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The influence of epidermal windows on the light environment within the leaves of six succulents

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Abstract

An omni-directional fibre optic microprobe was used to measure the quantity and quality of light within the leaves of six succulents having epidermal windows, three species having a subterranean growth habit (*Haworthia truncata, Lithops olivacea,* and *Opthalmophyllum longum*) and three growing above ground (*Peperomia dolabriformis, P. graveolens,* and the sprawling vine *Senecio rowleyanus*). Although light levels at most locations inside the leaves of all species were high, near those incident on the window surfaces, light levels inside the leaves of the two species of *Peperomia* often greatly exceeded incident light levels, indicating considerable light scattering and focusing by the leaf tissue. The spectral quality of light inside the leaves of all taxa reflected the absorption properties of chlorophyll, with most of the photons in the green wavelengths. Light quality and quantity inside the leaves did not correlate with the growth habit of the plants, the size of the window (as a proportion of the total leaf area), or location inside the leaf, although light levels generally declined and wavelengths increased deeper in the leaves. Application of reflective tape to the windows reduced internal light levels in *L. olivacea* and *S. rowleyanus*, although reductions were not always statistically significant. Although light levels throughout the leaves of *P. graveolens* were substantially and significantly reduced as a result of the application of reflective tape to its windows, the light levels even at the basal chlorenchyma on the abaxial side of the leaf remained high. In all species investigated, the levels of near-infrared radiation inside the leaves were surprisingly high, yet also declined deeper inside the succulent leaves. This near-infrared radiation may add to the heat load of these plants. Furthermore, application of reflective tape to the windows also reduced the amount of near-infrared radiation inside the leaves of the three succulents examined. These results led to a novel, testable hypothesis that may help to explain previous findings that application of reflective tape to the windows of the leaves of these succulents did not effect a reduction in photosynthetic activity.

Key words: Chlorenchyma, *Haworthia truncata*, hydrenchyma, infrared radiation, internal light environment, leaf windows, light focusing and scattering, *Lithops olivacea*, omni-directional fibre optic microprobe, *Peperomia dolabriformis*, *Peperomia graveolens*, *Opthalmophyllum longum*, photon flux density, *Senecio rowleyanus*, succulents, visible radiation.

Introduction

In the past two decades, it has been possible to measure the light environment (quantity and quality) within leaf tissues directly by using optical microfibres (Vogelmann *et al*., 1988, 1989, 1996*a*; Vogelmann, 1989, 1993; Cui *et al*., 1991; Martinez v Remisowsky *et al*., 1992*a*). Several such studies have also included measurements of the photosynthetic responses to variations in the leaf internal light environment (Terashima and Inoue, 1984; Terashima and Saeki, 1985; Poulson and Vogelmann, 1990; Myers *et al*., 1994; Vogelmann *et al*., 1996*b*; Evans and Vogelmann, 2006). Light quantity and quality are altered inside a leaf as a result of the absorption and scattering of photons by cells and their adjacent air spaces. Reflection and scattering can result in a longer photon pathlength allowing increased chances for absorption by the chlorophyllous tissue (Vogelmann, 1993). Furthermore, internal light levels can be increased over 4-fold

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from those impacting the leaf as a result of focusing by the curved surfaces of leaf cells (Martinez v Remisowsky *et al.*, 1992*a, b*; Seyfried and Fukshansky, 1983; Martin *et al.*, 1989; Poulson and Vogelmann, 1990; Richter and Fukshansky, 1996; Vogelmann *et al.*, 1996*a*, 1989).

For desert succulents with uncommonly thick leaves or stems, the opportunities for tissue-light interactions are enhanced as a result of longer path lengths for light inside the tissue of these thick leaves, relative to those in thinner leaves, potentially resulting in pronounced effects on the photosynthetic capacity of these plants (Pearman, 1966; Sinclair and Thomas, 1970; Ehleringer, 1981; Nishio *et al.*, 1993). This may be especially important in succulents with leaf 'windows', which are transparent or translucent sections of epidermis on the adaxial surfaces of the leaves of certain succulents and which are especially prominent in a variety of South African succulents with a subterranean growth habit, for example, species of *Lithops, Conophytum, Frithia, Haworthia*, *Opthalmophyllum,* etc. (Dugdale, 1970; Hillson, 1979; Krulik, 1980; Simpson and Moore, 1984; Moore and Langenkamp, 1991). These windows are often the only portion of the plant visible at the surface of the ground. They potentially allow light to penetrate the thick, underground leaves, illuminating the green photosynthetic tissue, or 'chlorenchyma', below. Leaf windows are typically subtended by clear water-storage parenchyma, or 'hydrenchyma'. The chlorenchyma usually lies below or often surrounds the hydrenchyma (often below and along the sides of the leaf). Windows also occur on leaves of other species of succulents that grow above ground. These windows have been considered an adaptation that, in effect, increases the surface area of the leaf for light absorption and, hence, photosynthesis, by exposing two sides (internal and external) of the chlorenchyma layer to light (Rauh and Hutchinson, 1973; Christensen-Dean and Moore, 1993; Moore *et al.*, 1998). Although leaf windows in both types of plants provide light to the chlorenchyma layer deep inside the leaf, potentially enhancing photosynthetic rates, recent evidence indicates that such windows may allow too much light inside the leaf, resulting in apparent photoinhibition (Egbert and Martin, 1999, 2000; but see Egbert and Martin, 2002). The latter puzzling finding is based on experimental evidence in which photosynthetic activity increased, or did not decrease, when the amount of light penetrating the leaf windows was presumably minimized by covering the windows with reflective tape (Egbert and Martin, 1999, 2000). On the other hand, no direct evidence for reductions in leaf internal light levels was presented by Egbert and Martin (1999, 2000, 2002). Given the possibility of light focusing by cells inside the leaf, as well as light scattering by illuminated cells near the untaped portions of the leaf surfaces (Martin *et al.*, 1989; Poulson and Vogelmann, 1990; Vogelmann *et al.*, 1996*a*), it is possible that the reflective tape did not substantially reduce the light levels inside the leaves in the previous studies, thereby possibly explaining the lack of reduced photosynthetic rates after covering the leaf windows with reflective tape. The use of fibre optic microsensors allows direct measurement of the light levels in the photosynthetic tissues of window-leaved succulents before and after application of reflective tape, potentially providing greater insight into the results obtained by Egbert and Martin (1999, 2000, 2002).

