

## Photosynthetic symmetry of sun and shade leaves of different orientations

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**Summary.** The photosynthetic responses to light of leaves irradiated on the adaxial or abaxial surfaces, were measured for plants with contrasting leaf orientations. For vertical-leaf species of open habitats (*Eryngium yuccifolium* and *Silphium terebinthinaceum*), photosynthetic rates were identical when irradiated on either surface. However, for horizontal-leaf species of open habitats (*Ambrosia trifida* and *Solidago canadensis*), light-saturated rates of photosynthesis for adaxial irradiation were 19 to 37% higher than rates for abaxial irradiation. Leaves of understory plants (*Asarum canadense* and *Hydrophyllum canadense*) were functionally symmetrical although they had horizontal orientation. Photosynthetic rates were measured at saturating CO<sub>2</sub>, thus differences in the response to incident irradiance presumably resulted from complex interactions of light and leaf optical properties rather than from stomatal effects. Differences in absorptance (400–700 nm) among leaf surfaces were evident for horizontal-leaf species but the primary determinant of functional symmetry was leaf anatomy. Functionally symmetrical leaves had upper and lower palisade layers of equal thickness (vertical leaves of open habitats) or were composed primarily of a single layer of photosynthetic cells (horizontal leaves of understory habitats). Photosynthetic symmetry of vertical-leaf species may be an adaptation to maximize daily integrated carbon gain and water-use efficiency, whereas asymmetry of horizontal-leaf species may be an adaptation to maximize daily integrated carbon gain and photosynthetic nutrient-use efficiency.

**Key words:** Leaf anatomy – Photosynthesis – Functional symmetry – Leaf orientation – Optical properties

In natural light environments leaves may receive direct or diffuse light on abaxial and adaxial leaf surfaces. This

is evident especially for vertically-oriented foliage, yet few studies have examined the quantity and quality of light incident on each surface or the ability of leaves to process this light. The direct effects of sunlight interception on leaf temperature and the water vapor gradient between leaf and air are well known. Vertical leaf display, evident in many plants of hot dry environments, causes substantial reductions in transpiration (Ehleringer and Werk 1986; Geller and Smith 1982) and permits greater light penetration into the canopy (Niklas and Owens 1989). However, reduced daily photosynthetic photon flux density (PPFD) resulting from steep leaf angle can significantly decrease net carbon gain and growth even in open habitats (Nobel 1986). Efficient light processing by the abaxial surface of vertically-oriented leaves may lessen this potential reduction in daily carbon gain.

Studies of agronomic species suggest that leaves are functionally *unilateral* (asymmetrical) or *bilateral* (symmetric). Moss (1964) compared photosynthetic rates of maize, sugar cane, tobacco, and sunflower when irradiated on the ad- or abaxial sides of leaves. Maize and sugar cane leaves have no palisade cell layers and are amphistomatous. These species have similar photosynthetic responses to light when irradiated on either side of the leaf and are thus functionally bilateral. These results were confirmed for maize by Vaclavik (1984). In contrast, photosynthesis at intermediate irradiances was approximately 30–40% lower when tobacco and sunflower leaves were irradiated on the abaxial surface. These functionally unilateral species possess well developed palisade layers under the adaxial epidermis, and the majority of stomata are located on the abaxial surface. Day *et al.* (1990) observed photosynthetic symmetry (bilateral function) for the vertical leaves of *Populus tremuloides* and asymmetry (unilateral function) for horizontal leaves of *Mahonia repens*. Data for *Rumex densiflorus*, however, pose an interesting dilemma. The leaves of this species are steeply angled yet photosynthetic rates are ca. 40% lower when irradiated on a north-facing abaxial surface. Foliage of *Rumex densiflorus* has primarily north-south orientation (*i.e.*, leaf lamella faces N–S), so that irradiance is highest on the south-facing leaf surface despite vertical orientation (Geller and Smith 1982).

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In this study we test the prediction that vertically-oriented leaves with an east-west azimuth are photosynthetically bilateral in their ability to drive photosynthesis when irradiated on either leaf surface, whereas horizontally-oriented leaves maintain higher photosynthetic rates when irradiated on the adaxial surface and are functionally unilateral. Horizontal-sun and horizontal-shade leaves (horizontal leaves in open and understory habitats, respectively) were also compared to examine the contribution of light environment to photosynthetic symmetry. We selected several  $C_3$  dicots common in tallgrass prairie and forest understory that represented extremes in leaf orientation and light environment. Photosynthetic measurements were made with a gas-phase  $O_2$  electrode at high  $CO_2$  concentrations. Thus, differences in the photosynthetic response to ad- versus abaxial irradiation did not arise from stomatal distribution or the differential response of stomata on each leaf surface, but were presumably a function of leaf optics and structure.

