

Epidermal cells functioning as lenses in leaves of tropical rain-forest shade plants

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A ray tracing model has been developed to investigate the possible focusing effects of the convexly curved epidermal cell walls which characterize a number of shade-adapted plants. The model indicates that such focusing occurs, resulting in higher photosynthetic photon flux densities at certain locations within the leaf. It is postulated that there will be a corresponding increase in the rate of photosynthesis. In addition, leaf reflectance measurements indicate that this is generally less for the shade plants compared with sun species and would be advantageous in increasing the efficiency of energy capture. Either effect is important for plants which must survive at extremely low light levels.

I. Introduction

Among the striking features of plants growing in the extreme shade of tropical rain forests is the frequently observed velvety sheen of leaf surfaces^{1,2} due to reflection from the convexly curved outer epidermal cell walls. Although the light-focusing properties of these cells were suggested earlier,^{3,4} subsequent research has primarily involved their taxonomic significance⁵ and their role in the regulation of chloroplast movement.^{6,7} Haberlandt⁸ demonstrated the light-focusing properties of these cells with paradermal sections of leaves viewed under the microscope (*linsenverch*). We have repeated these methods (see Fig. 1), but the complexity of leaf structure and the natural light environment in forest shade make it technically unfeasible to measure the distribution of refracted light within the leaf. It has been hypothesized that these convexly curved cells could increase flux densities within the leaf and thereby increase the rates of photosynthesis and growth.^{3,4} Haberlandt⁸ stressed the importance of light refraction in controlling the orientation of leaves (see also Smith⁹) but, in earlier editions of his book, also placed credence on the idea of increased light absorptance due to refractive properties. However, light refraction obviously does not increase the limited quantities of radiation

arriving at the leaf surfaces, and the advantages of refraction are not obvious. Here we describe a ray tracing model for predicting light levels within these leaves and discuss the implications of light refraction, and also reflection, for photosynthesis and survival of these plants at extremely low quantum flux densities.

II. Leaf Samples

Plants examined in this study (Table I) were obtained from nurseries at Florida International University and Fairchild Tropical Garden. Freshly prepared hand sections of leaves were photographed (Fig. 1), and the negatives were used for tracing epidermal cell shapes and chloroplast locations for use in the optical model (illustrated in Fig. 2). z - x coordinates (see Fig. 2) from five cell shapes were used from each species.

Leaf reflectances at different angles of incidence were measured in an integrating sphere attached to a spectrophotometer.¹⁰ Reflectances of leaves of seven sun species freshly collected from the FIU campus (Table I) were also measured for comparison with the shade plants.

III. Ray Tracing Model

A best fit was obtained between each epidermal cell surface and the surface of revolution

$$f(x,y,z) = z - \sum c_n(x^2 + y^2)^n = 0, \quad n = 0-5. \quad (1)$$

For the case of diffuse illumination, we first considered a beam of (~1500) parallel uniformly spaced rays incident on the surface of a horizontal leaf from a specific zenith angle θ and azimuthal angle ϕ (see Fig. 2). The directions of the refracted rays were obtained by applying Snell's law at the surface using a cell refractive index of 1.35. This value, fractionally above that of water, is based on a dilute (0.3-M) sucrose solution¹¹ to

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Received 30 November 1984.

0003-6935/85/101408-05\$02.00/0.

