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# Contribution of intercellular reflectance to photosynthesis in shade leaves

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## ABSTRACT

**The potential contribution of intercellular light reflectance to photosynthesis was investigated by infiltrating shade leaves with mineral oil. Infiltration of leaves of *Hydrophyllum canadense* and *Asarum canadense* with mineral oil decreased adaxial leaf reflectance but increased transmittance. As a result of the large increase in transmittance, infiltration caused a decrease in absorptance of 25% and 30% at 550 and 750 nm, respectively. Thus, intercellular reflectance increased absorptance in these species by this amount. In a comparison of sun and shade leaves of *Acer saccharum* and *Parthenocissus quinquefolia*, oil infiltration decreased absorptance more in shade than in sun leaves. This difference suggests that the higher proportion of spongy mesophyll in shade leaves may increase internal light scattering and thus absorptance. The importance of the spongy mesophyll in increasing internal reflectance was also evident in comparisons of the optics of *Populus* leaves and in the fluorescence yield of oil-infiltrated leaves of several sun and shade species. Oil infiltration decreased the quantum yield of fluorescence ($F_o$) by 39–52% for shade leaves but only 21–25% for sun leaves. We conclude that the greater proportion of spongy parenchyma in shade leaves increased intercellular light scattering and thus absorptance. Direct measurements with fibre-optic light probes of the distribution of light inside leaves of *Hydrophyllum canadense* confirmed that oil infiltration decreased the amount of back-scattered light and that most of the light scattering for this species occurred from the middle of the palisade layer to the middle of the spongy mesophyll. We were not, however, able to assess the potential contribution of reflectance from the internal abaxial epidermis to total internal light scattering in these experiments. Using a mathematical model to compare the response of net photosynthesis ($O_2$ flux) to incident irradiance for control leaves of *H. canadense* and theoretical leaves with no intercellular reflectance, we calculated that intercellular reflectance caused a 1·97-fold increase in photosynthesis at 20 $\mu$mol m$^{-2}$ s$^{-1}$ (incident photon flux density). This enhancement of absorption and photosynthesis by intercellular reflectance, without additional production and maintenance of photosynthetic pigments, may maintain shade leaves above the photosynthetic light compensation point between sunflecks and maintain the light induction state during protracted periods of low diffuse light.**

*Key-words:* absorptance; fibre-optic probe; intercellular reflectance; leaf anatomy; leaf optics; light gradients; light scattering; oil infiltration; photosynthesis; shade adaptation; spongy mesophyll.

## INTRODUCTION

The leaves of terrestrial plants vary dramatically in their morphology and anatomy. This variation is evident across species and habitats, and many adaptive scenarios have been proposed to explain these differences. Morphological features, such as leaf display and shape, influence light interception and boundary layer thickness, and thus affect light absorption, energy balance and gaseous diffusion. Despite the potential importance of leaf anatomy to the internal distribution of light (Vogelmann 1993) and gases (Parkhurst 1994), little is known about the consequences of variation in internal structure.

Perhaps the most profound differences in leaf anatomy, phenotypically and between species, are evident for plants growing in different light regimens. Typical sun-type leaves of mesophytes utilizing $C_3$ photosynthesis are thick with single or multiple layers of palisade parenchyma beneath the adaxial epidermis, followed by a relatively thin spongy parenchyma (Boardman 1977; Bjorkman 1981). The columnar-shaped cells of the palisade parenchyma facilitate light penetration through to the spongy tissue (Vogelmann & Martin 1993; Vogelmann 1993). These cells act like light guides, propagating light through the tube-shaped vacuoles and intercellular air spaces. The distribution of light through the depth of leaves in high-light habitats, combined with acclimation of chloroplasts to their localized light environment, may maximize rates of photosynthesis (Terashima & Inoue 1984a,b) and reduce susceptibility to photoinhibition (Nishio, Sun & Vogelmann 1993).

Shade-type leaves, in contrast, are thinner with a greatly reduced number of palisade layers and proportionately

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more spongy parenchyma (Lee *et al.* 1990). The functional significance of the high proportion of spongy mesophyll in shade leaves is unclear. Collimated light entering a leaf becomes highly scattered, partially by multiple reflections from the numerous cell wall–air space interfaces encountered as light passes through the leaf. The greater proportion and rounded shape of the spongy parenchyma of shade leaves may increase light scattering and increase the mean pathlength of light in leaves. Because light absorption is a function of both pigment content and pathlength, an increase in absorption may also occur. This increase in absorption due to scattering may be adaptive in light-limited understorey habitats.

To test the hypotheses that light scattering within shade leaves increases absorption and photosynthesis under light-limiting conditions, we measured the absorptance spectra of control leaves and leaves that were vacuum-infiltrated with mineral oil. Mineral oil has a refractive index that is very similar to the refractive index of plant cell walls (Woolley 1971; Gausman *et al.* 1974). Thus, internal reflections caused by cell wall–air space interfaces should be removed by oil infiltration. For shade-adapted *Hydrophyllum canadense*, we calculated that air space reflectance may increase photosynthesis at low light (*c.* 197%) relative to theoretical leaves without intercellular reflectance.

## MATERIALS AND METHODS

### Plant material

Initial characterization of the contribution of intercellular reflectance to light absorption was conducted with two shade species, *Hydrophyllum canadense* (L.) and *Asarum canadense* (L.), growing in the understorey of a maple-oak forest in central Illinois (University of Illinois Ecological Research Area, 6 km north-east of Urbana, IL, USA). These species are rhizomatous herbs that have typical shade-type leaf morphology (DeLucia *et al.* 1991). Both species have bicoloured leaves with a poorly developed palisade layer and most of the leaf mesophyll is composed of loosely packed spongy parenchyma. To examine the effect of growth light environment on internal reflectance, measurements were made on leaves of *Acer saccharum* (Marshall) and *Parthenocissus quinquefolia* (L.) Planchon from this same forest, which developed under full sun at the forest edge or under full shade.

Sun–shade comparisons were also made with leaves of *Thermopsis montana* Nutt. collected from the understorey of a *Populus–Salix* canopy or in the open in an adjacent *Artemisia* stand in the Laramie Mountains, Wyoming, USA. *Thermopsis montana* forms multiple palisade cells when grown in the open (Vogelmann & Martin 1993). *Smilacina stellata* (L.), a monocot that produces only spongy palisade, was also collected from the forest understorey.

To examine the effect of leaf anatomy on intercellular reflectance independently from the potentially confounding effects of sun–shade acclimation, we compared the optical properties of high-light-grown leaves of two species of *Populus*. This genus includes species with widely varying leaf orientations and internal anatomies. Measurements were made on leaves from *Populus trichocarpa* (T. & G.), which has highly bicoloured leaves with a prominent palisade layer under the adaxial epidermis and a very large spongy mesophyll, and *P. deltoides* (L.), which has symmetrical leaf colour with palisade parenchyma under the adaxial and abaxial leaf surfaces. Plants were grown from cuttings in the OEB glasshouse at Harvard University, Cambridge, MA, USA.

For all species measurements were made on fully expanded leaves that appeared healthy. Hydrated leaves were transported to the laboratory and measurements were completed within 1 h of collection.

### Optical measurements

Reflectance (diffuse and specular) and transmittance of control and oil-infiltrated leaves were measured with a Taylor-type integrating sphere (model 1800-12, LiCor, Lincoln, NE, USA). The sphere was connected to a dual-grating spectroradiometer (model 752, Optronic Laboratories, Orlando, FL, USA) via a quartz fibre-optic bundle. The spectroradiometer was configured with 0·25, 0·50 and 0·25 mm slits in the optical path, providing a nominal half-bandwidth of 0·5 nm. Wavelength calibration of the spectroradiometer was conducted frequently by scanning the emission line at 546·1 nm from a fluorescent source.

