* are measurements made directly on the chlorenchyma of the leaf tissue (from the leaf side), and the *solid bars* are measurements made on non-window leaf tissue when radiant energy provided by the window was excluded. *n* = 4 plants for each species. *Lines extending from the bars* indicate standard deviations. Sample sizes for the aboveground (AG) and belowground (BG) growth habits were *n* = 12 plants.

window, while only 4 % of the incident radiant energy remained at a depth of 2.5 cm.

At a depth of 1.0 cm, where only 41 % of the incident PPFD remained, there was a significant increase in Chl concentration ($p = 0.03$) relative to the Chl concentration of the top 0.5 cm (Fig. 1*B*). Chl *a/b* ratios were not significantly different in *L. olivacea* at the various depths (Fig. 1*C*). An increase in Chl concentration is a typical response to decreasing PPFD in most plants, whereas Chl *a/b* ratios may or may not change as growth PPFD changes (Björkman 1981, Boardman 1977). Accompanying the increase in Chl content, there was also a significantly greater water content ($p = 0.04$) in the leaf tissue below 1.0 cm relative to the top portion of the leaf (Fig. 1*D*). A higher water content may increase radiant energy focusing in the bottom portion of the leaf where PPFD are greatly reduced (Vogelmann 1993). Other studies have shown increased radiant energy scattering and attenuance in the spongy parenchyma of leaves compared to the palisade parenchyma (Terashima and Saeki 1983, Vogelmann *et al.* 1988).

After covering the windows reduced the penetration of radiant energy into the leaf, F_v/F_m and F_v'/F_m' were not significantly different from values for leaves with unobstructed windows for any of the species examined: *H. truncata, L. olivacea, O. longum*, and *P. graveolens* (Fig. 2). These results indicate that the additional radiant energy provided by the windows did not cause photoinhibition in these four species with leaf windows under the environmental conditions in this study.

P and D fractions of radiant energy were also not significantly different in leaves with obstructed and with unobstructed windows in *H. truncata* and *O. longum* (Fig. 2). In other words, the radiant energy provided by the windows did not significantly change the percentage of radiant energy utilized in photosynthesis or thermally dissipated in these two species. In contrast, P decreased and D increased in *L. olivacea* (CAM; Egbert and Martin 2000) and in *P. graveolens* (CAM with some daytime CO_2 uptake under well-watered conditions; Egbert and Martin 2000) when radiant energy was excluded from the leaf by covering the window compared to leaves with unobstructed windows (Fig. 2). Thus, blocking the windows apparently decreased the percentage of radiant energy utilized in photosynthesis and increased the percentage of energy thermally dissipated. Because there are no stomata located on the windows, CO_2 uptake was not inhibited by covering the windows. Reasons for these unusual results are presently unclear.

The proportion of total leaf surface area occupied by the window (Table 1) is similar for the three belowground species. In contrast, the species with an aboveground growth habit have a wide range of window sizes. Regardless, *P. graveolens* (with one of the largest windows per leaf surface area) responded similarly to *L. olivacea* when the windows were covered (Fig. 2).

Table 1. Percent of the total leaf surface area occupied by window tissue in four succulents. *n* = number of leaves.

Species	*n*	Mean % total leaf surface area occupied by the window	Standard deviation	Range
Haworthia truncata	14	5.92	2.87	3.20-12.90
Lithops olivacea	8	7.18	0.86	6.00-8.40
Opthalmophyllum longum	20	7.71	1.90	5.20-9.10
Peperomia graveolens	100	19.30	8.80	<1.00-30.00

The intrinsic efficiency of energy conversion in PS2, F_v/F_m, was significantly greater in the three *Peperomia* species when the leaf was measured through the window (above the leaf) compared to measurements taken directly from the chlorenchyma tissue on the side of the leaf (Fig. 2). In addition, a greater F_v'/F_m' was observed in *P. graveolens* and a larger P (fraction of photons used in photochemistry), accompanied by a smaller D, was measured for *P. dolabriformis* and *P. pulchella* when fluorescence was measured through the window compared to measurements taken directly from the chlorenchyma (Fig. 2). In all but one species with a belowground growth habit, Chl fluorescence parameters were the same when measured through the window or from the chlorenchyma directly. In *L. olivacea*, F_v'/F_m' and P were significantly smaller, and D was significantly greater, when the tissue was measured through the window compared to measurements made directly from the chlorenchyma (Fig. 2).

Despite the different leaf morphologies between older (glabrous and thick) and younger (heavily pubescent and thin) leaves of *P. pulchella*, all the Chl fluorescence parameters were the same except F_v/F_m, which was significantly lower in older leaves when comparing the abaxial surface to the adaxial surface ($p = 0.02$) (Fig. 2).

When the species were segregated according to growth habit, a distinct difference in the efficiency of utilization of radiant energy was observed. F_v/F_m values were the same for species with aboveground and belowground growth habits, however, the remaining three Chl fluorescence parameters differed significantly (Fig. 2). While the architecture of species with aboveground growth habits may result in self-shading, these plants clearly receive more incident radiant energy then the belowground species. The mean mid-day F_v'/F_m' value for aboveground species (0.43) was significantly lower than the mean of the belowground species (0.72). In addition, the species with belowground growth habits utilized a much larger fraction of radiant energy for photosynthesis (70 %) relative to the aboveground species (28 %), and the aboveground species dissipated more radiant energy (57 %) than did the belowground species (28 %). These findings may reflect an adaptive response of species with a belowground habit associated with a lower availability of radiant energy below the soil, relative to the levels available to species with an aboveground growth habit. The species with aboveground growth habits appear to be saturated with photons at mid-day based on their low F_v'/F_m' values and the low percentages of radiant energy utilized for photosynthesis (Fig. 2). Similarly, Nobel *et al.* (1994) reported that the outermost leaf tissue was PPFD-saturated and exhibited photoinhibition in the desert succulent *Opuntia ficus-indica.*

Leaves of species with an aboveground growth habit had a greater percent water content (95.3 %) compared to the species with a belowground growth habit (89.8 %). Therefore, radiant energy focusing or scattering may increase the radiant energy available inside the leaf and, hence, the probability of photoinhibition within the leaves of the aboveground species (Egbert 2000). While no evidence of photoinhibition was found in the aboveground species, *P. graveolens*, some evidence for photoinhibition was reported by Egbert and Martin (1999) in *Senecio rowleyanus*, another species with an aboveground growth habit.

In summary, the radiant energy penetrating the leaves *via* the windows does not cause photoinhibition in *H. truncata*, *L. olivacea*, *O. longum*, and *P. graveolens*, although photosynthesis in *P. graveolens* was apparently PPFD-saturated at mid-day. The species with a belowground growth habit had greater efficiencies of radiant energy capture and utilization compared to the species with an aboveground growth habit, reflecting the lower PPFD available to succulents growing primarily below ground.

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