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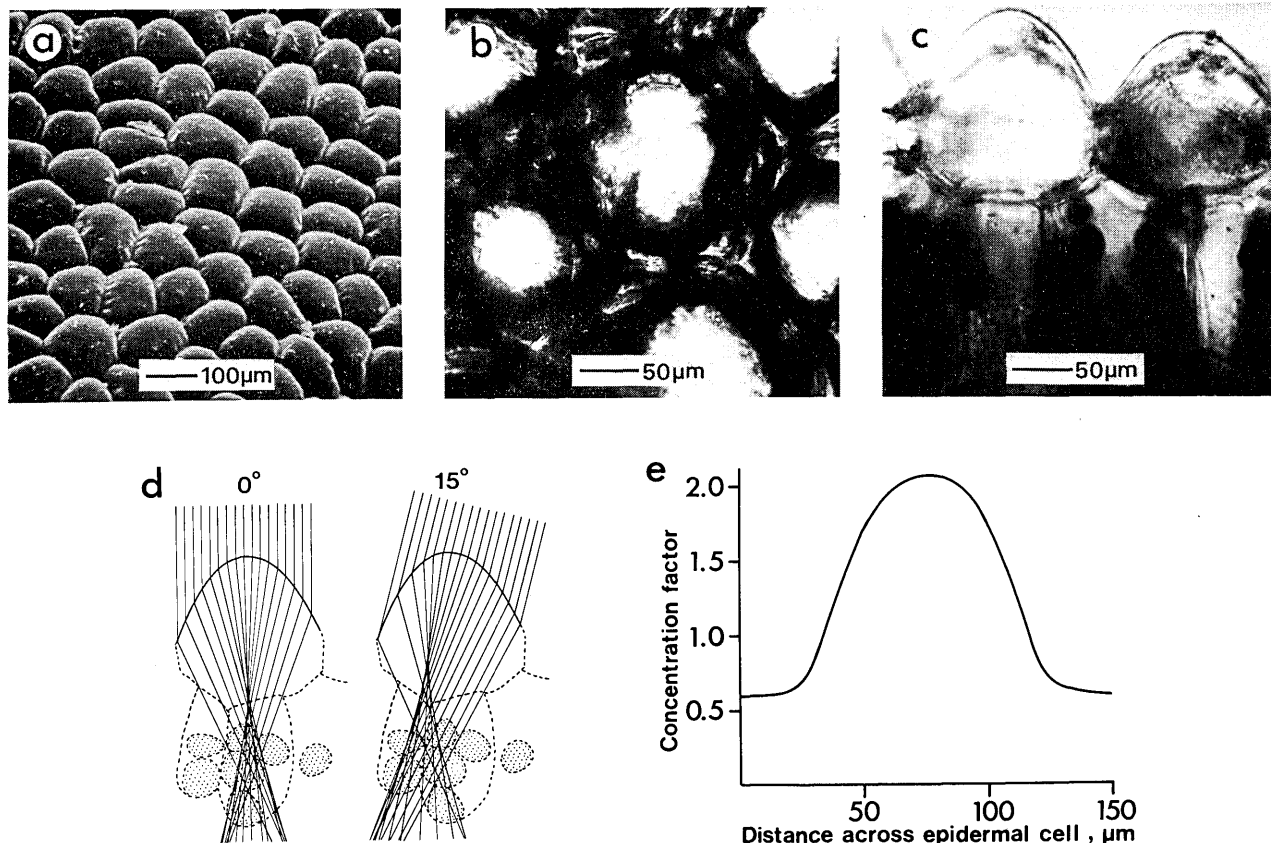


Fig. 1. Light refraction by leaf epidermal cells of *Anthurium warocqueanum*: (a) SEM photograph of epidermal surface; (b) paradermal sections of leaf showing light focusing by epidermal cells; (c) light microscope transverse section showing convexly curved epidermal cells and locations of chloroplasts; (d) ray diagram showing refraction at zenith angles of 0 and 15°; (e) distribution of refracted light beneath an epidermal cell (150 µm wide) at the top of the chloroplast layer (88 µm beneath lowest exposed surfaces of epidermis). The concentration factor has been normalized by dividing it by the concentration factor for a flat cell surface. The distribution is for a diffuse light environment.

Table I. Concentration Factors Due to Epidermal Cell Refraction and Diffuse Reflectances of the Eight Shade Species and Seven Sun Species Examined in this Study

Species	Origin	Diffuse reflectance ^a (%) 0°/60°	Concentration factor (CF)/level			
			Diffuse radiation		Direct radiation	
			CF/chloroplast ^b	CF/optimal ^c	CF/chloroplast ^b	CF/optimal ^c
<i>Anthurium warocqueanum</i> Moore	Columbia	2.6 ± 0.2/36.2 ± 12.6	1.96/88	2.16/42	10.11/88	19.21/160
<i>Begonia pavonina</i> Ridl.	Malayan Peninsula	5.6 ± 0.5/21.1 ± 1.0	1.04/51	1.66/15	8.99/51	19.59/82
<i>Calathea makoyana</i> Nichols	Brasil	2.1 ± 0.3/10.6 ± 2.3	0.96/94	2.40/0	1.41/94	9.85/32
<i>Hoffmania refulgens</i> (Hook.) Hemsl.	Central America	3.4 ± 0.4/19.5 ± 3.9	1.22/48	2.26/12	11.40/48	11.76/40
<i>Kaempferia pulchra</i> Ridl.	Southeast Asia	3.9 ± 0.1/13.0 ± 1.8	1.15/116	2.12/13	7.81/116	8.99/103
<i>Ludisia discolor</i> A. Rich	Southeast Asia	2.0 ± 0.3/10.2 ± 1.6	1.32/75	2.25/10	8.59/75	8.59/75
<i>Scindapsus pictus</i> Hassk.	Southeast Asia	4.4 ± 0.2/24.1 ± 4.4	1.38/64	1.59/26	3.49/64	9.65/288
<i>Selaginella uncinata</i> Spr.	South China	6.3 ± 1.1/26.2 ± 6.5	1.22/22	1.45/12	1.93/22	19.63/91
Seven sun ^d species	—	8.3 ± 1.7/37.5 ± 7.2	0.961/—	0.961/—	0.971/—	0.971/—

^a Diffuse reflectances measured in an integrating sphere with incident light at 0 and 60° from the perpendicular to the leaf surface.