Spectral reflectance ($R$) was calculated as $(I_s - I_d)/(I_r - I_d)$, where $I_s$ is the output from the sphere when the leaf was illuminated and $I_r$ is the output when a reference standard (100% reflectance) is illuminated. Before measuring each leaf, scattered light ($I_d$) within the sphere was measured. This was necessary because the tungsten source used to illuminate the leaf was not perfectly collimated and, although $I_d$ was typically <0·7% of light entering the sphere, this led to a significant error in the calculated reflectance for high-transmitting samples. Spectral transmittance ($T$) was calculated as $I_t/I_r$, where $I_t$ is the sphere output when light is passed through the leaf before entering the sphere. Absorptance was calculated as $1 - R - T$.

Measurements were made on controls and leaves with the intercellular air space infiltrated with mineral oil (paraffin oil, heavy; Fischer Scientific, New Brunswick, NJ, USA). Leaf discs were floated on mineral oil in a desiccator and a gentle vacuum was applied and released several times until infiltration was complete. The loss of buoyancy and colour change following infiltration indicated that all intercellular air space had become filled with oil. Three to five scans from 380 to 800 nm at 2 nm intervals were averaged for each measurement.

### Light gradients inside leaves

The penetration of light into shade leaves of *Hydrophyllum*

*canadense* and *Asarum canadense* was measured with a fibre-optic microprobe system as described in Vogelmann *et al.* (1991). Microprobes were made from 125-$\mu$m-diameter (OD) multimode step-index fibres made of fused silica (Polymicro Technologies, Phoenix, AZ, USA). Heated fibres were drawn to a tip diameter of *c.* 5 $\mu$m, and the tapered region of the probes was then coated with evaporated chromium and truncated with a diamond knife. Light entry was confined to the tip of the probes that had a near-perfect Gaussian acceptance angle (50% acceptance half-bandwidth) of 27–34°. Photons captured by the probe were measured with a calibrated spectroradiometer (model 742, Optronic Laboratories) and stored in a computer via an A/D converter.

Small leaf sections were clamped between plastic coverslips through which small holes had been drilled, and leaves were illuminated with a collimated beam from a 150 W xenon arc lamp (Hanovia 901c-1). The microprobe was passed through the leaf by a computer-controlled stepper motor (Stepper-mike, model 18515, Oriel, Stratford, CT, USA) at a rate of 6 $\mu$m s$^{-1}$. Depending on leaf thickness each scan took between 25 and 40 s. By orienting the probe relative to the leaf, measurements were made of the penetration of transmitted, forward-scattered and back-scattered light. Transmitted light gradients were measured by directing light perpendicular to the adaxial leaf surface and passing the probe through the leaf, from the abaxial to the adaxial surface, parallel to the light beam. The penetration of forward-scattered light was measured by orienting the path of the probe at 150° from the light beam, and the penetration of back-scattered light was measured by passing the probe through the adaxial surface at a 30° angle from the light beam. Measurements were made at 550, 680 and 750 nm. Light at 680 nm represents an absorption peak of chlorophyll and most of the photosynthetically active radiation (PAR) at depth in leaves centres on 550 nm. Measurements in the near-infrared (750 nm) provided the opportunity to examine the effect of scattering on absorption independently of gradients of chlorophyll within leaves.

After the microprobe measurements, fresh sections (*c.* 20 $\mu$m) were prepared close to the point of entry of the probe and leaf dimensions were measured with a calibrated ocular micrometer at 80×.

## Fluorescence and photosynthesis measurements

To assess the potential contribution of intercellular reflectance to photosynthesis, the initial fluorescence ($F_o$) emitted from the adaxial and abaxial surfaces was measured from: (1) control leaves, (2) leaves with a thin layer of mineral oil on the epidermis and (3) oil-infiltrated leaves. After a 10 min dark acclimation period, fluorescence was detected at wavelengths greater than 700 nm following excitation with a modulated measuring beam (PAM-2000, Walz, Effeltrich, Germany). $F_o$ is proportional to light absorbed by the pigment bed of the photosystem II reaction centre and provides an indication of the amount of light that is available to drive photosynthesis. Because $F_o$ is measured at very low irradiances [<1 $\mu$mol m$^{-2}$ s$^{-1}$, photon flux density (PFD); peak irradiance at 650 nm], electron flow is not initiated. This measurement can therefore be made on oil-infiltrated leaves. Light emitted by fluorescence is detected at wavelengths where leaf absorptance is low ($\lambda > 700$ nm), and because it is emitted from chloroplasts close to the irradiated leaf surface reabsorption of fluorescence should be small and not substantially alter the interpretation of the results. The quantum yield of fluorescence was calculated from $F_o$ measured at several low irradiances.

The photosynthetic response ($O_2$ flux) to adaxial irradiance at saturating $CO_2$ for *H. canadense* and *A. canadense* was measured with a leaf disc oxygen electrode (LD2, Hansatech, Kings Lynn, UK) as described by Delieu & Walker (1981). The leaf disc was illuminated with a fixed-output metal halogen lamp (LS2, Hansatech) in conjunction with neutral-density filters (Melles-Griout, Irvine, CA, USA). Photosynthetically active radiation passing through the filters was measured with a quantum sensor (LI-190SB, LiCor), and photosynthesis was measured at a range of irradiances from 0 to 2000 $\mu$mol m$^{-2}$ s$^{-1}$ (PFD). Before measurement, leaf discs (10 cm$^2$) were exposed to an induction cycle of repeated exposure to 0 and 250 $\mu$mol m$^{-2}$ s$^{-1}$, and measurements were made from low to high irradiance. Four replicate leaves were measured for each species.

A power function was used to describe the photosynthetic response to irradiance:

$$y = (a \times x)/\{1 + [(a \times x)/b]^{1/c}\} + d,$$

where $y$ is net photosynthesis, $a$ is the initial slope or maximum quantum yield, $x$ is incident irradiance, $d$ is dark respiration and $c$ is a convexity term that describes the degree of curvature at intermediate irradiances. This equation, which was fitted to the data by a least-squares method, provided a better fit at low irradiances than the non-quadratic hyperbola discussed in Zhang (1989) and Baker & Ort (1992).

The rate of photosynthesis at different irradiances that would occur without intercellular reflectance was calculated from the photosynthetic response to irradiance expressed on an absorbed light basis and the absorptance of oil-infiltrated leaves. For each photosynthetic measurement, absorbed light was calculated by multiplying the spectral absorptance of the adaxial leaf surface (absorptance at each wavelength) by the spectral irradiance of the light source. An equation describing the photosynthetic response to absorbed irradiance was then calculated using the power function described previously. For each incident light level the amount of light absorbed by the leaf without intercellular reflectance was calculated from the absorptance spectra of the oil-infiltrated leaf. This absorbed light was then used to calculate the photosynthetic rate from the function describing the photosynthetic response to absorbed light. The photosynthetic response to incident irradiance for a leaf without intercellular reflectance was

then calculated by plotting this photosynthetic rate against the initial incident irradiance. A simplifying assumption of this analysis was that changes in the light gradient inside leaves caused by infiltration, and the resulting differences in the photosynthetic contribution of different cell layers, had a small effect on the overall photosynthetic–light response function. This may not be the case, as indicated by Fukshansky & Martinez v. Remisowsky (1992).

