

LEAF ARCHITECTURE AND DIRECTION OF INCIDENT LIGHT INFLUENCE MESOPHYLL FLUORESCENCE PROFILES¹

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Light propagation and distribution inside leaves have been recognized as important processes influencing photosynthesis. Monochromatic light absorption across the mesophyll was measured using chlorophyll fluorescence generated from illumination of the cut edge (epi-illumination), as well as the adaxial or abaxial surfaces of the leaf. Species were selected that had basic leaf types: laminar leaf with adaxial palisade layer (*Rhododendron catawbiense*), needle with palisade (*Abies fraseri*), and needle without palisade (*Picea rubens*). Fluorescence was more evenly distributed across the mesophyll for adaxially illuminated leaves with a palisade cell layer, as well as for the needles (cylindrical) without palisade, when compared to fluorescence generated by abaxial illumination. Moreover, fluorescence from green light illumination remained high across the mesophyll of adaxially illuminated *R. catawbiense*, indicating a possible influence of mesophyll structure on internal light distribution beyond that of chlorophyll levels. These data support the idea that light propagation within the mesophyll is associated with asymmetric mesophyll structure, in particular the presence of palisade cell layers. In addition, we propose that the evolution of a more cylindrical leaf form, such as found in conifer species, may be a structural solution to excessive sunlight that replaces the highly differentiated mesophyll found in most laminar-leaved species.

Key words: *Abies fraseri*; chlorophyll; Ericaceae; high-altitude species; leaf orientation; photosynthesis; *Picea rubens*; Pinaceae; *Rhododendron catawbiense*.

Leaf orientation may be coordinated with external leaf morphology and internal anatomy for efficient light capture, according to different sunlight environments and stress levels (see Smith et al., 1997, for review). Typically, sun leaves of laminar-leaved plants are smaller and/or more deeply lobed, thicker, and lighter in color compared to shade leaves. Also, sun leaves are commonly more amphistomatous with well-developed palisade layers, while shade leaves are typically thinner, primarily hypostomatous, and without palisade layers. Sun leaves also tend to be more inclined in their orientation, away from horizontal. In contrast, numerous coniferous species often have cylindrical- or diamond-shaped needles in cross section and have significant morphological responses to sun (thicker and rounder) and shade (thinner and flatter). Due to the more cylindrical geometry, the intensity of sunlight incident on the conifer needle surface is reduced, while the more circular cross section concentrates absorbed photons inside the leaf as a result of radial diffusion (Smith et al., 1997).

The evolution of the conifer leaf form has also been associated phylogenetically with the early evolution of gymnosperms into higher sun environments and during colder, glacial periods (Field et al., 2003), possibly in response to increased stress associated with low-temperature photoinhibition. Similarities also persist between the conifer and typical laminar leaf, including the presence of palisade cells in conifer genera with more flattened needles (Fig. 1). In the gymnosperm fam-

ily Pinaceae, palisade layers occurred in the more laminar-like leaves of four of the six genera, but not in the more needle-like leaves of *Picea* or *Pinus* spp. (Esau, 1965). There are also substantial structural differences found commonly between cotyledons vs. primary leaves of most species, although studies of light propagation and photosynthetic effects do not appear to exist.

Chlorophyll fluorescence can be used to measure light absorption in leaves (e.g., Takahashi et al., 1994) and to indicate photosynthetic potential (Koizumi et al., 1998; Vogelmann and Evans, 2002). As indicated by monochromatic- light-induced fluorescence, green wavelengths (515–550 nm) penetrated deeper into leaves than either red (650 nm) or blue (450–488 nm). Also, the strongest absorption of blue and red light corresponded to the area of highest chlorophyll content, Rubisco concentration, and ¹⁴C fixation in spinach (Evans, 1999; Evans and Vogelmann, 2003). Although considerable work has dealt with differences in leaf morphology and anatomy associated with sun exposure, only one study (to our knowledge) has measured differences in absorbed light profiles inside sun and shade leaves. In this study, Cui et al. (1991) used a fiber-optic microprobe and found that green light penetrated deeper in shade leaves than in sun leaves.