The fibre optic microsensors used in the studies cited above sense light primarily from one direction (radiance type sensor). By contrast, omni-directional fibre optic probes sense light from all directions simultaneously and have been used for studies of the three-dimensional nature of light inside animal tissues (Cheong *et al.*, 1990) and inside microbial benthic mats (Lassen *et al.*, 1992). Until now, such sensors have been too large for use in most plant tissues. The unusually thick leaves of succulents, however, allow the use of these sensors to characterize the three-dimensional nature of the light environment (quantity and quality) within such leaves. Such omni-directional measurements should provide greater insight into the effect of windows, as well as their blockage, on the internal light environment of these succulent leaves.

In this study, the quantity and quality of the light inside the leaves of six species with leaf windows were measured with an omni-directional fibre optic sensor. Three of the species investigated, *Haworthia truncata, Lithops olivacea,* and *Opthalmophyllum longum*, exhibit a subterranean growth habit and have thick, succulent leaves with large, distinct, adaxial windows that are typically the only part of the plants visible above ground. Nearly the entire adaxial surface of the leaves of *Peperomia graveolens* and *P. dolabriformis*, terrestrial species native to South America, constitute one large window. By contrast, the window on the spherical leaves of the trailing vine *Senecio rowleyanus* is a narrow crescent. In addition to describing the quantity and quality of light at various locations inside the leaves of the six window-leaved succulents, two specific questions were addressed in this study: (i) do larger windows allow more light into the leaf ('larger' defined as occupying a greater proportion of the leaf surface area)?; and (ii) does the application of reflective tape to the window substantially reduce light levels inside the leaf?

Materials and methods

Plant material

Six species with highly succulent leaves having adaxial 'windows' were used in this study. *H. truncata, L. olivacea,* and *O. longum* are succulent herbs that grow naturally with all but the tops of their leaves underground and are native to arid regions of southern and western South Africa (Jacobsen, 1960), *S. rowleyanus* is a sprawling terrestrial vine native to the same general region (Jacobsen, 1960),

while *P. graveolens* is a terrestrial herb with succulent leaves and is native to Peru (Rauh and Barthlott, 1975), and *P. dolabriformis* also has succulent leaves and is a small terrestrial shrub native to Ecuador (Jacobsen, 1960). Plants were purchased from commercial suppliers (Burks' Nursery, Benton, AR; Highland Succulents, Gallipolis, OH; and Living Stones Nursery, Tucson, AZ) and grown prior to experimentation for a minimum of 5 weeks in a growth chamber with a 14/10 h photo- and thermo-period, 30/20 °C and 2.5/0.9 kPa average day/night air temperature and vapour pressure deficit, and average photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} (range at plant level: 330–550 μmol m^{-2} s^{-1}). Plants were watered twice a week, and fertilizer [a dilute solution of 18% of each of total N, P_2O_5, and K_2O (trace elements also included)] was applied monthly. All plants were growing vigorously before use in the experiments.

Positioning of the plants during measurements

Detached leaves were used for the internal light measurements with the three species having above-ground growth habits, whereas whole plants were used for the three species with below-ground growth habits. For the latter, each plant was uprooted and held suspended by a clamp. In all cases, leaves were held in their natural orientation for the internal light measurements. When measuring the subterranean species, the vertical leaf surfaces, which are normally buried in the soil, were shielded from the light source by wrapping the sides of the leaves (leaving the top exposed) with aluminium foil. This shielding was provided to test the effectiveness of the reflective tape in preventing light penetration through or around the window and the leaf surface areas surrounding the window. No shielding was used for the leaves of the species with above-ground growth habits.

Light measurements inside the leaves with the optic probe

An omni-directional fibre optic probe (constructed according to Garcia-Pichel, 1995) was used to measure incident light within 5% accuracy (Fig. 1) on all surfaces of the light-sensitive glass sphere (approximately 1 mm diameter; see Vogelmann *et al.*, 1991). The probe was calibrated in air and then in water with known light levels and wavelengths as illustrated in Fig. 1 before each set of leaf measurements. Light levels at each wavelength were then measured just above the leaf before measurements were made inside the leaf. To prevent damage to the probe upon insertion in the leaves, the probe was placed inside a 16-gauge stainless steel needle (1.3 mm diameter) which was then inserted into the leaf at a 45° angle from the light source for all species, except *S. rowleyanus* (Fig. 2). Insertion of the apparatus at an angle avoided light piping down the needle. For most species, the needle containing the probe was first inserted into the adaxial surface at the edge of the window, then pushed down and through the central hydrenchyma, then into the chlorenchyma tissue at the base of the leaf. As the needle/probe was retracted, light was measured every 2 mm, but data are presented only for three locations: the basal chlorenchyma, the centre of the leaf hydrenchyma, and the edge of the window (Fig. 2). Light readings were made as the needle/probe was retracted from the tissue, as opposed to during insertion, for two practical reasons: (i) water or cellular debris was less likely to clog the open end of the needle, and (ii) the spherical sensor of the probe at the needle tip was less susceptible to damage.

The pea-shaped leaves of *S. rowleyanus* are small and spherical, each having a narrow, crescent-shaped window. Whereas the light measurements at the basal chlorenchyma as described above determined the maximal light available to photosynthetic tissue deep within the tissue directly below the window in the other succulents, the shape of the leaves and windows of *S. rowleyanus* provided an opportunity to measure the minimal amount of light