## RESULTS

### The effect of oil infiltration on leaf optics

Oil infiltration altered the optical properties of shade leaves of *Hydrophyllum canadense* and *Asarum canadense* similarly; therefore data are presented only for *H. canadense*. Below approximately 700 nm, oil infiltration caused a small decrease in reflectance from the adaxial leaf surface (Fig. 1). This decrease was greatest for poorly absorbed wavelengths in the green portion of the spectrum (*c.* 550 nm) and above 725 nm, and smallest in the regions of maximum absorptance at 680 nm and below 500 nm. A pronounced increase in transmittance (233% at 550 nm) was observed for oil-infiltrated leaves (Fig. 1) and, as for reflectance, oil infiltration increased transmittance most at 500–600 nm and above 725 nm. The increase in transmittance and decrease in reflectance reduced absorptance across the spectrum, but the differences were greatest at 500 nm and above 700 nm, where oil infiltration caused an approximate 25% and 30% decrease in absorptance, respectively.

Absorptance of shade-grown leaves of *Acer saccharum* and *Parthenocissus quinquefolia* was reduced more by oil infiltration than that of sun-grown leaves (Fig. 2), and the absorptance difference [(control − oil infiltrated)/control] at 550 nm was approximately 20% greater for shade than for sun leaves.

Leaves of *Populus deltoides* were relatively symmetrical in internal anatomy, whereas leaves of *P. trichocarpa* were highly asymmetrical, resulting in pronounced bicolouration. Below the adaxial epidermis (12 ± 4 μm thick) of *P. deltoides* were two layers of palisade parenchyma (84 ± 12 μm), followed by a relatively thin spongy mesophyll (24 ± 8 μm) and another two layers of palisade parenchyma (76 ± 16 μm). The lower palisade cells were more rounded than the highly columnar cells of the adaxial palisade. The lower epidermis was 16 ± 4 μm and the mean thickness of the entire leaf was 216 ± 20 μm. For *P. tri-*

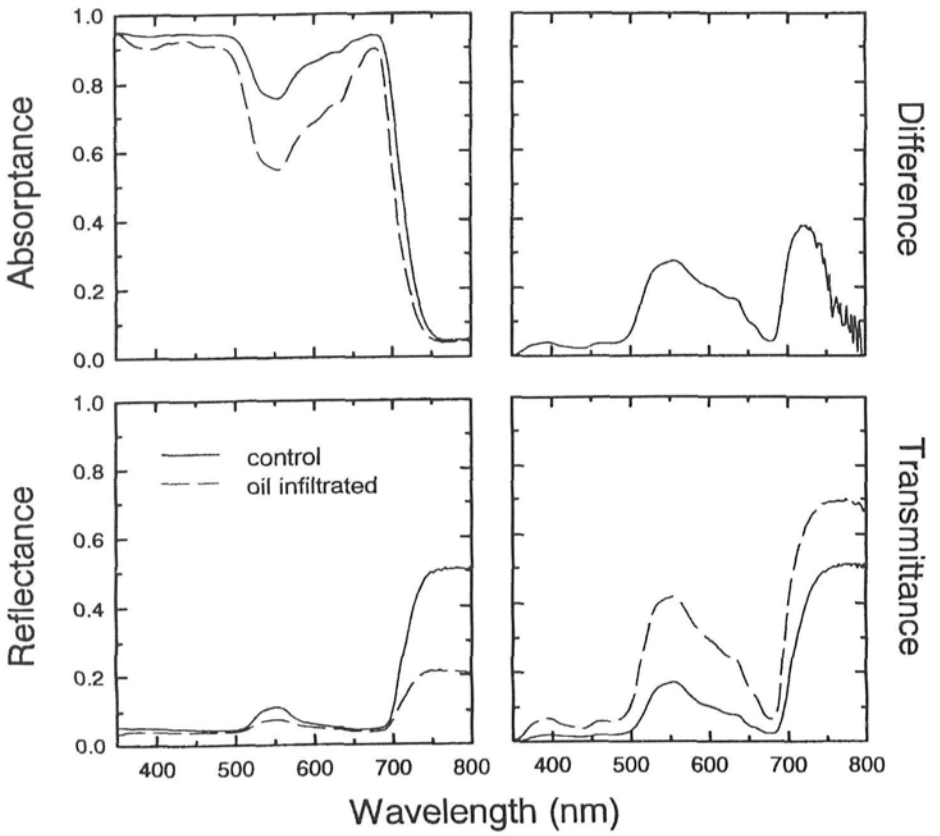


**Figure 1.** Absorptance, reflectance and transmittance spectra for the adaxial leaf surface of *Hydrophyllym canadense*. Data for control leaves are indicated by the solid lines, and the dashed lines are for leaves infiltrated with mineral oil to remove intercellular reflectance (see 'Materials and methods'). The fractional difference in absorptance between control and treatment leaves [$(A_{control}-A_{oil})/A_{control}$] is shown in the panel marked 'Difference'. Each line represents a mean of 3–5 scans from different leaves and the coefficient of variation was typically less than 5%.

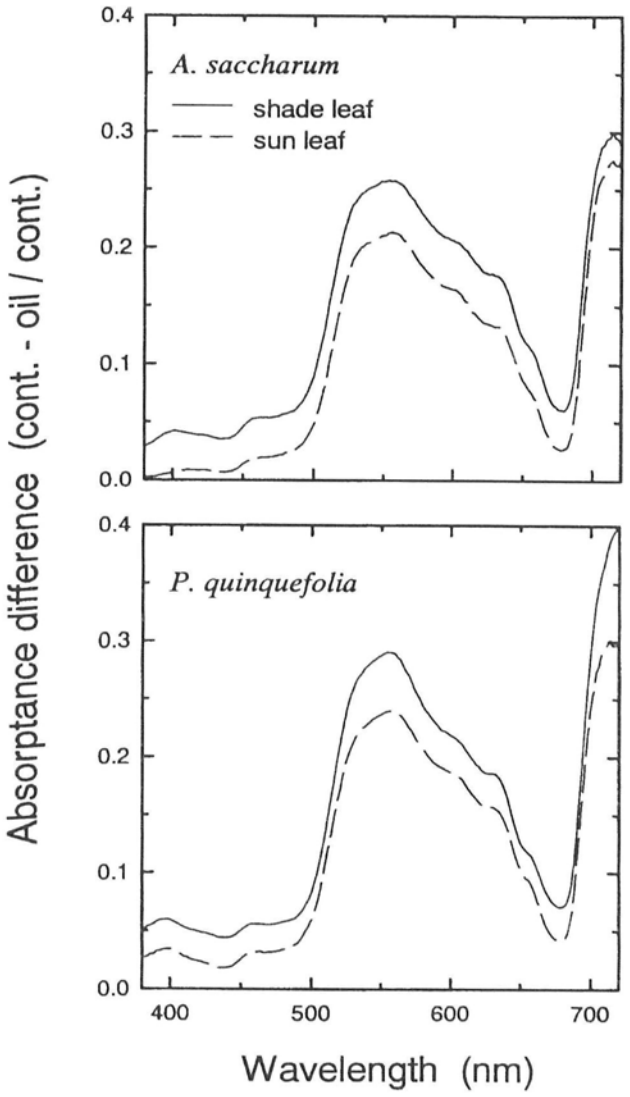


**Figure 2.** The fractional difference in absorptance between control and oil-infiltrated leaves $[(A_{control}-A_{oil})/A_{control}]$ for shade-grown and sun-grown leaves of *Acer saccharum* and *Parthenocissus quinquefolia*. Data for shade leaves are indicated by the solid lines, and the dashed lines are for sun leaves. Each line represents a mean of five scans from different leaves and the coefficient of variation was typically less than 5%.