The general purpose of the present study was to measure chlorophyll fluorescence profiles (also used as an accurate proxy for chlorophyll content) across the leaf mesophyll of broadly representative leaf types (conifer needle with and without palisade, conifer cotyledon, and a typical laminar-leaf type). Specifically, fluorescence in the needle-like leaves of the coniferous species, *Abies fraseri* (Pursh) Poir. and *Picea rubens* Sarg. was compared to a laminar-leaf shrub (*Rhododendron catawbiense* Mich.). Because studies of light processing inside leaves have involved only mature leaves with a single leaf phenotype, both sun and shade leaves were evaluated in

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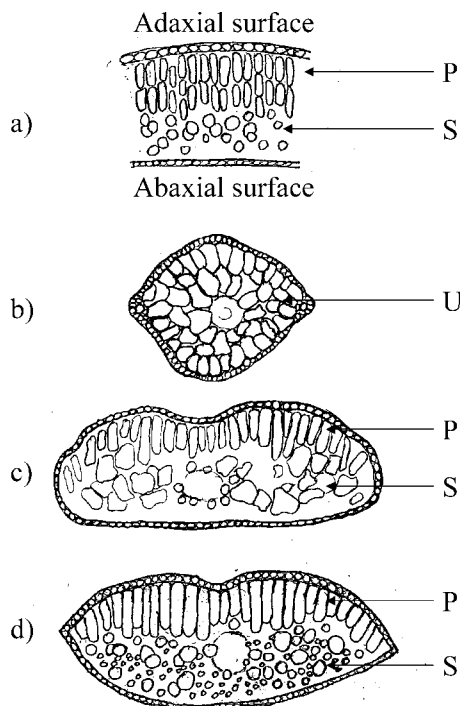


Fig. 1. Schematic of different leaf types used in this study. (a) *Rhododendron catawbiense* leaf, (b) *Picea rubens* needle, (c) *Abies fraseri* primary needle, (d) *A. fraseri* cotyledon. P, S, and U represent palisade, spongy and undifferentiated mesophyll types, respectively. Bar = 100 μm .

P. rubens and at different developmental stages (cotyledons vs. primary needles) in *A. fraseri*. Finally, the effect of leaf orientation and light incidence (adaxial vs. abaxial) on internal fluorescence was assessed in *A. fraseri* and *R. catawbiense*. These data were then used to interpret the possible influence of leaf architecture (e.g., presence of palisade layer) on internal light propagation and distribution, either because of differences in structure or chlorophyll distribution.

Smith et al. (1997) proposed that the conifer needle form is a structural and orientational solution for growth under high sun exposure that precludes the mesophyll cell differentiation necessary in typical sun-type, laminar leaves. More specifically, both leaf types with mesophyll cell differentiation (e.g., spongy and palisade cell layers) and cylindrical conifer needles (without mesophyll differentiation) were predicted to have uniform fluorescence intensity across the leaf, but only under abaxial illumination. If palisade acts to propagate light across the leaf into the spongy mesophyll, which then scatters light, then adaxial illumination (passing first through palisade) would result in fluorescence deeper into the leaf. In contrast, abaxial

illumination (passing first through the spongy layer) would result in greater fluorescence in the spongy vs. palisade layer.

MATERIALS AND METHODS

Leaves representing two of the most contrasting leaf structural forms, needle-like vs. laminar, were selected for these initial comparisons of internal fluorescence profiles. In addition, fluorescence profiles inside needles with and without palisade mesophyll were compared to assess the potential light propagation function of palisade mesophyll and the possibility that more cylindrical (circular cross-section) leaf forms do not require a palisade layer in order to propagate light deeper into the leaf (e.g., Smith et al., 1997). Finally, fluorescence in sun vs. shade needles, as well as for cotyledons vs. primary needles, were compared because of their ubiquity in terrestrial plants, as well as the lack of internal light processing measurements in general. Moreover, fundamental differences in internal light processing might be expected in these contrasting leaf forms due to natural differences in sunlight availability (Smith et al., 1998). Fluorescence after epi-illumination was also compared to that generated by axial illumination to evaluate chlorophyll activation potential under simulated, natural illumination conditions. In this manner, the quantitative effects of leaf structure and light direction on chlorophyll activation could be evaluated.

Abies fraseri seedlings (<5 cm, with 4–6 cotyledons and the initial set of primary needles) used for primary needle and cotyledon comparisons were collected from Roan Mountain, Tennessee (USA) in April 2003 and grown in a greenhouse until the beginning of the study in September 2003. This field collection site had 23% canopy openness (fisheye photographs) with a maximum photosynthetic photon flux density (PPFD) of $580 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, while the greenhouse had approximately 50% full sunlight and a maximum PPFD of approximately $800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Both primary needles (8–10) and cotyledons (4–6) were present on seedlings at the time of measurement. *Abies fraseri* seedlings (same size as above) used for light orientation comparisons were collected from Roan Mountain and transported on ice to the University of Vermont where they were analyzed 3 days later. Cross sections of needles stored on ice for 3 days were compared to fresh sections ($N = 10$), and no differences in internal anatomy were observed. Sun and shade needles of *Picea rubens* were collected from exposed south and north sides, respectively, of individual trees growing near the University of Vermont campus in Burlington, Vermont, USA. Although sun needles appeared to be oriented more vertically than shade needles, differences in individual needle morphology (e.g., length, width, thickness) were not apparent (Table 1). Leaves of *Rhododendron catawbiense* were collected from an intermediate branch position in the upper canopy from plants growing near the campus of University of Vermont. All measurements were replicated, with 4–6 individual leaves for each leaf type and light orientation.