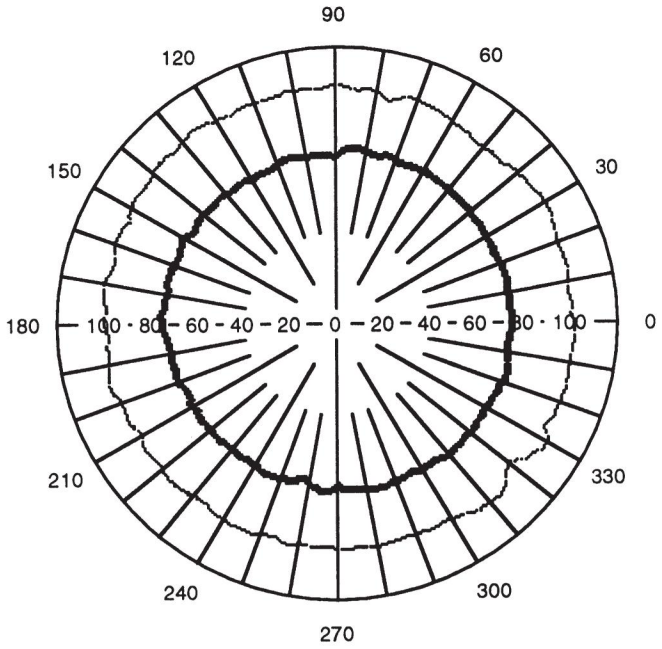

Fig. 1. Example of a calibration curve for the omni-directional fibre optic microprobe. The probe was rotated around a stationary light source to measure its accuracy in sensing incident light. Equatorial readings refer to measurements of probe sensitivity as light was applied to different points along an imagined equator. The equatorial readings of the sensor are represented by short dashes, and the polar readings of the sensor are represented by bold dashes. The numbers across the middle of the circle represent the percentage of incident light measured. The equatorial readings show accuracy of 95% (deviations along the 100% mark indicate minor imperfections in the spherical sensor of the probe). The outer numbers represent the angle from the light source. Polar readings are perhaps more accurately called 'latitudinal readings' in which probe sensitivity was measured as light was applied to the north pole and the orientation of the probe changed such that sensitivity was measured as light followed an imaginary line going from the north to the south pole. Collimated light was focused on the equator of the probe sensor, so the polar readings of incident light decrease accordingly due to the shape of the sphere. The accuracy at the poles also shows less than a 5% error in detecting incident light (deviations along the 80% mark also indicate minor imperfections in the spherical sensor). The probe was calibrated first in air, then in water, prior to each set of leaf measurements.

available to the chlorenchyma inside a succulent leaf along the lateral sides of the leaf. Thus, the probe was inserted at a 90° angle from the light source into the chlorenchyma along one side of leaf, then into the middle of the leaf hydrenchyma. Then, the probe was retracted, and light was measured in the chlorenchyma tissue just inside the leaf epidermis near the point of insertion (Fig. 2). The latter measurement was repeated for the opposite side of the spherical leaf. Thus, there are two 'basal' (actually lateral) measurements for this species. Light levels were not measured near the narrow window in *S. rowleyanus*.

Windows of all leaves were positioned perpendicularly to a 150 W xenon arc lamp (Bornman and Vogelmann, 1988; Martin *et al.*, 1989), and the probe was inserted as described above (Fig. 2). PPFD incident on the leaves for all measurements was approximately 570 μmol m^{-2} s^{-1} (at the top of the round probe; PPFD at the bottom part of the probe, facing away from the light source, was approximately 170 μmol m^{-2} s^{-1}). The distal end of the optical fibre was coupled to a triple grating spectrograph (SpectraPro 275, Action Research, Acton, MA), and leaf spectra were captured with a cryogenically cooled CCD camera (CH270 camera head, CF200A

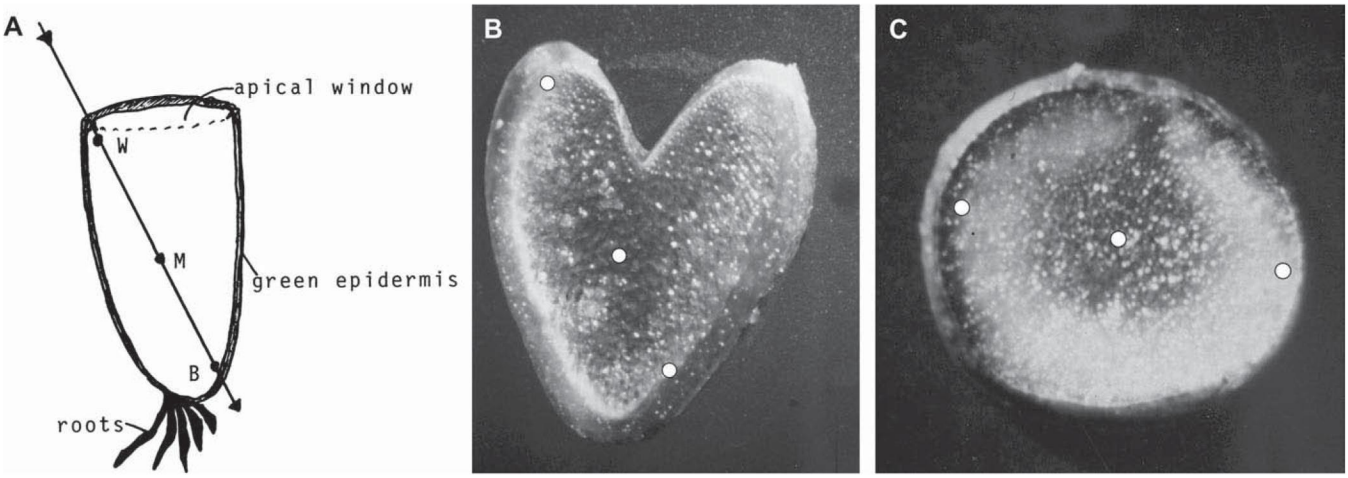

Fig. 2. The three locations at which light measurements were made within a succulent leaf of five of the six species of succulents (A, B), except *S. rowleyanus* (C). The probe was inserted into the leaf at a 45° angle from the light source. In (A), 'W' indicates the location of measurements 0.2 mm beneath the epidermis adjacent to the window margin; 'M' indicates measurements in the middle of the leaf in the hydrenchyma; and 'B' indicates measurements at the basal part of the leaf just above the chlorenchyma layer, 0.2 cm before exiting the leaf. In *S. rowleyanus*, the probe was inserted at a 90° angle, and 'M' was measured as above; two 'B' measurements were made just inside the chlorenchyma at both sides of the leaf, and a 'W' measurement was not made. In (B) and(C), the locations of the light measurements are indicated by white dots. In all figures, the leaves are approximately 1 cm wide.

16/40 camera electronics unit, AT 200 controller board; Photometrics, Tucson, AZ). Light quantity, measured as energy, but converted to quanta, and quality (spectral range from 250 nm to 950 nm) inside the leaves were measured as described above (data given for three locations for each species) and recorded using a computerized data acquisition system.

Effectiveness of reflective tape in blocking light penetration through the windows

To test the effectiveness of covering the window with reflective tape in preventing light penetration into the leaf interior, the quantity and quality of light inside the leaves of *L. olivacea, P. graveolens,* and *S. rowleyanus* were measured as above but with the window covered with silver, reflective adhesive tape (3M Scotch Silver, St Paul, MN), cut to match the shape of the window exactly. The reflective tape transmitted 0% and reflected 94% of the incident PPFD (measured with an integrating sphere). The same leaves were measured with and without the reflective tape for *L. olivacea*, whereas different leaves of the same plant were used for the two measurements for the other two species.

Statistical analyses

Three plants per species were used for all measurements. Means for plants with and without reflective tape affixed to their windows were compared using the Student's *t* test. Window area:leaf area ratios were compared among the six species with an Analysis of Variance, coupled with the Tukey Pairwise Multiple Comparison Procedures test (Sokal and Rohlf, 1981). All statistical tests were performed with the SigmaStat (SPSS, Inc., Chicago, IL) software package. Statistically significant differences between or among means were inferred when $P < 0.05$.