^b Concentration factor at the upper edge of the chloroplast layer, the latter measured in microns beneath the lowest exposed surface of the epidermal cells.

^c Concentration factor at the level where this is maximum, the level being expressed in microns beneath the lowest exposed surface of the epidermal cells.

^d These are all trees native to tropical regions and growing in Miami: *Bauhinia purpuria* L., *Brassaia actinophylla* Eudl., *Coccoloba wifera* L., *Ficus aurea* Nutt., *Mangifera indica* L., *Ravenala madagascarensis* Sonn., *Theobroma cacao* L. Reflectance values are means and standard deviations of five leaf measurements.

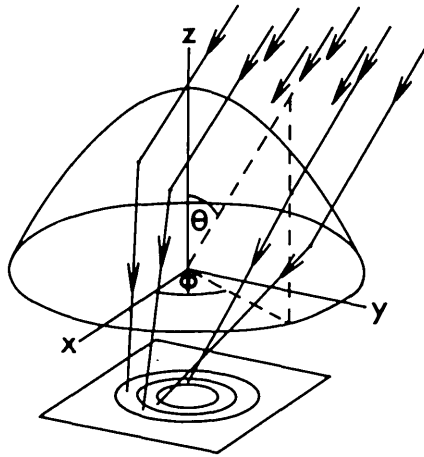


Fig. 2. Geometry of the ray tracing model. A beam of parallel rays approaching the epidermal cell surface is characterized by a zenith angle θ and an azimuthal angle ϕ . The flux distribution over a horizontal plane below the surface is determined by the model.

which the cell contents may be approximated. (Dilute aqueous ionic solutions, e.g., potassium chloride, also have values close to 1.35.) Hence the points of intersection of the rays with the plane, $z = \text{constant}$ (e.g., at the level of the chloroplasts), could be found. The plane was divided into annular zones about the z axis. In the absence of losses by reflection at the cell surface, the fraction of incident energy reaching each zone would be calculated by counting the rays intersecting the zone and expressing the total as a fraction of the number in the incident beam. To take account of reflection, each refracted ray was weighted by a factor equal to the transmittance. This was determined by Fresnel's laws of reflection, the outer portion of the cell wall being assigned a refractive index of 1.45.¹⁰

Since the same result would be obtained for any azimuthal angle ϕ , the total energy $E(\theta)$ related to the selected zenith angle θ was combined with the above fractions to give the corresponding energy in each zone. $E(\theta)$ itself was obtained from the incident energy flux density per unit solid angle given by Norman and Jarvis,¹² using a rain-forest structure from Malaysia.¹³ With this structure, which assumes azimuthal symmetry, the distribution of light beneath the forest canopy for a clear sky with sun at 45° elevation is not significantly different from that with a standard overcast sky. Therefore, an average was used for further calculations. To determine $E(\theta)$, the energy flux distribution was integrated over the azimuthal range, $\phi = 0-2\pi$, and over the zenith range, $\pm 5^\circ$ about the selected zenith angle, and combined with the calculated area of the incident beam intercepted by the refracting surface. The above calculations were repeated for $\theta = 5, 15, \dots, 55^\circ$, energy contributions from larger angles being negligible. Also, where appropriate, contributions from surrounding cells (up to 18 and assumed hexagonally close packed) were included in the calculations.

For comparison purposes, the above methods were applied to a flat horizontal refracting surface of the same area as that of the epidermal cell surface projected onto

a horizontal plane. Each concentration factor given in Table I represents the energy flux at a point on the axis of symmetry of the cell expressed as a ratio of the corresponding energy flux for the flat refracting surface.

For the case of direct illumination, for example, by sun flecks, a similar calculation to the diffuse case was carried out but with only one zenith angle (zero) being considered. Further details of the model may be found in the Appendix.

A complication, which for some leaves may be of considerable importance, is multiple scattering within a leaf.^{14,15} However, most of the scatter results from intercellular airspaces. All the leaves used in this study are characterized by an epidermal layer plus palisade layer, which are essentially devoid of such spaces. Immediately below is the layer of chloroplasts where light is rapidly attenuated. Hence scattering is considered of secondary importance and, due to the obvious computational difficulties of incorporating it in the model, has been ignored.