*chocarpa*, the adaxial epidermis was subtended by two layers of palisade parenchyma followed by the spongy mesophyll, an 'air-spongy' mesophyll and the abaxial epidermis. The air-spongy mesophyll was a relatively large area which contained very few lightly pigmented cells. Leaf thickness for *P. trichocarpa* was $272\pm12\,\mu$m, and the thickness of the respective cell layers, beginning with the adaxial epidermis, was $20\pm4\,\mu$m, $72\pm12\,\mu$m, $56\pm28\,\mu$m, $108\pm24\,\mu$m and $16\pm4\,\mu$m.

The adaxial surface of the highly bicoloured leaves of *Populus trichocarpa* responded in a qualitatively similar manner to oil infiltration as the shade leaves of *H. canadense* and *A. canadense*. Lower reflectance and higher transmittance for infiltrated leaves caused a decrease in absorptance at 550 nm from *c.* 80 to 63% (Fig. 3). Reflectance at 550 nm from the abaxial surface of this species was high (30%) compared with the adaxial surface (10%).

Oil infiltration caused a dramatic decrease in reflectance from the abaxial surface of *P. trichocarpa* leaves to levels similar to those measured for the adaxial surface. This decrease in reflectance had a proportionately greater effect on abaxial absorptance than the oil-induced increase in transmittance. For this species, oil infiltration reduced reflectance from the spongy mesophyll and from the internal abaxial epidermis and caused an increase in abaxial absorptance.

The colour asymmetry among leaf surfaces observed for *P. trichocarpa* was not evident for *P. deltoides* (Fig. 4). Reflectance was slightly higher from the abaxial than from the adaxial leaf surface, but transmittance through the two surfaces was similar. As with the other species, oil infiltration decreased reflectance and increased transmittance; however, these effects were largely off-setting and oil infiltration had only a small influence on absorptance from either leaf surface. Thus, the bicoloured leaf of *P. trichocarpa* was most strongly influenced by oil infiltration.

## Light gradients inside leaves

Light captured by the fibre-optic probe within leaves of *Hydrophyllum canadense* is influenced by absorption and the amount of light that is scattered out of the acceptance cone of the probe. At 550 and 680 nm, transmitted light was attenuated strongly in the palisade parenchyma, dropping to below 10% of incident levels by 70 $\mu$m into the adaxial leaf surface (Fig. 5). Oil infiltration reduced intercellular scattering and greatly decreased the attenuation of visible light in the palisade mesophyll. The differences in attenuation between infiltrated and control leaves, and thus the contribution of intercellular reflectance, was greatest from the middle of the palisade mesophyll to the middle of the spongy mesophyll (from approximately 40 to 120 $\mu$m).

Attenuation of 750 nm light was linear with depth in oil-infiltrated leaves (Fig. 5). The primary factor contributing to attenuation of this wavelength in infiltrated leaves is scattering originating from facets other than cell wall–air space interfaces, such as organelles and cytosol/vacuolar boundaries.

Forward-scattered light at 550 and 680 nm was also attenuated strongly in the palisade mesophyll (Fig. 6). After an initial drop in the first 40 $\mu$m, forward-scattered light remained constant through the remaining palisade and spongy mesophyll and only declined again through the abaxial epidermis. Unlike transmitted and back-scattered light, the pattern of attenuation of forward-scattered light was not affected by oil infiltration.

Back-scattered light at 550 nm was fairly constant across the adaxial epidermis and into the palisade mesophyll, and began to attenuate at the interface of the palisade and spongy tissues (Fig. 7). Removal of intercellular reflectance by infiltration caused back-scattered light to be distributed uniformly throughout the leaf.

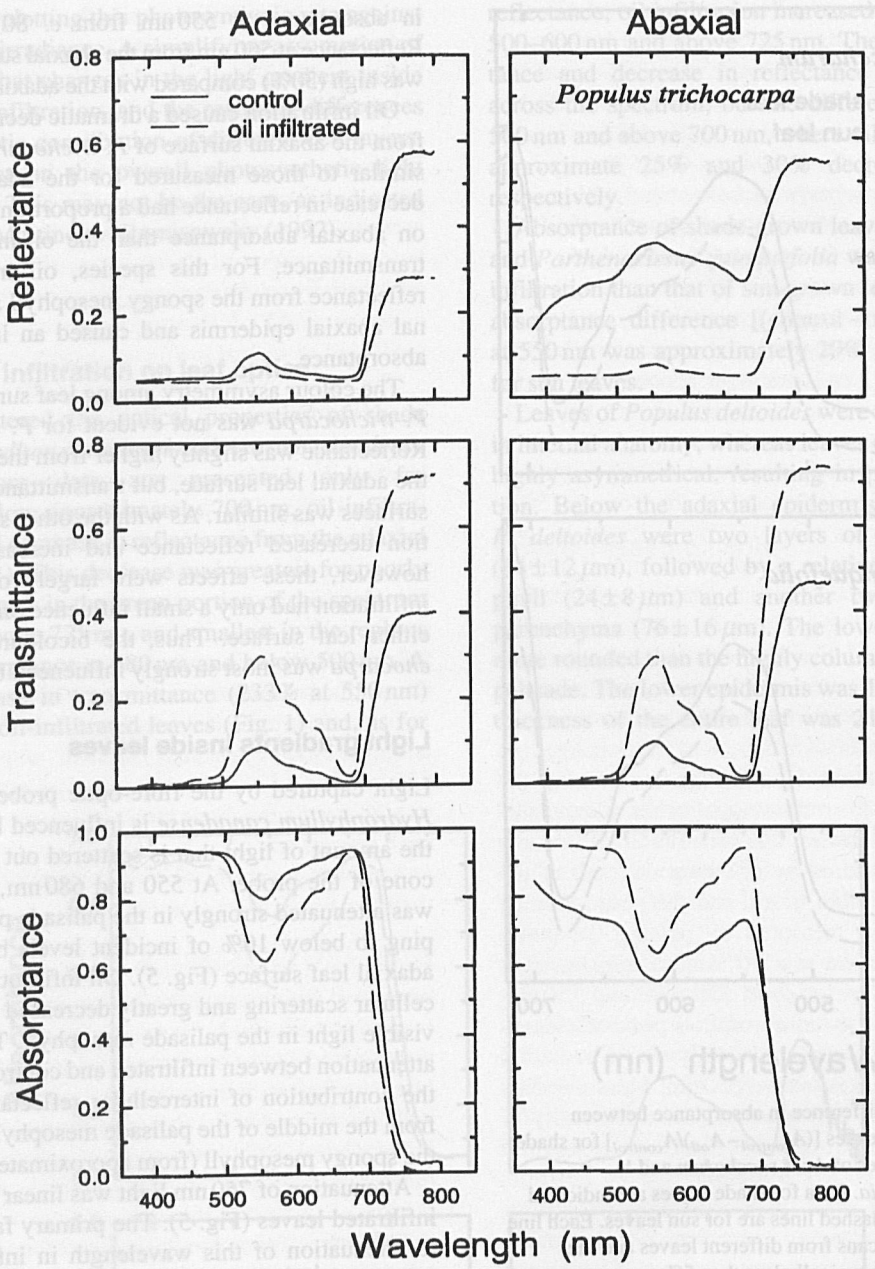


**Figure 3.** Absorptance, reflectance and transmittance spectra for the adaxial and abaxial leaf surfaces of *Populus trichocarpa*. Data for control leaves are indicated by the solid lines, and the dashed lines are for leaves infiltrated with mineral oil. Each line represents a mean of five scans from different leaves.