Results and discussion

Light levels inside the leaves

The amount of light, expressed as the ratio of internal to external (incident) PPFD ($PFD_{int/ext}$), at nearly all wavelengths decreased with depth inside the leaves of all species; however, the degree of reduction with depth varied among the species (Fig. 3; Table 1). The maximum $PFD_{int/ext}$ (primarily green wavelengths around 550 nm) inside the leaves of the subterranean species (Table 1; Fig. 3) ranged from about 0.7 to nearly 1.5 at 0.2 cm, immediately below the window margin, indicating that nearly all the incident light penetrated into the leaf tissue adjacent to the window margin. This was true, however, only for light in the green portion of the visible radiation spectrum (Table 1; Fig. 3), while the amounts of blue and red light inside the leaves near the window were considerably lower (Table 1; Fig. 3). Values of $PFD_{int/ext}$ exceeding 1.0 indicate light levels inside the leaves greater than the light levels impacting the external surfaces of the leaf windows, reflecting light focusing by epidermal or subepidermal cells or concentration through light scattering. Such focusing and scattering are not uncommon in leaves of other, non-succulent taxa (Martin *et al*., 1989; Vogelmann, 1989, 1993; Poulson and Vogelmann, 1990; Myers e*t al.,* 1994; Vogelmann *et al*., 1996*a*). Light levels were substantially reduced in the hydrenchyma tissue at the centre of the leaves (1.2–1.5 cm from the adaxial window). Relative to the irradiance incident on the leaves, reductions in light levels in the hydrenchyma were over 90% in *H. truncata*, nearly 70% in *L. olivacea*, and almost 40% in *O. longum* (Table 1). Very little light penetrated to the basal chlorenchyma of the leaves (2.4–2.9 cm deep in the leaves) in *H. truncata* and *L. olivacea*, while light levels at the basal chlorenchyma in the leaves of *O. longum* were still almost one-quarter or even greater than one-half, depending on the wavelength, of levels incident on the leaves (Table 1; Fig. 3).

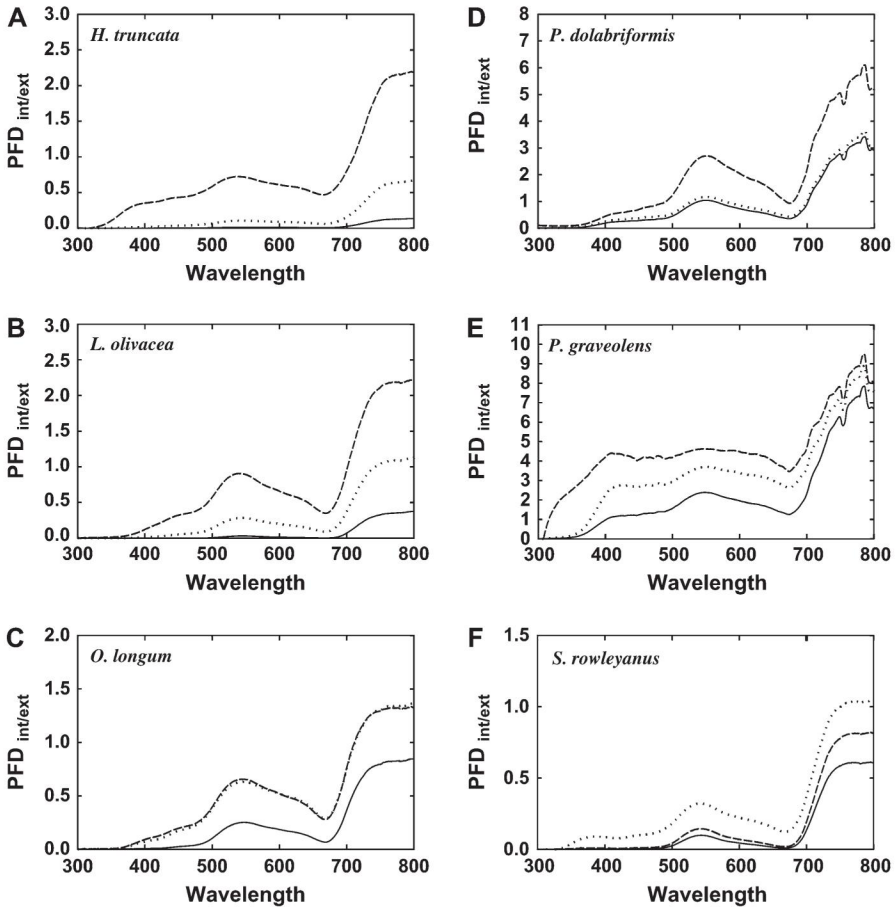

Fig. 3. The amount of light, expressed as the ratio of internal to external (incident) PFD ($PFD_{int/ext}$), for each wavelength from 300 nm to 800 nm at three different locations inside a succulent leaf of six succulents *H. truncata* (A), *L. olivacea* (B), *O. longum* (C), *P. dolabriformis* (D), *P. graveolens* (E), and *S. rowleyanus* (F) with four different growth habits (see Table 1) and with windows unobstructed. The dashed line represents $PFD_{int/ext}$ just inside and adjacent to the window margin (except in *S. rowleyanus*, in which the dashed line represents $PFD_{int/ext}$ at the chlorenchyma on one side of the spherical leaf (a window measurement was not made in this species); while the dotted line represents $PFD_{int/ext}$ in the hydrenchyma in the middle of the leaf, and the solid line represents $PFD_{int/ext}$ at the chlorenchyma at the bottom of the leaf (or, in *S. rowleyanus*, on the other side of the leaf). See Fig. 2 for more information about the measurement locations inside the leaves. Although data were obtained from three different plants for each species (selected mean values are provided in Tables 1 and 3), results presented here comprise a representative data set from one of those plants.

Although all three species with subterranean growth habits had remarkably clear windows, the amount of light penetrating deep into the leaves varied among these taxa, with the greatest amount of visible light penetrating the leaves of *O. longum* and the least in *H. truncata* (Fig. 3; Table 1). The greater decrease in light levels within leaves of *H. truncata* might be attributable to scattering and reflection of the incident light as a result of the rough surface texture of the window (but see Tanner and Eller, 1986), which is papillose with a regular array of low bumps (Ehleringer *et al.*, 1976; Reicosky and Hanover, 1978; Mulroy, 1979; Krulik, 1980). By contrast, the window surfaces of *L. olivacea* and *O. longum* are smooth (Krulik, 1980). Although the windows of *O. longum* appeared remarkably transparent, which may explain, at least in part, the greater penetration of light into the leaves of this species, detailed morphological comparisons of the windows of *O. longum* and *H. truncata* are not available.