Leaf thickness, percentage of cross-sectional area occupied by palisade mesophyll, number of palisade cell layers, and mean palisade and spongy mesophyll cell length were measured from images of leaf cross sections using Image Pro Plus software (version 4.5, Media Cybernetics, Silver Spring, Maryland, USA). Schematic representations of each leaf type were traced from images of leaf cross sections.

Monochromatic-light-induced fluorescence was measured according to the techniques described in Vogelmann and Han (2000), while chlorophyll abundance and/or activity inside leaves were estimated using direct illumination of the cut face (epi-illumination, Vogelmann and Evans, 2002). Cross sections

TABLE 1. Leaf anatomical characteristics of study species. Leaf thickness and cell lengths are in μm . Numbers in parentheses are standard errors.

Leaf type	Mean leaf thickness	Leaf area palisade (%)	No. of palisade layers	Mean palisade cell length	Mean spongy cell length
<i>Abies fraseri</i> primary	331.3 (23.5)	24.2 (0.7)	1	45.7 (1.8)	46.2 (2.0)
<i>A. fraseri</i> cotyledon	354.5 (11.0)	14.7 (1.7)	1	55.6 (4.1)	46.4 (1.6)
<i>Picea rubens</i> sun	511.2 (1.9)	—	—	—	37.0 (1.1)
<i>P. rubens</i> shade	519.5 (14.0)	—	—	—	35.7 (1.6)
<i>Rhododendron catawbiense</i>	485.0 (8.4)	32.4 (0.3)	2–3	44.1 (2.4)	42.3 (1.4)

of leaves were cut by hand (razor blade) and then placed into a holder that allowed either abaxial or adaxial illumination, as well as cross-sectional viewing (see Vogelmann and Han, 2000, for details). To determine light absorption gradients across the mesophyll, abaxial, or adaxial surfaces of leaf specimens were illuminated perpendicularly with monochromatic light (using interference filters corresponding to 450, 550, or 650 nm; 50 nm half bandwidth for 450 and 550 nm, 10 nm half bandwidth for 650 nm) and cross-sectional fluorescence at 680 nm was viewed with a CCD camera (SDS 9000, Photometrics, Tucson, Arizona, USA). Chlorophyll profiles across the leaf mesophyll were estimated from blue light epi-illumination and resulting fluorescence at 680 nm (captured with the CCD camera). Light absorption and chlorophyll content is proportional to this fluorescence (Vogelmann and Evans, 2002).

Images of abaxially, adaxially and epi-illuminated leaf cross-sections were analyzed quantitatively using Image Pro Plus software to generate relative pixel intensities. To minimize error due to non-parallel leaf surfaces, narrow transects of approximately 30 μm in width were used for analysis of all samples. Individual measurements consisted of 70–90 data points recorded at approximately 5- μm intervals. Transects did not include vascular tissue or resin ducts. Relative chlorophyll fluorescence (RCF) was computed for each of the corresponding leaf types and wavelengths of monochromatic light by dividing individual fluorescence values by the maximum fluorescence measured, and then averaging for each wavelength, leaf type, and specific tissue depth. Standard errors were also calculated for each wavelength, leaf type, and specific tissue depth. Mean standard errors for each wavelength and leaf type were calculated to evaluate statistically the variation among measurements. Estimates of absolute values of fluorescence intensity among different samples were not possible.

Regression curves were fitted for each data set (wavelength, leaf type, and incident light direction) and were tested for significant differences. The best-fit regression model was predicted using Sigma Plot (SPSS Science, Chicago, Illinois, USA), then model equations were fitted to the actual data sets. Model equations for red-, blue-, and green-light-induced RCF in *R. catawbiense*, *P. rubens* sun and shade needles, and *A. fraseri* primary needles (adaxially illuminated) and cotyledons were of the type $y = ae^{-bx}$, where x = depth into the leaf and a and b are best-fit regression parameters. Curves for comparisons of *P. rubens* sun and shade needle epi-illumination induced RCF were of the form $y = y_0 + ax$, and curves for comparisons of *R. catawbiense* and *A. fraseri* primary needle and cotyledon epi-illumination-induced RCF were of the form $y = y_0 + ax + bx^2$. Model equations for *R. catawbiense* and *A. fraseri* abaxially illuminated samples were also of the form $y = y_0 + ax + bx^2$. Significant differences between parameters for pairs of curves were tested using F tests to determine if curves were statistically indistinguishable (Motulsky and Christopoulos, 2003). No comparisons were attempted between adaxial and abaxial pairs because one model could not be fitted to both treatments (the same model would not converge on both data sets). Curve-fitting and statistical analysis were performed using GraphPad Prism (version 4.0, GraphPad software, San Diego, California, USA).