## The effect of intercellular reflectance on photosynthesis

The initial level of chlorophyll fluorescence ($F_o$) increased linearly with increasing incident irradiance up to approximately 0·3 $\mu$mol $m^{-2}$ $s^{-1}$ (Fig. 8). An actinic effect would have caused a decrease in $F_o$ at higher irradiances, and therefore these low irradiances provided insufficient energy to cause electron flow. For *Hydrophyllum canadense*, surface oil and oil infiltration caused a decrease in fluorescence yield ($\Delta F_o/\Delta$ irradiance) from the adaxial leaf surface (Fig. 8 & Table 1). The decrease in fluorescence yield with the application of surface oil may have resulted from the elimination of the lens effect of epidermal cells (Poulson & Vogelmann 1990; Myers, Vogelmann & Bornman 1994). Oil infiltration caused a further 39% decrease in fluorescence yield. A similar decline was caused by infiltration with water (54%; Table 1) so it is unlikely that oil decreased fluorescence yield by entering cells and solubilizing photosynthetic membranes. We interpret the decrease in fluorescence yield resulting from oil infiltration to be a

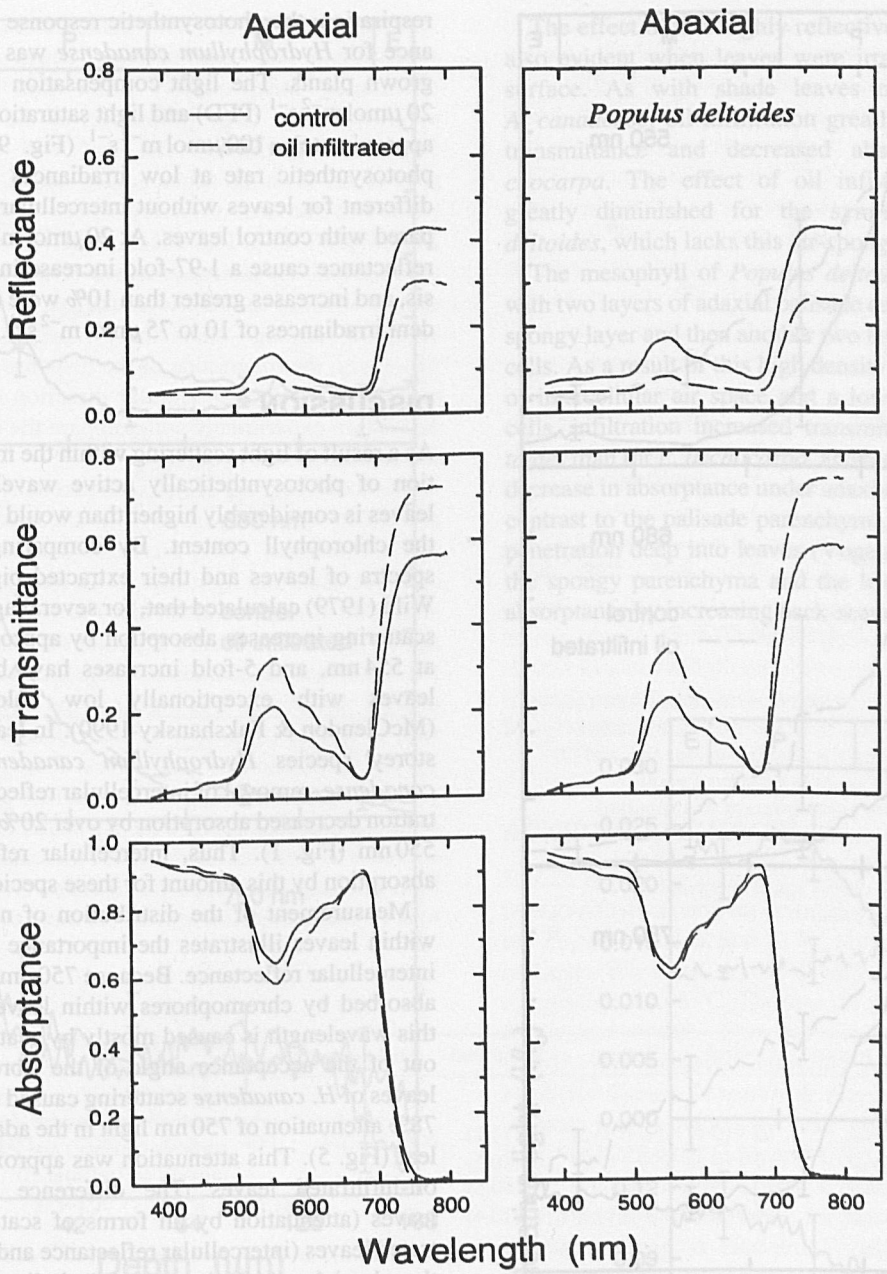


**Figure 4.** Absorptance, reflectance and transmittance spectra for the adaxial and abaxial leaf surfaces of *Populus deltoides*. Data for control leaves are indicated by the solid lines, and the dashed lines are for leaves infiltrated with mineral oil. Each line represents a mean of five scans from different leaves.

result of a reduction in intercellular reflectance and thus reduced light absorption by chlorophyll.

Reduction of intercellular reflectance caused an approximately 39–52% reduction in fluorescence yield for the adaxial surface of shade leaves, but only a 21–25% reduction in sun leaves. Thus, the contribution of intercellular reflectance to light absorption, at least in the first 40–50 $\mu$m, where most fluorescence originates, was greater for shade than sun leaves. With the exception of *Smilacina stellata*, infiltration caused a greater reduction in fluorescence yield when illuminated from the adaxial than from the abaxial leaf surface. As the only monocot included in this study, *S. stellata* has structurally symmetrical leaves composed only of spongy mesophyll.

A power function provided a good fit to the measured photosynthetic response to incident irradiance (Fig. 9). Although fitting statistics were not calculated, the measured values were within 10% of the predicted photosynthetic rate at all irradiances. The fitting parameters were: *a* (initial slope), 0·1318; *b* (maximum photosynthetic rate), 6·58; *c* (convexity), 2·195; and *d* (dark respiration), –2·642. With the exception of relatively high rates of dark