Light levels (visible wavelengths) in the central hydrenchyma 0.3 cm below the crescent-shaped window on the pea-shaped, succulent leaves of the vine *S. rowleyanus* were up to one-third of those incident on the leaf exterior for green wavelengths, while penetration of blue and red light deep into the leaf was substantially less (Fig. 3F; Table 1). Light levels in the green portion of the spectrum decreased to 10–20% of incident light levels at the chlorenchyma tissue on the outermost interior portions of these leaves (Table 1), while light levels at blue and red wavelengths were less than 5% here, relative to the irradiance incident on the leaves (Table 1).

The maximum (green wavelengths) $PFD_{int/ext}$ values measured at all three locations inside the leaf tissue of both species of *Peperomia* were exceptionally high, up to three to four times those of the incident light (Fig. 3; Table 1). In *P. graveolens*, even light levels in the blue and red exceeded incident light levels at these

Table 1. *Mean (± standard deviation;* n=*3) maximum light level ($PFD_{int/ext}$); see text) in the visible portion of the spectrum, wavelength of that light (primarily green), light level at the wavelength of minimal irradiance in the red region of the visible portion of the spectrum (670 nm), light level at the wavelength of minimal irradiance in the blue region of the visible portion of the spectrum (440 nm), light level at the wavelength of peak irradiance in the infrared (780 nm), and measurement location inside the leaves of six succulents* (Haworthia truncata, Lithops olivacea, Opthalmophyllum longum, Peperomia graveolens, Peperomia dolabriformis, *and* Senecio rowleyanus) *with four different growth habits (the first three are subterranean herbs,* P. graveolens *is an above-ground herb,* P. dolabriformis *is an above-ground shrub, and* S. rowleyanus *is an above-ground vine) and with windows unobstructed*

Light levels are expressed as a fraction of that incident on the leaves ($PFD_{int/ext}$). For all species except *S. rowleyanus*, measurement locations are expressed as the depth, in cm, below the tops of the windows of the leaves; measurements were made just inside the edge of the adaxial window, in the hydrenchyma at the centre of the leaf, and at the basal chlorenchyma on the abaxial side of the leaf (see Fig. 2). In *S. rowleyanus*, the 'depths' are relative to a side of the spherical leaves (see Fig. 2); measurements were made at the outermost chlorenchyma on one side of the spherical leaf (this value appears under 'inside window' in the table), in the hydrenchyma at the centre of the leaf, and at the outermost chlorenchyma at the other side of the leaf (this value appears under 'basal chlorenchyma' in the table). A window measurement was not made for *S. rowleyanus*.

Species	Window depth	Max. $PFD_{int/ext}$ inside window	Wavelength of max $PFD_{int/ext}$ inside window	Red (670 nm) $PFD_{int/ext}$ inside window	Blue (440 nm) $PFD_{int/ext}$ inside window	Infrared (780 nm) $PFD_{int/ext}$ inside window	Hydrenchyma depth	Max. $PFD_{int/ext}$ in hydrenchyma	Wavelength of max. $PFD_{int/ext}$ in hydrenchyma	Red (670 nm) $PFD_{int/ext}$ in hydrenchyma	Blue (440 nm) $PFD_{int/ext}$ in hydrenchyma	Infrared (780 nm) $PFD_{int/ext}$ in hydrenchyma	Chlorenchyma depth	Max. $PFD_{int/ext}$ at chlorenchyma	Wavelength of max. $PFD_{int/ext}$ at chlorenchyma	Red (670 nm) $PFD_{int/ext}$ at chlorenchyma	Blue (440 nm) $PFD_{int/ext}$ at chlorenchyma	Infrared (780 nm) $PFD_{int/ext}$ at chlorenchyma
H. truncata	0.2	0.72±0.07	700.0±0	0.42±0.03	0.42±0.03	1.72±0.37	1.2[a]	0.18±0.06	544.67±0.58	0.12±0.15	0.08±0.04	0.70±0.06	2.4[e]	0.073±0.04	699.33±1.16	0.03±0.02	0.01±0.02	0.26±0.11
T . olivacea	0.2[a]	1.25±0.54	539.0±2.65	0.56±0.37	0.56±0.37	2.47±0.51	1.2[a]	0.33±0.03	543.33±0.58	0.11±0.02	1.27±0.31	1.22±0.12	2.4	0.08±0.08	544.67±1.53	0.01±0.02	0.33±0.58	0.59±0.30
O. longum	0.2	1.45±0.78	547.33±5.86	0.63±0.44	0.63±0.44	1.96±0.67	1.5	0.64±0.12	546.67±0.58	0.33±0.14	0.17±0.09	1.21±0.14	2.9[h]	0.21±0.06	545.67±1.53	0.05±0.03	0.01±0.02	0.70±0.12
P. graveolens	0.2	2.23±2.00	549.33±3.06	1.62±1.97	1.62±1.97	6.75±1.97	0.8[c]	2.32±1.27	549.67±2.52	1.64±0.87	1.69±0.86	7.61±0.74	1.6[f]	2.22±0.15	549.67±1.53	1.18±0.10	1.06±0.14	7.47±0.32
P. dolabriformis	0.2	1.43±1.10	544.67±4.73	0.47±0.15	0.47±0.15	2.92±2.51	0.5[d]	0.88±0.26	544.33±4.16	0.37±0.06	0.36±0.02	2.05±1.22	0.9[g]	0.71±0.30	545.00±4.58	0.28±0.09	0.24±0.05	1.84±1.23
S. rowleyanus	0.5[b]	0.10±0.01	542.67±1.53	0.01±0.01	0.01±0.01	0.66±0.06	0.3[a]	0.26±0.05	541.33±1.16	0.06±0.03	0.06±0.03	1.01±0.06	0.5[c]	0.19±0.06	542.33±0.58	0.04±0.04	0.03±0.03	0.94±0.16

[a] Depth for infrared measurement was 0.1 mm greater.
[b] Depth for infrared measurement was 0.4 mm less.
[c] Depth for infrared measurement was 0.2 mm less.
[d] Depth for infrared measurement was 0.2 mm greater.
[e] Depth for infrared measurement was 0.3 mm greater.
[f] Depth for infrared measurement was 0.6 mm less.
[g] Depth for infrared measurement was 0.4 mm greater
[h] Depth for infrared measurement was 0.1 mm less.