RESULTS

Leaf anatomy—Leaves of *R. catawbiense* had 2–3 palisade mesophyll layers (Table 1, Fig. 1a), while *A. fraseri* primary needles and cotyledons had one palisade layer (Fig. 1c, d) and *P. rubens* had none (Fig. 1b). *Rhododendron catawbiense* leaves also had a higher percentage leaf area occupied by palisade mesophyll than any of the other leaf types ($P < 0.001$ vs. *A. fraseri* primary needles and cotyledons). There were no significant differences in mean palisade cell lengths between leaf types, but spongy mesophyll cells were smaller in *P. rubens* (both sun and shade) than in *R. catawbiense* leaves, *A. fraseri* primary needles, or cotyledons ($P < 0.01$ for all comparisons).

Epi-illumination—Under epi-illumination of the cut edge, RCF in leaves of the laminar-leaf *R. catawbiense* increased

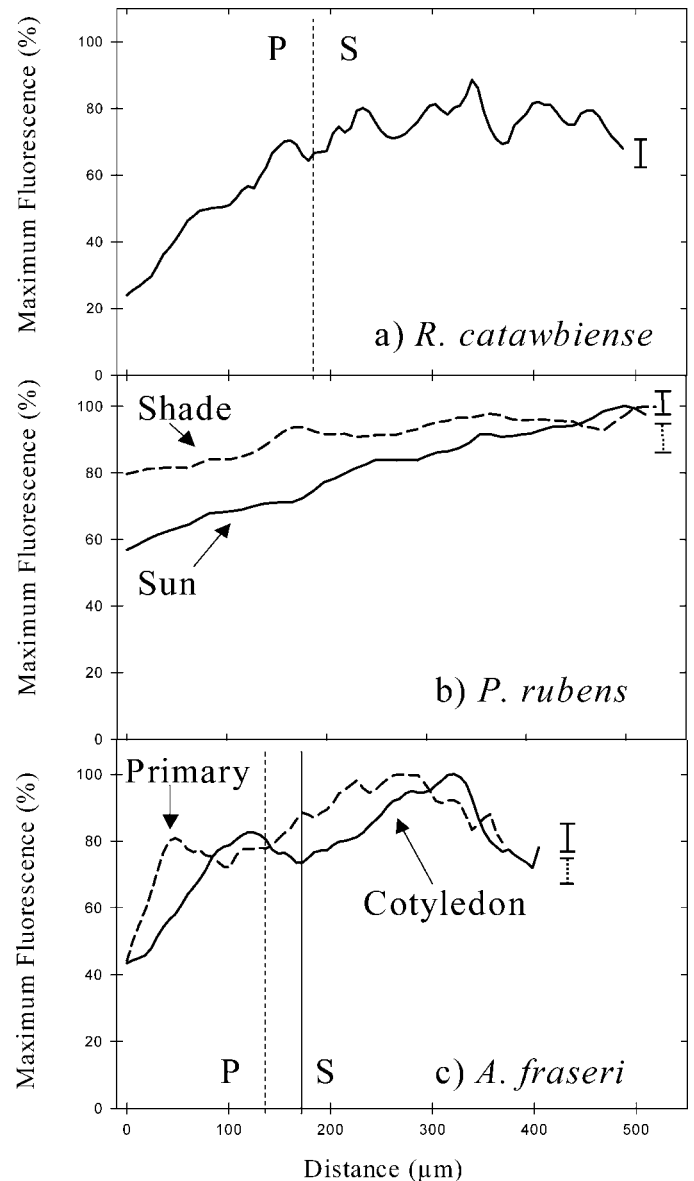


Fig. 2. Mean epi-illumination-induced fluorescence vs. depth in the mesophyll for leaf cross sections of different leaf types expressed as a percentage of maximum fluorescence. Error bars represent mean standard error calculated across all depths. (a) *Rhododendron catawbiense* leaves. (b) *Picea rubens* sun and shade needles; dashed line/error bar indicate shade needles and solid line/error bar indicate sun needles. (c) *Abies fraseri* cotyledons and primary needles; dashed line/error bar indicates primary needles and solid line/error bar indicates cotyledons. Dashed (*R. catawbiense* and *A. fraseri* primary needle) and solid (*A. fraseri* cotyledon) vertical lines indicate approximate tissue boundaries between palisade (P) and spongy mesophyll (S). Adaxial surface is at zero μm in all panels.