IV. Results and Discussion

Light environments within rain forests are heterogeneous^{16,17} consisting of (1) low-level diffuse background radiation, (2) intermediate levels arising from penumbral effects of the solar disk, and (3) occasional sun flecks. The shade-adapted plants grow on sites of few or no light flecks and at photosynthetic photon flux densities (PPFD) of $\sim 5 \mu\text{mol sec}^{-1} \text{m}^{-2}$ at midday (0.3% of full sunlight or just above the photosynthetic light compensation points reported for other shade-adapted vascular plants^{18,19}). Photosynthetic organisms with chlorophylls *a* and *b* (including Chlorophyta and all vascular plants) appear to be less able to survive than other photosynthetic organisms, a limitation compounded for land plants by the higher respiratory costs associated with maintaining support tissue for displaying leaves.^{19,20} Limits to the photosynthetic responses of extreme shade plants suggest that the properties of epidermal cells, as quantified by this model, may be biologically important at low light levels in several ways.

All the species studied concentrate a beam of parallel rays (as with direct sunlight) within the leaf (Table I, Fig. 1). The degree of maximum concentration is diminished and the depth where this occurs is increased as light strikes the leaf surface at more oblique angles. Such high concentrations could contribute to the physical damage of chloroplasts should the plant be exposed to full sunlight. For example, a light fleck (at $\sim 200 \mu\text{mol sec}^{-1} \text{m}^{-2}$ of PPFD) incident normally on a leaf of *Anthurium warocqueanum* (Table I, Fig. 1) would produce an intensity equivalent to full sunlight ($2020 \mu\text{mol sec}^{-1} \text{m}^{-2}$) within the leaf. Thus these plants should be confined to the deepest shade (a distribution confirmed by field observations in Malaysia and Costa Rica but requiring additional study). In all the species examined, the level of maximum concentration does not coincide with the chloroplasts, thereby alleviating potential damage by high light levels. For example, in *Calathea makoyana* the maximum con-

centration factor is 9.85 but occurs in clear hypodermal cells well above the chloroplast layer.

Since light flecks could potentially damage these species, and they appear to grow in diffuse light environments where sun flecks are not important, it is more realistic to use a model of diffuse radiation within the forest. In all the species studied, diffuse radiation is still concentrated within the leaf, the maximal focusing effect being closer to the levels of the chloroplasts. The concentration effect is smaller than with direct radiation but still significant. For *Anthurium warocqueanum* the concentration factor at the top of the chloroplast layer is 1.96, the maximum value of 2.16 being just above this level. For this species the actual flux density at the level of some chloroplasts is calculated to be almost twice that at the leaf surface. In addition, direct radiation from penumbral effects with clear sky and sun at zenith may account for ~20% of the total incident radiation.¹² This would lead to an effective concentration factor of ~5.

Light concentration may be significant for extreme shade-adapted plants in at least two ways. Some species may not distribute chloroplasts uniformly in the palisade layer but in discrete clusters near the zone of maximum focus. Such a strategy may lower metabolic costs of producing light harvesting machinery in a diffuse and shady light environment. *Selaginella uncinata* produces two chloroplasts at the bottom of its inverted cone-shaped epidermal cells,²¹ and these chloroplasts do not form a continuous layer. Light is focused onto the chloroplasts with a concentration factor of 1.22 (1.67 for clear sky and sun at zenith). Jagels²² measured light compensation points for these plants at 1.5–10 $\mu\text{mol sec}^{-1} \text{m}^{-2}$, depending on respiration rates for individual plants. The light concentration effect could be responsible for the difference between net carbon gain and loss for the plant. A discontinuous layer of chloroplasts was also observed in *Begonia pavonina*, but the other six species produce a continuous and discrete layer of chloroplasts in the palisade layer, meaning that different portions of the chloroplasts are more intensely illuminated at different times of the day. The advantage to these plants may be that of providing fewer chloroplasts at any one time with higher PPFD, minimizing photosynthetic inefficiencies at low quantum flux densities caused by leakage of H^+ from photosynthetic membranes and slippage by unstable photosystem II intermediates.^{19,23}

An additional implication for the shape of epidermal cells of these extreme shade plants is that the curvature may decrease specular reflectance from the leaf surface at oblique angles by presenting cell surfaces at angles more nearly perpendicular to a major proportion of the incident light.^{3,8} Measured reflectances at the oblique angle of 60° in comparison to the sun species are reduced for all the species except *Anthurium warocqueanum* (Table I). An ideal surface for reducing reflectance at an oblique angle would be a cone (as for *Ludisia discolor* and *Kaempferia pulchra*) but, on the other hand, would be less efficient in focusing light. Thus there is an inverse relationship between light-

focusing properties and reflectance at oblique angles among these species. Some plants may have been under natural selection for cell surfaces optimizing light-focusing properties and others for diminishing reflectance at oblique angles. Leaves displayed vertically, such as the climbing arid *Scindapsus pictus*, would be predicted to do the latter.