wavelengths near the window and, surprisingly, in the centre and even at the basal chlorenchyma of the leaves (Table 1; Fig. 3). These high internal light levels are most likely a result of light trapping due to scattering and focusing as seen in other studies (Martin *et al*., 1989; Vogelmann 1989, 1993; Poulson and Vogelmann, 1990; Myers *et al*., 1994; Vogelmann *et al*., 1996*a*). The maximum $PFD_{int/ext}$ just inside the window in leaves of *P. dolabriformis* was nearly 1.5 times the incident light levels, slightly more than the incident light level in the central hydrenchyma of the leaf at a depth of 0.8 cm, and nearly the same as incident light levels at the chlorenchyma at the base of the leaf at a depth of 1.6 cm (Table 1). Windows of the leaves of this species are exceptionally clear, and the cells of the central hydrenchyma are 'huge' (Kaul, 1977), which probably contributes to light scattering and focusing. In *P. graveolens,* the maximum $PFD_{int/ext}$ was more than twice the incident light levels at the edge of the window, as well as in the central hydrenchyma of the leaf (0.5 cm from the window), and still over twice the incident light levels at the chlorenchyma at the abaxial side of the leaf (0.9 cm from the window; Table 1). Although one might expect that such high internal light levels might result in photoinhibition, no evidence of the latter was detected in *P. graveolens* measured at growth light levels under well-watered conditions, although light utilization efficiency was indeed low, relative to other window-leaved succulents (Egbert and Martin, 2002).

Amounts of near-infrared radiation (defined as 750–950 nm in this study; data presented for 780 nm only) just under the windows in the leaves, as well as in the central hydrenchyma in most of the species examined, always exceeded or greatly exceeded incident levels of infrared radiation impinging on the leaves (Table 1; Fig. 3). Although most leaves absorb poorly at these wavelengths (Gates, 1980), Eller *et al.* (1983) and Tanner and Eller (1986) provide evidence that this is not the case with many succulents. The results of the current study appear to support this contention, as evidenced by decreasing levels of near-infrared radiation at increasing tissue depths inside the leaves of all taxa except *S. rowleyanus* (Table 1; Fig. 3). This decrease in near-infrared radiation could be the result of absorption or progressive loss of radiation through light scattering. In addition, values of $PFD_{int/ext}$ in the near-infrared region of the spectrum well above one in these succulents, and up to nearly ten below the window and nearly eight at the chlorenchyma deep inside the leaf in *P. graveolens* (Fig. 3E), emphasize the high degree of light focusing in the infrared by the succulent leaf tissue of these taxa.

Light quality inside the leaves

The spectral properties of the light inside the leaves of all six succulent taxa reflected the absorption spectrum of chlorophyll, i.e. amounts of red (approximately 600–700 nm) and blue (approximately 400–500 nm) light were typically less than the amounts of green light (approximately 500–550 nm; Fig. 3; Table 1). This finding has been reported earlier (Bornman and Vogelmann, 1988; Vogelmann, 1989, 1993; Vogelmann *et al*., 1996*b*, 1989; Cui *et al*., 1991; Sun *et al*., 1998). Not surprisingly, the abundance of green light inside leaves may be of particular importance as a major source of energy driving photosynthesis in the chlorenchyma tissue (Sun *et al*., 1998). Generally, the irradiance spectra for all depths in each of the leaves were similar, although differences among species were observed (Fig. 3). For example, in *H. truncata* (Table 1; Fig, 3) and *P. graveolens* (Table 1; Fig. 3), blue and red light inside the leaf appeared to be less efficiently absorbed, relative to the other four species (Fig. 3). Reasons for this finding are unclear. The maximum wavelength of visible light changed little with depth in the leaves, remaining in the green, around 550 nm, although blue and red light appeared to attenuate to a greater degree with depth in the succulent leaves (Table 1), which most likely reflects absorption of these spectral bands by chlorophyll in the leaves.

Influence of window size on leaf internal light levels

The proportion of leaf area occupied by the adaxial window was greatest in *P. graveolens* (Table 2). As discussed above, light levels inside leaves of this species far exceeded those of the other species. In contrast, although the narrow windows of leaves of *S. rowleyanus* were the smallest, in terms of actual area and proportional area of the leaf (Table 2), the maximum $PFD_{int/ext}$ in the middle of the spherical leaves of this succulent vine was equal to or exceeded levels (Table 1) inside the leaves of *H. truncata* and *L. olivacea,* both species with windows of intermediate size (Table 2). Thus, window size, as

Table 2. *Mean (± standard deviation;* n=*3) areas, in* cm^2*, of the window and the leaf, and the ratio of window area to leaf area for six succulents* (Haworthia truncata, Lithops olivacea, Opthalmophyllum longum, Peperomia graveolens, Peperomia dolabriformis, *and* Senecio rowleyanus) *with four different growth habits (see Table 1) and with windows unobstructed*

Species	Area of window	Area of leaf	Window:leaf area ratio[a]
H. truncata	1.2±0.2	18.5±1.1	9.6±3.7c
L. olivacea	1.4±0.1	10.4±1.0	13.2±1.3c
O. longum	2.4±0.2	29.0±11.7	9.6±3.7c
P. graveolens	1.8±0.1	6.7±0.9	27.8±2.6a
P. dolabriformis	0.8±0.2	8.4±3.2	10.6±1.8c
S. rowleyanus	0.1±0.0	2.5±0.0	1.6±0.0b

[a] Letters indicate the results of ANOVA and Tukey Pairwise Multiple Comparison Procedures tests; means with different letters are significantly different at $P < 0.05$.

a proportion of the total leaf area, did not correlate well with the amount of light inside the leaves of the species examined.

Effectiveness of covering the windows with reflective tape in blocking light penetration into the leaves

Attachment of reflective adhesive tape to the leaf windows, cut to match the area of each window, reduced the amount of light inside the leaves of the three species investigated, although these reductions were not always statistically significant, and their magnitude varied among species and among locations within the leaf (Fig. 4; Table 3). In *L. olivacea*, internal light levels, quantified as maximum $PFD_{int/ext}$ at 550 nm), just inside the window and at the chlorenchyma at the base of the leaf were lower, but not significantly so, than those measured at these locations in leaves with unobstructed windows; while the light level at the centre of the leaves with reflective tape covering their windows was significantly lower, by two-thirds, than the light level at this location in leaves lacking tape on their windows (Table 3). In the succulent vine *S. rowleyanus,* the decrease in light level at the centre of the leaf was only about one-third, though this decrease was not statistically significant, when reflective tape was attached to its leaf windows (Table 3). Light levels measured at the internal chlorenchyma along the sides of the leaves of *S. rowleyanus* were substantially lower for one side of the spherical leaf in leaves with reflective tape on their windows, relative to light levels at the same locations in leaves lacking reflective tape, whereas the light level at the other side was inexplicably higher in leaves with reflective tape affixed to their windows, compared with leaves lacking such tape. Although these contrasting differences are both statistically significant, their biological significance is questionable,