gradually with increasing depth to near 250 μm , then leveled off (Fig. 2a). Epi-illumination generated RCF profiles from sun and shade *P. rubens* needles increased linearly from adaxial to abaxial, although the slope of the line for shade needles was less than for sun needles (Fig. 2b). Primary needles of *A. fraseri* also had increased fluorescence with depth into the mesophyll, but with two distinct peaks, one closer to the upper (adaxial) epidermis of the needle and one nearer the lower (abaxial) epidermis (Fig. 2c). Similar peaks occurred in

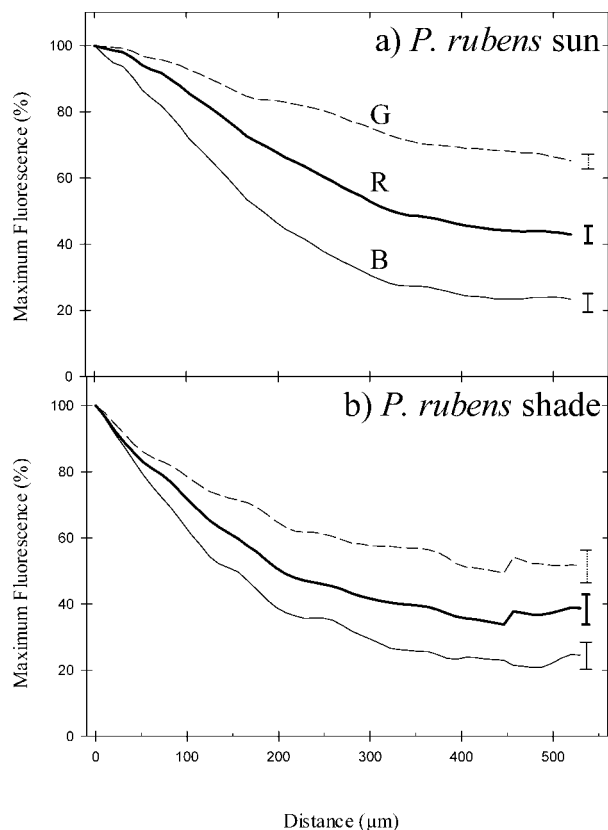


Fig. 3. Mean adaxial illumination-induced fluorescence using monochromatic light in sun (a) and shade (b) needles of *Picea rubens*. Solid heavy lines = 650 nm (R, red); dashed lines = 550 nm (G, green); solid light lines = 450 nm (B, blue). Error bars represent mean standard error calculated across all depths. Adaxial surface is at zero μm in both panels.

cotyledons of *A. fraseri*, but appeared to be shifted more toward the abaxial side compared to primary needles. Increases in RCF from adaxial to abaxial were greater in *A. fraseri* than in *P. rubens* (~40% of maximum at adaxial surface to 100% at 300 μm for *A. fraseri* and ~60% of maximum at adaxial surface to 100% at abaxial surface for *P. rubens*). Chlorophyll fluorescence profiles across the leaf mesophyll were significantly different in each species and leaf type (all $P < 0.001$, Fig. 2).

Adaxial vs. abaxial illumination—In both conifer tree species, *P. rubens* and *A. fraseri*, fluorescence generated by adaxially incident light declined substantially deeper in the leaf, especially for blue light (and to a lesser degree, red and green wavelengths) (Figs. 3, 4). Although RCF diminished in a non-linear, asymptotic pattern for needles of both species, initial declines appeared more rapid in cotyledons of *A. fraseri* than for primary needles of either species. In addition, cotyledons and primary needles of *A. fraseri* had more sigmoidal or bimodal profiles in RCF with a delay in initial diminution just beneath the adaxial epidermis, followed by a relatively steep decline, and then a leveling off (Fig. 4). The RCF in cotyledons of *A. fraseri* diminished approximately linearly with depth up to about 225 μm and then remained relatively constant (Fig. 4a). In contrast, primary needles had a distinct peak in fluorescence just beneath the adaxial epidermis at approximately 50 μm , which then decreased with depth to approxi-