Terrestrial plants are at a disadvantage in several ways compared with their green algal ancestors. Among these, maintaining a spatial orientation for capturing sunlight has meant greater capital investment in nonphotosynthetic tissue, which means higher light compensation levels for plant growth and reproduction. The air-cell interface has also meant a loss in efficiency of light absorptance due to specular reflectance, but its refractive properties have made selection on this basis a distinct possibility. At low light levels, strong selection pressures for increasing efficiency in energy capture has led to the evolution of convex cell surfaces, both reducing specular reflectance at oblique angles and focusing light to produce higher levels of PPFD at specific locations within the leaf and thus increasing the efficiency of photosynthesis. The model described in this paper makes quantitative predictions about the distribution of light within leaves, providing a better understanding of the ecology and physiology of these plants.

A useful extension of this work would be to test experimentally the predictions of our model. Such a test might involve a disruption of the epidermal cell's focusing properties by covering the leaf with a film of water. If the model is accurate, a reduction in the photosynthetic rate would be expected.

We thank Susan Brammeier and Sara Tarsis for technical assistance and John Raven for constructive criticism. This work was supported by a grant from the Whitehall Foundation.

Appendix

The refraction of a ray at an epidermal surface, beyond which the refractive index is μ , may be expressed by the equations

$$\hat{n} \times \hat{i} = \mu \hat{n} \times \hat{r}, \quad (\text{A-1})$$

$$\hat{r} = a\hat{i} + b\hat{n}, \quad (\text{A-2})$$

where \hat{i} , \hat{r} , and \hat{n} are unit vectors directed along the incident ray, the refracted ray, and the outward normal, respectively. These are solved to give

$$\hat{r} = \frac{\hat{i}}{\mu} - \left[\frac{\hat{i} \cdot \hat{n}}{\mu} + \sqrt{\left(\frac{\hat{i} \cdot \hat{n}}{\mu} \right)^2 + 1 - \frac{1}{\mu^2}} \right] \hat{n}.$$

For the case $\phi = 0^\circ$ (Fig. 2), for example, i_x , i_y , and i_z are, respectively, $\sin\theta$, 0, and $\cos\theta$, and \hat{n} is obtained straightforwardly from ∇f [Eq. (1)]. The coordinates x', y' , where the refracted ray intersects the plane $z' = 0$, are given by

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} x_c \\ y_c \end{pmatrix} + \begin{pmatrix} z' - z_c \\ r_z \end{pmatrix} \begin{pmatrix} r_x \\ r_y \end{pmatrix}, \quad (\text{A-3})$$

x_c , y_c , and z_c being the coordinates on the cell surface

where refraction is occurring. An algorithm was developed which selects these coordinates so that the incident rays are uniformly spaced. Having determined the corresponding values of x' and y' , the algorithm calculates the percentage of refracted rays, appropriately weighted by their transmittances, falling in different annular zones (see Fig. 2). Multiplying these percentages by the energy per unit time $E(\theta)$ incident on the cell from the zenith angle θ gives the energy per unit time transmitted to each zone. $E(\theta)$ is found by numerical evaluation of the integral

$$2\pi \int_{\theta-5^\circ}^{\theta+5^\circ} \gamma(\theta)A(\theta) \sin\theta \cos\theta d\theta, \quad (\text{A-4})$$

where $\gamma(\theta)^{11,12}$ is the incident energy flux density per unit solid angle and $A(\theta)$ is the projected area of the cell surface perpendicular to the incident rays. The latter is obtained from a second integral

$$A = \int \hat{i} \cdot d\mathbf{A}, \quad (\text{A-5})$$

also evaluated numerically, but with only positive terms in the sum being retained. The differential area $d\mathbf{A}$ is given by

$$d\mathbf{A} = \hat{n} \left[1 + \left(\frac{\partial z}{\partial x} \right)^2 + \left(\frac{\partial z}{\partial y} \right)^2 \right]^{1/2} dx dy. \quad (\text{A-6})$$

For the case of diffuse radiation, the above calculations are repeated over a range of zenith angles. Finally, the energy flux density for each annular zone is obtained by dividing the total energy per unit time reaching the zone by its area.

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