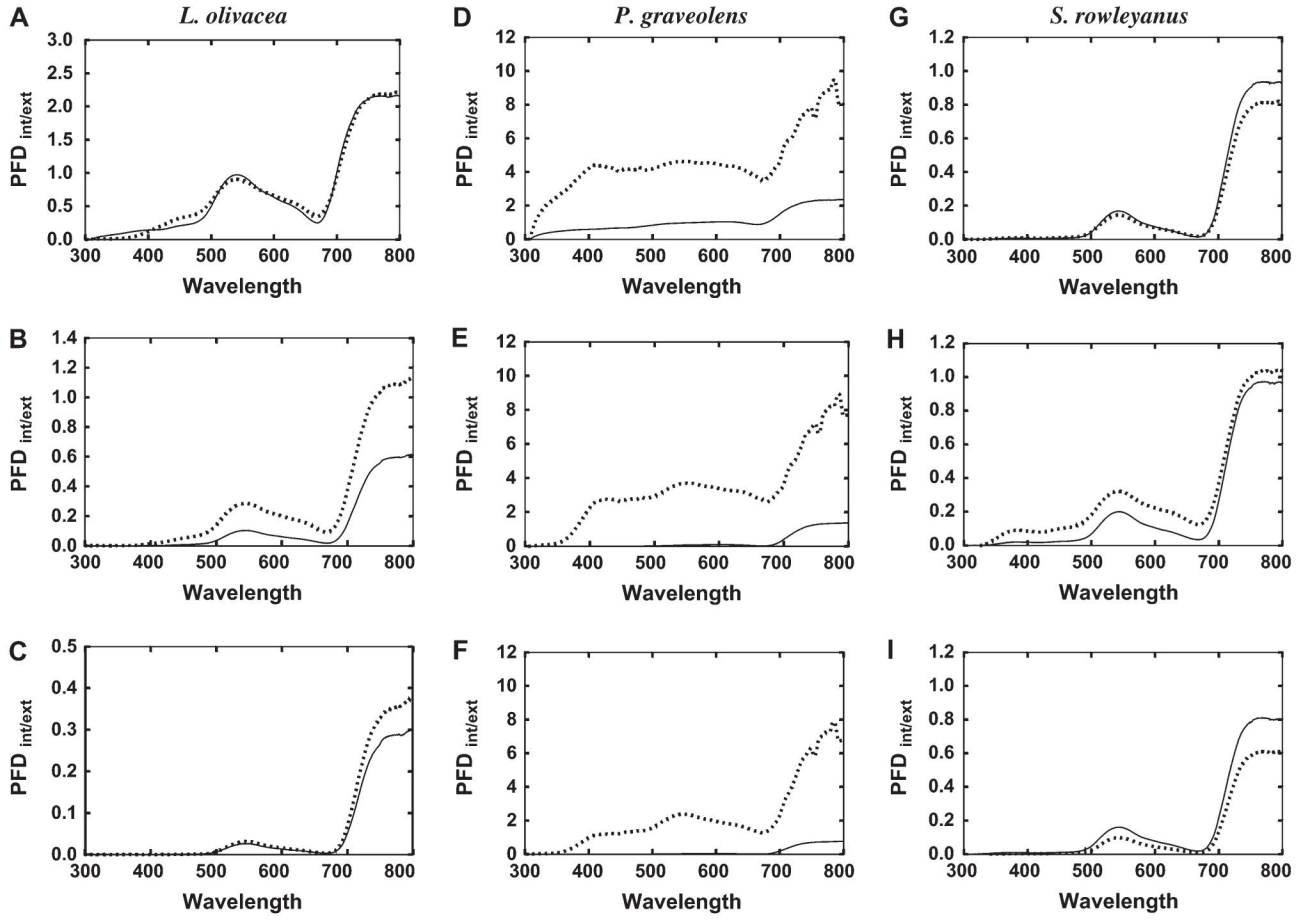

Fig. 4. The amount of light, expressed as the ratio of internal to external (incident) PFD ($PFD_{int/ext}$), for each wavelength from 300 nm to 900 nm at three different locations inside a succulent leaf of *L. olivacea* (A–C), *P. graveolens* (D–F), and *S. rowleyanus* (G–I) with (solid line) and without (dotted line) reflective tape covering the leaf window. Measurements of $PFD_{int/ext}$ were made just inside and adjacent to the window margin (A, D), in the hydrenchyma in the middle of the leaf (B, E, H), and at the chlorenchyma at the basal portion of the leaf (C, F, G, I; although G and I depict measurements made at the chlorenchyma along the opposite sides of the spherical leaf of *S. rowleyanus*). No window measurement was made for *S. rowleyanus*. See Table 1 for growth habits of the species and Fig. 2 for more information about the measurement locations inside the leaves. The same leaves were used for the tape and no-tape treatments only for *L. olivacea (*different leaves on the same plant were used for the other two species. Although data were obtained from three different plants for each species (selected mean values are provided in Table 1), results presented here comprise a representative data set from one of those plants.

Table 3. *Changes in mean (see Table 1 for standard deviations;* n=*3) maximum light level, wavelength at that level, and infrared light (at 780 nm) inside the leaves of three succulents* (Lithops olivacea, Peperomia graveolens, *and* Senecio rowleyanus) *with three different growth habits (see Table 1) after covering the leaf windows with reflective tape*

Light level is expressed as a fraction of that incident on the leaves at the wavelengths of peak irradiance ($PFD_{int/ext}$; see text). For measurement locations, see Fig. 2 and Table 1. The same leaves were used for the before and after taping measurements only for *L. olivacea* (different leaves on the same plant were used for the other two species). Letters indicate the results of *t*-tests comparing the mean values with and without reflective tape obscuring the leaf windows: 'ns' indicates that the means are not significantly different (P >0.05); * indicates that the means are significantly different at P <0.05; **indicates that the means are significantly different at P <0.01; and *** indicates that the means are significantly different at P <0.001).