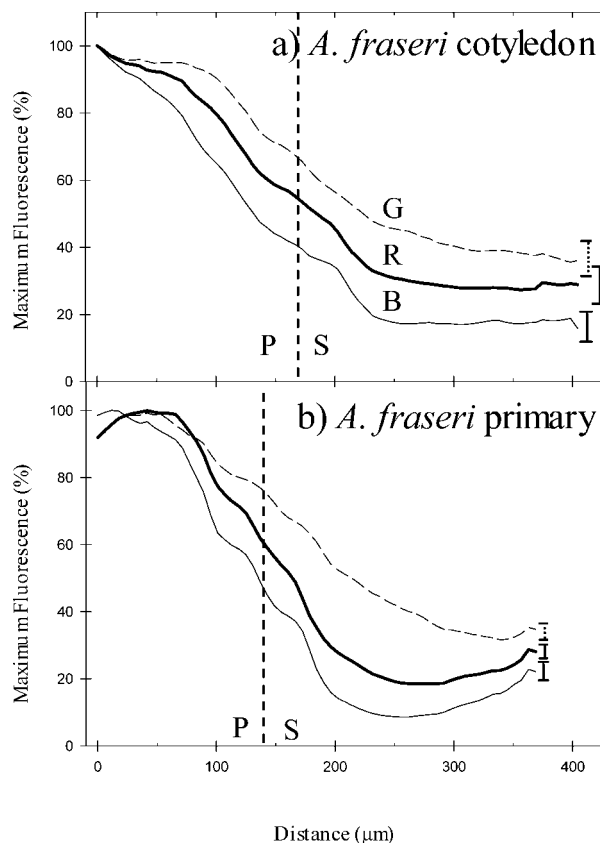


Fig. 4. Mean adaxial illumination-induced fluorescence using monochromatic light in cotyledons (a) and primary needles (b) of *Abies fraseri*. Solid heavy lines = 650 nm (R, red); dashed lines = 550 nm (G, green); solid light lines = 450 nm (B, blue). Error bars represent mean standard error calculated across all depths. Dashed vertical lines indicate approximate tissue boundaries between palisade (P) and spongy mesophyll (S). Adaxial surface is at zero μm in both panels.

mately 225 μm where a slight increase occurred (Fig. 4b). All three wavelengths induced similar RCF profiles in cotyledons and primary needles of *A. fraseri*, although abaxial and adaxial peaks in fluorescence observed in primary needles were absent in cotyledons.

Adaxial-light-generated fluorescence in *A. fraseri* primary needles for all three wavelengths deep into the spongy mesophyll (to approximately 225 μm), and then decreased toward the abaxial epidermis (Fig. 5a). Fluorescence generated from abaxial illumination decreased approximately linearly from the illuminated surface for all wavelengths (Fig. 5b).

In adaxially illuminated *R. catawbiense*, fluorescence from red and blue wavelengths declined rapidly through the palisade mesophyll, while green-light-induced fluorescence remained high across the palisade and into the spongy mesophyll (Fig. 5c). Profiles of RCF induced by abaxial red, blue, and green illumination were similar (Fig. 5d), peaking in the spongy mesophyll and then declining almost linearly away from the illuminated surface. Profiles of fluorescence were similar between adaxially and abaxially illuminated samples for red and blue wavelengths. However, green-light-induced fluorescence diminished with depth in abaxially illuminated leaves, but remained high throughout the leaf in samples illuminated adaxially.