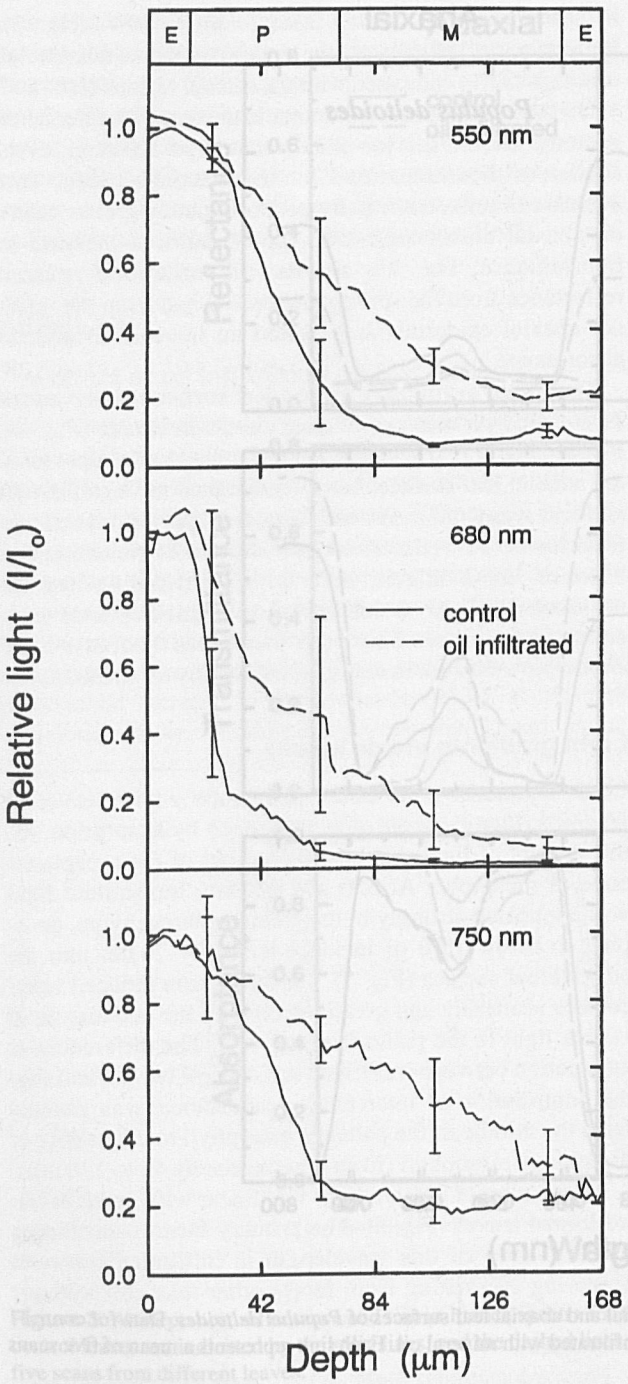


**Figure 5.** Distribution of transmitted 550, 680 and 750 nm light through leaves of *Hydrophyllum canadense*. The relative amount of light [measured in the leaf ($I$)/incident ($I_o$)] is shown as a function of depth into the leaf. The leaf was irradiated on the adaxial surface (represented by the left side of the panels) and the probe was passed from the abaxial epidermis toward the light (right to left in the graph). The bar at the top indicates the thickness of, from left to right, the adaxial epidermis (E), the palisade parenchyma (P), the spongy mesophyll (M) and the abaxial epidermis (E). Data for control leaves are indicated by the solid lines, and the dashed lines are for leaves infiltrated with mineral oil. Each line represents a mean of six scans from different leaves and the error bars represent one standard deviation.

respiration, the photosynthetic response to incident irradiance for *Hydrophyllum canadense* was typical of shade-grown plants. The light compensation point occurred at 20 $\mu$mol m$^{-2}$ s$^{-1}$ (PFD) and light saturation was achieved at approximately 100 $\mu$mol m$^{-2}$ s$^{-1}$ (Fig. 9). The estimated photosynthetic rate at low irradiances was dramatically different for leaves without intercellular reflectance compared with control leaves. At 20 $\mu$mol m$^{-2}$ s$^{-1}$ intercellular reflectance cause a 1·97-fold increase in net photosynthesis, and increases greater than 10% were evident from incident irradiances of 10 to 75 $\mu$mol m$^{-2}$ s$^{-1}$.

## DISCUSSION

As a result of light scattering within the mesophyll, absorption of photosynthetically active wavelengths by intact leaves is considerably higher than would be predicted from the chlorophyll content. By comparing the absorption spectra of leaves and their extracted pigments, Ruhle & Wild (1979) calculated that, for several agronomic species, scattering increases absorption by approximately 2·6-fold at 554 nm, and 5-fold increases have been reported for leaves with exceptionally low chlorophyll content (McClendon & Fukshansky 1990). In leaves of the understorey species *Hydrophyllum canadense* and *Asarum canadense*, removal of intercellular reflectance by oil infiltration decreased absorption by over 20% at approximately 550 nm (Fig. 1). Thus, intercellular reflection increased absorption by this amount for these species.

Measurement of the distribution of near-infrared light within leaves illustrates the importance of scattering and intercellular reflectance. Because 750 nm light is so poorly absorbed by chromophores within leaves, attenuation of this wavelength is caused mostly by scattering of photons out of the acceptance angle of the fibre-optic probe. In leaves of *H. canadense* scattering caused an approximately 78% attenuation of 750 nm light in the adaxial 80 $\mu$m of the leaf (Fig. 5). This attenuation was approximately 35% for oil-infiltrated leaves. The difference between control leaves (attenuation by all forms of scattering) and infiltrated leaves (intercellular reflectance and reflectance from the abaxial epidermis removed) indicates that approximately 50% of the light scattering in the palisade parenchyma of this species was caused by intercellular and epidermal reflectance—the remaining 50% is attributed to other cellular facets, plastids and starch grains (Ruhle & Wild 1979; Vogelmann 1993). For *Hydrophyllum canadense* and *Asarum canadense* (data not shown), intercellular reflectance increased absorptance by maintaining high levels of back-scattered light (Fig. 7).

The possible role of the spongy parenchyma and internal air space in maintaining a high level of internal reflectance is evident from the comparison of *Populus trichocarpa* and *P. deltoides*. The palisade parenchyma of *P. trichocarpa* is subtended by a spongy mesophyll and then a large air space with very few cells. As a result of this air-spongy layer and the internal surface of the abaxial epidermis, the abaxial surface is highly reflective. In excess of 30%

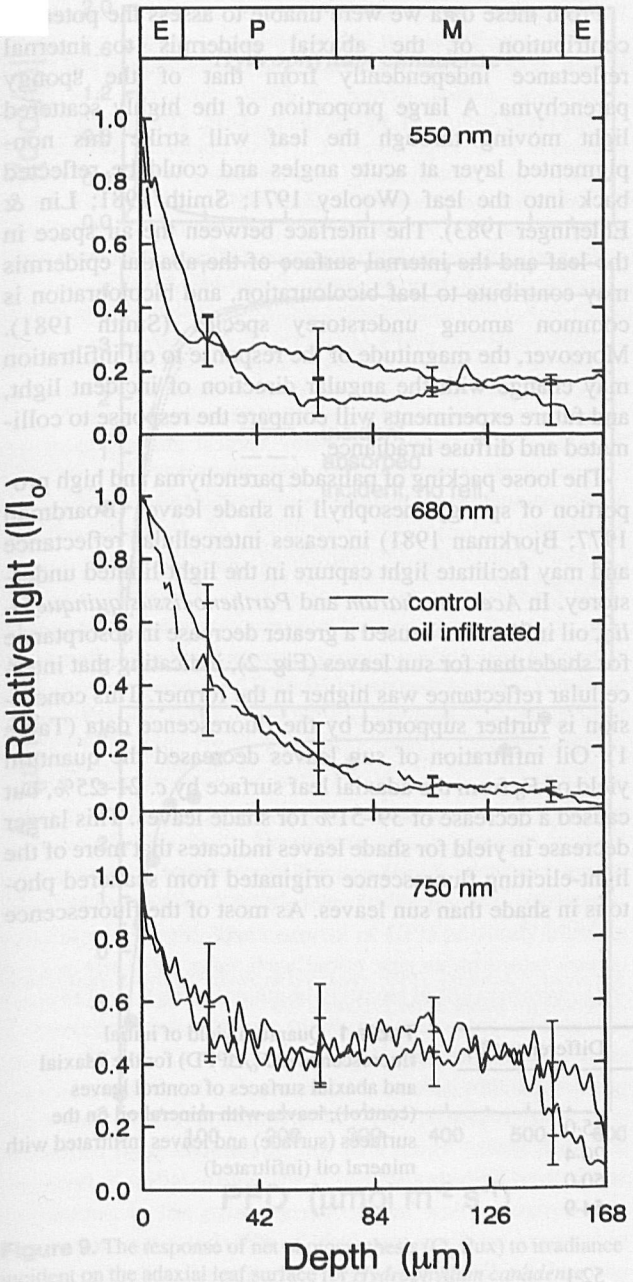


**Figure 6.** Distribution of forward-scattered 550, 680 and 750 nm light into leaves of *Hydrophyllum canadense*. The relative amount of light [measured in the leaf ($I$)/incident ($I_o$)] is shown as a function of depth into the leaf. The leaf was irradiated on the adaxial surface (represented by the left side of the panels) and the probe was passed from the abaxial epidermis toward the adaxial epidermis at 150° from the light beam (right to left in the graph).