Species	Change in maximum $PFD_{int/ext}$ inside window with reflective tape	Change in wavelength of maximum $PFD_{int/ext}$ inside window with reflective tape	Change in infrared $PFD_{int/ext}$ inside window with reflective tape	Change in maximum $PFD_{int/ext}$ at mid-leaf hydrenchyma with reflective tape	Change in wavelength of maximum $PFD_{int/ext}$ at mid-leaf hydrenchyma with reflective tape	Change in infrared PFDint/ext at mid-leaf hydrenchyma with reflective tape	Change in maximum $PFD_{int/ext}$ at basal chlorenchyma with reflective tape	Change in wavelength of maximum $PFD_{int/ext}$ at basal chlorenchyma with reflective tape	Change in infrared $PFD_{int/ext}$ at basal chlorenchyma with reflective tape
Lithops olivacea	1.25 to 0.69 (45% decrease) ns	539 to 542 (<1% increase)***	2.47 to 1.70 (31% decrease) ns	0.33 to 0.11 (67% decrease)***	543 to 543 (no change) ns	1.22 to 0.66 (47% decrease)*	0.08 to 0.03 (63% decrease) ns	545 to 550 (< 1% increase) ns	0.59 to 0.33 (44% decrease) ns
Peperomia graveolens	2.23 to 0.46 (79% decrease) ns	549 to 614 (12% increase)***	6.75 to 1.70 (75% decrease)*	2.32 to 0.09 (96% decrease)*	550 to 607 (10% increase)***	7.61 to 0.66 (91% decrease)**	2.22 to 0.05 (98% decrease)***	550 to 604 (10% increase)***	7.47 to 0.86 (88% decrease) ns
Senecio rowleyanus	0.10 to 0.15 (50% increase)*	543 to 543 (no change) ns	0.66 to 0.73 (11% increase) ns	0.26 to 0.16 (38% decrease) ns	541 to 543 (<1% increase) ns	1.01 to 0.84 (17% decrease) ns	0.19 to 0.16 (16% decrease) ns	542 to 542 (no change) ns	0.94 to 0.86 (9% decrease) ns

given the opposite findings for similar locations in the leaf, the high variability in the data, and the very low light levels measured in the leaves.

The light levels at all three locations in the leaves of *P. graveolens* were substantially lower when their windows were covered with reflective tape (Table 3; Fig. 4). The maximum $PFD_{int/ext}$ decreased by over three-quarters at the window margin and decreased nearly 10-fold in the central hydrenchyma in the middle of the leaf when the leaf windows were obstructed by tape (Table 3). Furthermore, although values of maximum $PFD_{int/ext}$ (visible radiation) were over twice the incident light levels at the base of leaves with unobstructed windows, covering the windows with reflective tape reduced light levels at the base of the leaves to nearly zero (Table 3). Given these findings, one would expect that the reduction in light provided by the application of reflective tape to the windows should effect a profound reduction in photosynthetic rates in *P. graveolens*. Carbon assimilation rates for plants of *P. graveolens* with windows covered and uncovered, however, did not differ substantially (Egbert and Martin, 2000). Reasons for these puzzling results are presently unclear (but see below).

Reducing the ingress of light into the leaves of the three succulents examined by covering the windows of their leaves with reflective tape resulted in no or only modest changes in the spectral quality of the internal light; in some instances, the wavelength of maximum $PFD_{int/ext}$ shifted from about 550 nm to over 600 nm, depending on depth in the tissue and species (Table 3; Fig. 4).

Not only were levels of visible light inside the leaves substantially reduced when leaf windows were covered with reflective tape, but levels of near-infrared radiation were also diminished inside the leaves by covering the windows with reflective tape (Table 3). Although such apparent changes were not significant in leaves of *S. rowleyanus*, levels of near-infrared radiation decreased by half in the central hydrenchyma of the leaves of *L. olivacea* and by three-quarters just inside the window of the leaves of *P. graveolens* (Table 3).

Overall, although exceptions were noted, covering the windows of the leaves with reflective tape was highly effective in reducing the amount of light, both visible and near-infrared, inside the leaves of these succulents. Whereas the reduction in availability of visible light inside the leaves of these succulents might be predicted to lead to reductions in photosynthetic activity when reflective tape is applied to the windows of these succulent leaves, the reduction in infrared radiation inside the leaves might effect lower leaf temperatures. Given that leaf temperatures of succulents with a below-ground growth habit are among the highest reported for vascular plants (Eller and Nipkow, 1983; Nobel, 1989), reductions in daytime leaf temperatures might prove exceedingly beneficial for these plants. For example, if such reductions in leaf

temperatures maintained leaf temperatures closer to their optima for maximal photosynthetic rates, this new finding may provide a plausible explanation for the puzzling results of Egbert and Martin (Egbert and Martin, 1999, 2000, 2002), in which photosynthetic rates of these window-leaved succulents did not decline, and appeared to increase, when the leaf windows are covered with reflective tape. This possibility, that the application of reflective tape to the windows reduces the input of infrared radiation into the leaf, thus maintaining leaf temperatures more favourable for photosynthetic activity, is currently being investigated.

Conclusions

This is the first study in which an omni-directional fibre optic microprobe was used in plant tissue to measure the light environment within the leaves of six species of window-leaved succulents. Although light quality varied little among the species tested and locations inside the leaves, the quantity of light at the various depths below the windows on the adaxial surfaces of the leaves of these six species was highly variable and did not exhibit clear correlations with differences in window size (proportional to leaf area), location inside the leaf, or growth habit (Tables 1, 2; Fig. 3). Levels of near-infrared radiation were high in the leaves of the six succulents, although levels decreased with depth in the leaves, suggestive of absorption of this thermal energy by the leaf tissue. Differences in the quantity of light at the different locations within the leaves of the six succulents were most likely the result of different epidermal and intercellular properties of each species. The leaves of *P. graveolens* were highly unusual in the extraordinary light-focusing and scattering properties of the leaf tissue, resulting in light levels inside the leaves that were many times higher than those impacting the surface of the leaves.

When reflective tape was applied to the windows of the leaves of two of the three species tested (*L. olivacea* and *S. rowleyanus*), no differences in the quantity or quality of light were observed just inside the window margin or at the chlorenchyma at the base (or sides) of the leaf, although light levels were reduced in the central hydrenchyma of these leaves (Fig. 4; Table 3). Proportionally large reductions in light levels at all locations inside the leaves of *P. graveolens* were noted, although, given the light-focusing nature of the leaf tissue in this species, light levels deep in the tissue remained relatively high (near those incident on the leaves or even higher). Covering the leaf windows with reflective tape also substantially reduced the levels of near-infrared radiation in these leaves, thus presumably decreasing the heat load in these leaves (Fig. 4; Table 3). Consideration of the latter yields a novel and testable hypothesis potentially explaining the puzzling results obtained previously by Egbert and Martin (1999, 2000, 2002) in which photosynthetic rates of these succulents did not decrease after affixing reflective tape to their leaf windows. This hypothesis will be the subject of a future investigation.

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