Differences in curves were significant for all treatment com-

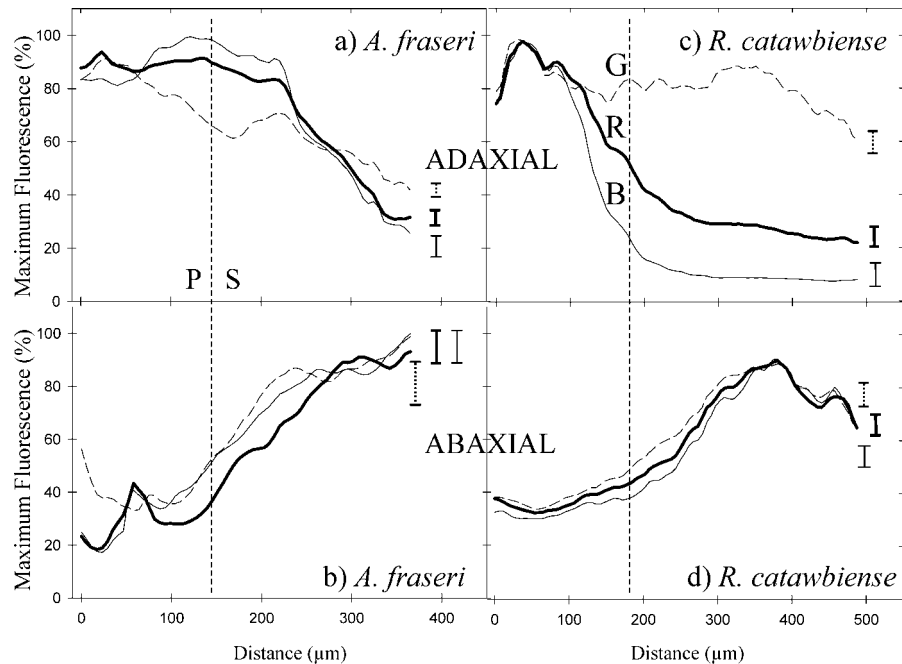


Fig. 5. Mean adaxial (a, c) and abaxial (b, d) fluorescence induced by monochromatic wavelengths in mature needles of *Abies fraseri* (a, b) and *Rhododendron catawbiense* (c, d). Solid heavy lines = 650 nm (R, red); dashed lines = 550 nm (G, green); solid light lines = 450 nm (B, blue). Error bars represent mean standard error calculated across all depths. Dashed vertical lines indicate approximate tissue boundaries between palisade (P) and spongy (S) mesophyll. Adaxial surface is at zero μm in all panels.

parisons. For all comparisons, P values were less than 0.001 except for the red vs. blue wavelengths in *A. fraseri* cotyledons ($P = 0.02$) and for *P. rubens* sun vs. shade needles illuminated with blue light ($P = 0.02$).

DISCUSSION

Differences in relative fluorescence patterns between epi-illumination and adaxial/abaxial illumination could come from at least two sources—the amount of chlorophyll at a given location inside the mesophyll or the corresponding exposure of the chloroplasts to incoming photons (assuming equal absorption potential among chloroplasts). However, the propagation of absorbed light across the mesophyll could also be due to architectural effects on optical properties of the different cell types, as well as their spatial organization (see Smith et al., 1997, for review).

Using the same techniques employed here, Vogelmann and Evans (2002) demonstrated that light absorption and chlorophyll content were directly proportional to observed fluorescence patterns. Also, structural characteristics of laminar leaves that appear to impact internal light and CO_2 profiles have been previously identified (see Parkhurst, 1994; Vogelmann et al., 1996; Smith et al., 1997; Evans et al., 2005, for reviews). Specifically, Smith et al. (1997, 1998) proposed that conifer needle structure may be an evolutionary solution for avoiding high light stress, but which also supplants the light processing function of specialized mesophyll cells (e.g., palisade and spongy mesophyll) found in sun leaves of most laminar-leaved species.

Epi-illumination and fluorescence profiles—There were distinct differences in relative chlorophyll fluorescence (RCF) patterns across the mesophyll among the species and leaf types

considered here. In the laminar-leaf species (*R. catawbiense*), the gradual incline to a plateau in fluorescence is in contrast with the linear increase in *P. rubens*. Also, sun needles of *P. rubens* had a substantially lower RCF at the adaxial epidermis (compared to shade needles), followed by a linear increase across the entire mesophyll in both sun and shade needles.

The RCF from epi-illumination in cross sections of *A. fraseri* cotyledons and primary needles had a large increase across the mesophyll from the adaxial to abaxial epidermis, although with two distinct peaks—one near the adaxial surface and one just before the lower epidermis. Also, these two peaks were shifted toward the abaxial side of the leaf in the slightly thicker cotyledons compared to primary needles. Overall, measured increases in RCF in the *A. fraseri* needles and cotyledons were greater than measured in *P. rubens*. These differences between *P. rubens* and *A. fraseri* needles, as well as the differences observed between primary needles and cotyledons, need further evaluation as to possible functional significance in light processing. Possibly, the lower sunlight microenvironment typical of cotyledonous seedlings, their more horizontal leaf orientation, and greater developmental constraints could all be involved (Germino and Smith, 1999).

Knapp et al. (1988) measured chlorophyll content directly in paradermal sections of *Cucurbita* cotyledons and found the greatest amount of chlorophyll toward the adaxial surface, decreasing with depth into the leaf. Vogelmann and Evans (2002) found that chlorophyll concentration in *Spinacia* increased from the adaxial epidermis into the palisade mesophyll, remained nearly constant throughout the spongy mesophyll, and then decreased rather abruptly near the abaxial epidermis. The epi-illumination fluorescence profile measured here for *R. catawbiense* was similar to that reported for *Spinacia* (also a laminar-leaf species) in that both showed an increase in fluo-

rescence across the palisade layer, followed by a plateau in the spongy mesophyll. This observed fluorescence pattern provides additional evidence that palisade mesophyll may act to propagate light deeper into the leaf, extending the light absorption profile across the full breadth of the mesophyll (Vogelmann and Martin, 1993; Smith et al., 1997).