(550 nm) of the light incident on this surface is reflected and the leaf underside appears white (Fig. 3). By eliminating intercellular reflectance, oil infiltration increased transmittance and greatly reduced reflectance from the abaxial surface — the bottom surface of infiltrated leaves became the same green as the adaxial surface. Because the decrease in reflectance was larger than the increase in transmittance, oil infiltration increased abaxial absorptance for this species.

The effect of this highly reflective air-spongy layer was also evident when leaves were irradiated on the adaxial surface. As with shade leaves of *H. canadense* and *A. canadense*, oil infiltration greatly increased in adaxial transmittance and decreased absorptance for *P. trichocarpa*. The effect of oil infiltration was, however, greatly diminished for the symmetrical leaves of *P. deltoides*, which lacks this air-spongy layer.

The mesophyll of *Populus deltoides* is densely packed, with two layers of adaxial palisade cells followed by a dense spongy layer and then another two layers of abaxial palisade cells. As a result of this high density of cells, a low volume of intercellular air space and a low proportion of spongy cells, infiltration increased transmittance less for *P. deltoides* than for *P. trichocarpa*, and there was only a marginal decrease in absorptance under adaxial irradiation (Fig. 4). In contrast to the palisade parenchyma, which facilitates light penetration deep into leaves (Vogelmann & Martin 1993), the spongy parenchyma and the lower epidermis increase absorptance by increasing back-scattering.

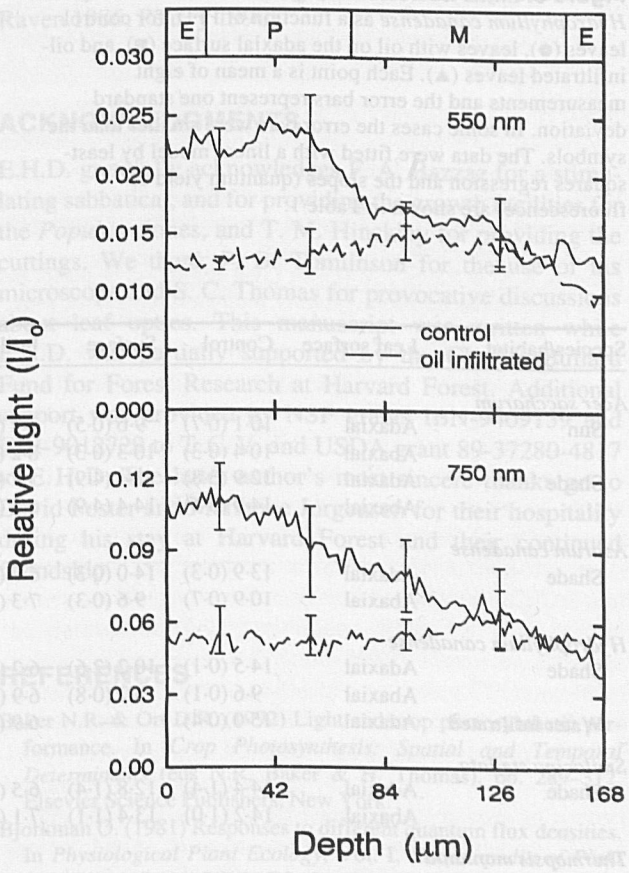


**Figure 7.** Distribution of back-scattered 550 and 750 nm light into leaves of *Hydrophyllum canadense*. The relative amount of light [measured in the leaf ($I$)/incident ($I_o$)] is shown as a function of depth into the leaf. The leaf was irradiated on the adaxial surface (represented by the left side of the panels) and the probe was passed from the adaxial epidermis toward the abaxial epidermis at 30° from the light beam (left to right in the graph).

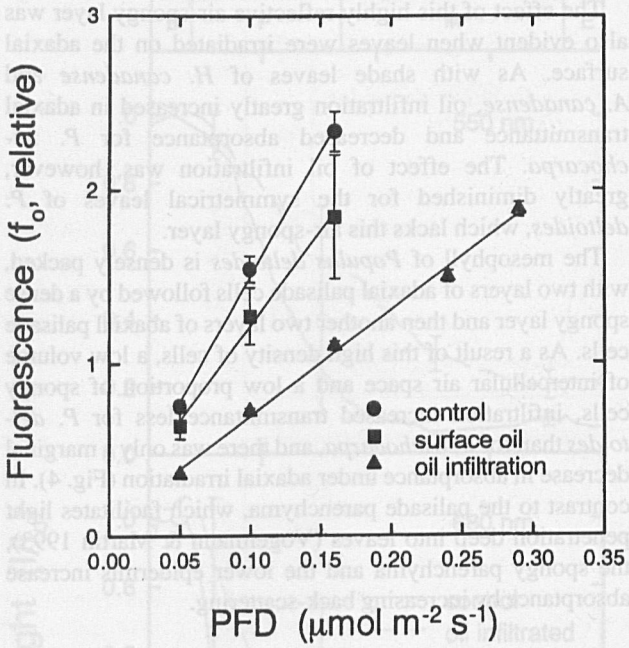


**Figure 8.** Initial fluorescence ($F_o$) from the adaxial surface of *Hydrophyllum canadense* as a function of PFD, for control leaves (●), leaves with oil on the adaxial surface (■), and oil-infiltrated leaves (▲). Each point is a mean of eight measurements and the error bars represent one standard deviation. In some cases the error bars were smaller than the symbols. The data were fitted with a linear model by least-squares regression and the slopes (quantum yield of fluorescence) are shown in Table 1.

From these data we were unable to assess the potential contribution of the abaxial epidermis to internal reflectance independently from that of the spongy parenchyma. A large proportion of the highly scattered light moving through the leaf will strike this non-pigmented layer at acute angles and could be reflected back into the leaf (Wooley 1971; Smith 1981; Lin & Ehleringer 1983). The interface between the air space in the leaf and the internal surface of the abaxial epidermis may contribute to leaf bicolouration, and bicolouration is common among understorey species (Smith 1981). Moreover, the magnitude of the response to oil infiltration may change with the angular direction of incident light, and future experiments will compare the response to collimated and diffuse irradiance.

The loose packing of palisade parenchyma and high proportion of spongy mesophyll in shade leaves (Boardman 1977; Bjorkman 1981) increases intercellular reflectance and may facilitate light capture in the light-limited understorey. In *Acer saccharum* and *Parthenocissus quinquefolia*, oil infiltration caused a greater decrease in absorptance for shade than for sun leaves (Fig. 2), indicating that intercellular reflectance was higher in the former. This conclusion is further supported by the fluorescence data (Table 1). Oil infiltration of sun leaves decreased the quantum yield of $F_o$ from the adaxial leaf surface by *c.* 21–25%, but caused a decrease of 39–51% for shade leaves. This larger decrease in yield for shade leaves indicates that more of the light-eliciting fluorescence originated from scattered photons in shade than sun leaves. As most of the fluorescence

**Table 1.** Quantum yield of initial fluorescence ($\Delta F_o/\Delta$PFD) for the adaxial and abaxial surfaces of control leaves (control), leaves with mineral oil on the surfaces (surface) and leaves infiltrated with mineral oil (infiltrated)

| Species/habitat | Leaf surface | Control | Surface | Infiltrated | Difference (%) |
|---|---|---|---|---|---|
| *Acer saccharum* | | | | | |
| Sun | Adaxial | 10·1 (0·7) | 9·6 (0·5) | 7·2 (1·1) | 25·0 |
| | Abaxial | 10·4 (0·3) | 10·3 (0·3) | 8·2 (0·5) | 20·4 |
| Shade | Adaxial | 12·9 (3·8) | 11·4 (4·9) | 5·7 (0·5) | 50·0 |
| | Abaxial | 14·9 (3·4) | 14·4 (4·9) | 6·5 (0·4) | 54·9 |
| *Asarum canadense* | | | | | |
| Shade | Adaxial | 13·9 (0·3) | 14·0 (0·3) | 6·7 (0·1) | 52·1 |
| | Abaxial | 10·9 (0·7) | 9·6 (0·3) | 7·3 (0·1) | 24·0 |
| *Hydrophyllum canadense* | | | | | |
| Shade | Adaxial | 14·5 (0·1) | 10·2 (2·6) | 6·2 (0·1) | 39·2 |
| | Abaxial | 9·6 (0·1) | 8·2 (0·8) | 6·9 (0·1) | 15·9 |
| Water infiltrated | Adaxial | 15·0 (0·4) | — | 6·9 (0·4) | 54·0 |
| *Smilacina stellata* | | | | | |
| Shade | Adaxial | 14·4 (1·4) | 12·8 (1·4) | 6·5 (0·6) | 49·2 |
| | Abaxial | 14·2 (1·0) | 13·4 (1·1) | 7·1 (0·5) | 47·0 |
| *Thermopsis montanta* | | | | | |
| Sun | Adaxial | 10·2 (1·3) | 8·9 (0·5) | 7·0 (0·6) | 21·3 |
| | Abaxial | 10·9 (1·1) | 9·5 (0·5) | 7·6 (0·6) | 20·0 |
| Shade | Adaxial | 10·2 (1·2) | 8·3 (0·9) | 4·6 (0·4) | 44·6 |
| | Abaxial | 11·1 (1·4) | 7·1 (4·4) | 5·4 (0·8) | 23·9 |

Each value is a mean of eight leaves followed by the standard deviation in parentheses. The decrease in fluorescence yield with oil infiltration (% difference) was calculated as $(\Delta F_{o\,surface} - \Delta F_{o\,infiltrated})/(\Delta F_{o\,surface}) \times 100$.

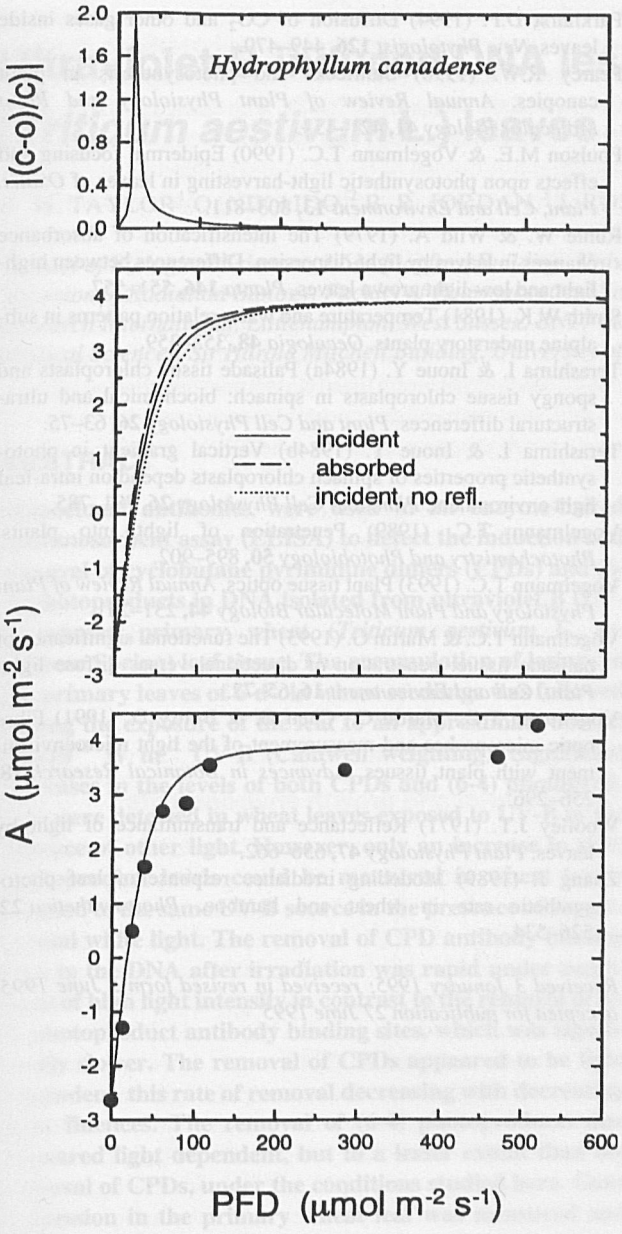


**Figure 9.** The response of net photosynthesis ($O_2$ flux) to irradiance incident on the adaxial leaf surface for *Hydrophyllum canadense* (bottom panel). Each point is a mean of five measurements made on different leaves but the curve was fitted to the scatter of all points. The coefficients describing the fitted curve are shown in 'Materials and methods'. The photosynthetic responses to absorbed PFD (long dashes) and incident PFD for a theoretical leaf with no intercellular reflectance (short dashes) are shown in the middle panel, and the relative difference in photosynthesis at each irradiance for the control leaf and theoretical leaf is shown in the top panel. The relative difference was calculated as $[(A_{control}-A_{oil})/A_{control}]$.

escaping from the adaxial leaf surface originates in the upper portion of the palisade layer (Bornman, Vogelmann & Martin 1991), the effect of infiltration on $F_o$ yield is influenced primarily by air space reflectance in the palisade parenchyma.

Plants adapted to light-limited understorey habitats, where incident PFD is often below 30 $\mu$mol m$^{-2}$ s$^{-1}$ (Chazdon & Fletcher 1984; Messier & Bellefleur 1988), maintain high levels of light capture with small metabolic expenditures in photosynthetic pigments and leaf area. In a broad array of tropical species, for example, shade species had slightly higher absorptance of PAR than sun species, 90·2% compared to 88·6%, but significantly lower chlorophyll content (42 versus 56 mg cm$^{-2}$; Lee & Graham 1986; Lee *et al.* 1990). This reduction in chlorophyll content may decrease the construction and maintenance costs per unit area of shade leaves, thereby decreasing the photosynthetic light compensation point. The maintenance of high levels of back-scattering and high levels intercellular reflectance in shade leaves provides a mechanism for increasing absorbance of PAR without additional investment in photosynthetic pigments, and may therefore increase the efficiency of shade leaves. Our model indicates that intercellular reflectance and possibly bicolouration may contribute to an increase in net photosynthesis compared with leaves with no intercellular reflectance. This enhancement of internal light may play a crucial role in maintaining leaves above photosynthetic light compensation between sunflecks and the light induction state of leaves during protracted periods of low incident irradiance (Osborne & Raven 1986; Pearcy 1990).

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