Incident light direction and wavelength effects—Fluorescence patterns in both *R. catawbiense* and *A. fraseri* differed according to the direction of incident light. Under adaxial illumination, fluorescence decreased with depth among all three species, as well as sun and shade leaf types. However, green-light-induced fluorescence in *R. catawbiense* decreased only slightly across the leaf compared to the larger reductions in *P. rubens* and *A. fraseri*. The fact that green-light-induced fluorescence remains high across leaves of *R. catawbiense* may be due to strong internal reflection of green light by both the light-colored lower epidermis and the spongy mesophyll. This “light-trap” effect has been shown in other species and may be especially effective in bicolored leaves (as in *R. catawbiense*) (Woolley, 1971; Smith, 1981; Lin and Ehleringer, 1983).

Also, when illuminated abaxially, *A. fraseri* and *R. catawbiense* responded similarly, with fluorescence decreasing with depth from the illuminated surface. In spinach leaves, fluorescence decreased more with depth under abaxial than adaxial illumination (Vogelmann and Evans, 2002), similar to the profiles from *A. fraseri* primary needles in this study. This is further evidence that palisade mesophyll acts to propagate light into the spongy mesophyll and that light propagation is inhibited when illuminated from the leaf side opposite the palisade layer (abaxially).

In all species and leaf types measured, green light generated fluorescence deeper into the leaf than either red or blue, supporting earlier studies on spinach (Vogelmann and Evans, 2002), although this effect was much more dramatic in *R. catawbiense* than in the two conifers. With the exception of adaxial green light in *R. catawbiense*, blue light absorption decreased most rapidly across the leaf followed by red and then green light. These results are in general agreement with previous studies showing greater green vs. red light penetration (Terashima and Saeki, 1983, 1985; Vogelmann et al., 1989). In addition, *A. fraseri* primary needles had a peak in fluorescence near the adaxial leaf surface for all three wavelengths, while Terashima and Saeki (1985) found increased absorption of 550 nm (green) light at the palisade-spongy mesophyll interface in leaves of *Camellia japonica*. Vogelmann and Evans (2002) found a similar peak in spinach leaves, although it occurred at the interface between epidermal and palisade tissues.

Summary and conclusions—The differences in mesophyll fluorescence measured here corresponded to differences in leaf anatomy and cross-sectional shape (laminar vs. cylindrical), as well as the direction of illumination (leaf orientation) (Smith et al., 1997, 1998). For example, leaves of the laminar-leaved *R. catawbiense* and the conifer needle of *A. fraseri* have both palisade and spongy mesophyll cell layers, whereas *P. rubens* needles do not (Fig. 1, Table 1; see also Kozłowski and Pallardy, 1997). However, in all cases, adaxial illumination resulted in penetration of fluorescence deeper into the mesophyll. Also, chlorophyll was less concentrated in the more abaxial portions of leaves having palisade mesophyll, but was more evenly distributed in leaves without palisade. It is pos-

sible that the propagation function of the palisade cell layer might necessitate fewer chloroplasts. However, the decreased propagation of incident light deeper into the mesophyll under abaxial illumination also suggests that more than just chlorophyll concentration is involved. Several structural factors at the chloroplast level and below could be contributing to this asymmetry in fluorescence profiles according to the direction of illumination (see Evans et al., 2005, for review).

The early proliferation of the conifer leaf form has been linked phylogenetically to the occupation of sunnier and more drought-stressed habitats and was derived most likely from ancestral species that occupied more shaded habitats and possessed laminar-type leaves (Field et al., 2003; Brodribb and Hill, 2004). This evolutionary trend also occurred during drought stress periods of both warm and particularly cold conditions of interglacial and glacial maximum periods, respectively. The more cylindrical cross-sectional shape of conifer needles, as well as other cylindrical leaf (and photosynthetic stem) shapes (e.g., numerous desert species) may represent an architectural solution for both avoiding high sunlight levels (photoinhibition) and enhanced internal light propagation. Not only does the more curved leaf surface reduce incident sunlight at the needle surface due to the cosine effect on surface reflectance, but the radial cross-sectional geometry would also act to concentrate penetrating photons with depth into the needle interior (Smith et al., 1997). A more cylindrical leaf shape (more circular cross-section) may act to eliminate the necessity for mesophyll cell differentiation (e.g., light-propagating palisade cells and light-scattering spongy cells) found in typical laminar-leaf species, or even in flattened, needle-leaved species (e.g., *Abies*, *Pseudotsuga*, *Tsuga*). Moreover, the differences and asymmetry in mesophyll fluorescence patterns may also indicate the involvement of chloroplast structure or movements (Evans et al., 2005). Further studies are needed to assess the possible contributions of leaf structure and chloroplast structure/movement on internal light processing, e.g., species with variegated leaves (areas without chlorophyll) could be particularly elucidative.

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