## Materials and methods

We selected six species that represented extremes of leaf orientation and light regime. *Ambrosia trifida* L. and *Solidago canadensis* L. were chosen as horizontal-leaf plants of a high-light habitat (horizontal-sun), and *Silphium terebinthinaceum* Jacq. and *Eryngium yuccifolium* Michx. were chosen as vertical-leaf plants of a high-light habitat (vertical-sun leaves). *Hydrophyllum canadense* Michx. and *Asarum canadense* L. grow in the forest understory and were selected as horizontal-leaf plants from a shaded habitat (horizontal-shade leaves). Henceforth, plants are referred to by their generic epithets. All plants grew in hardwood forest, recently abandoned agricultural field, or restored tallgrass prairie in the University of Illinois Trelease Woods Ecological Research Area, 6 km northeast of Urbana, Illinois. Leaf orientation was characterized by measuring angle (angle in degrees from horizontal) and azimuth (angle in degrees between magnetic north and the normal to the leaf blade) of the adaxial surface of approximately 100 leaves (5–40 plants) for each species. The tangent at mid-leaf and parallel to the main axis was used to characterize leaf angle.

The photosynthetic response to irradiance was measured with a leaf-disk oxygen electrode (LD2, Hansatech Ltd., Norfolk, England) according to Delieu and Walker (1981). Light was provided by a fixed output metal halogen lamp (LS2, Hansatech), and a range of irradiances was generated using combinations of neutral-density filters (Melles-Griot, Irvine, CA, USA). Transmittance of photosynthetically active radiation (PAR, 400–700 nm) through different combinations of filters was measured with a quantum sensor (LI-190SB, LI-COR, Lincoln, NE). Excessive heating of foliage was observed when the light source and the temperature-controlled sample chamber were configured as recommended by the manufacturer. To minimize heating we used an infrared filter consisting of 1 L of 5%  $CuSO_4/H_2SO_4$  in a round bottom flask placed between the light source and chamber, thus enabling us to maintain foliage temperature in the chamber at  $25 \pm 0.2^\circ C$  (range) over a wide range of irradiances.

Leaf disks (10  $cm^2$ ) for light response measurements were cut in the field from the most recently expanded leaf that represented the median leaf angle for each species. Leaves with an east-west orientation were selected for vertical foliage of *Silphium* and *Eryngium*. Different disks from adjacent leaves or from either side of the midrib of a single leaf were used for adaxial and abaxial light-response measurements. Foliage was sampled during early to mid-morning, after exposure to direct light for at least one hour but presumably prior to the onset of water stress. Three or four leaves

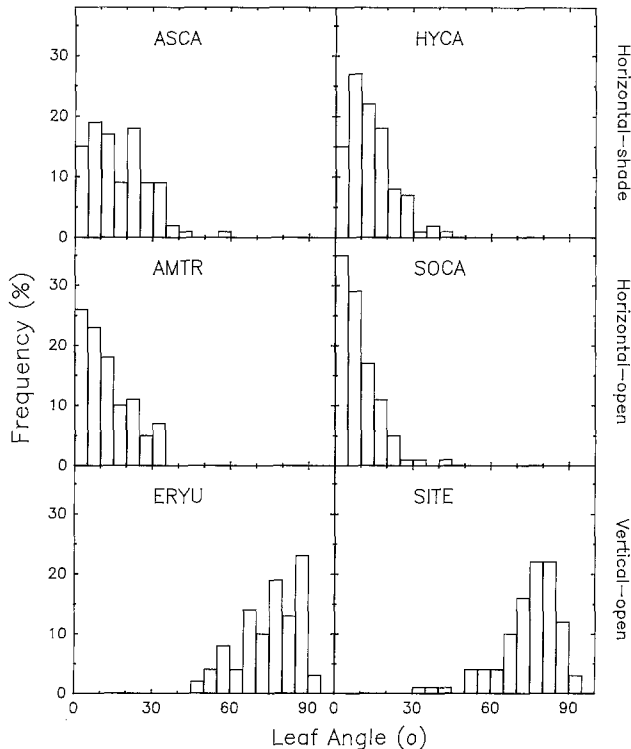
of each species were measured. Tissue was transported to a field laboratory on moist filter paper in a petri plate, and measurements were initiated within 2 min of cutting. Photosynthetic-light response curves were measured from low to high irradiance after an initial induction period of repeated cycles (1 to 4 min/cycle) of moderate irradiance (ca. 500  $\mu mol m^{-2} s^{-1}$ ) and darkness. The sample chamber was flushed with hydrated 5%  $CO_2$  (v/v) for approximately 2 min prior to measurement at each irradiance.

Curves were fit to the net photosynthesis versus irradiance data using a nonlinear least-squares fitting technique that employed the Marquardt algorithm (Schreiner *et al.* 1985). The model was a non-quadratic hyperbola adapted from Prioul and Chartier (1977) and recently discussed by Leverenz (1987), Terashima and Saeki (1985), and Zhang (1989). The equation was:

$$\theta P^2 - (\alpha I + P_{max})P + \alpha I P_{max} = 0 \quad (1)$$

$\theta$  ranges from 0 to 1 (inclusive) and describes the sharpness of the bend or convexity of the curve.  $P$  is gross photosynthesis calculated as net photosynthesis plus the rate of dark respiration.  $\alpha$  is the slope of the initial linear region of the curve.  $I$  is irradiance (absorbed or incident), and  $P_{max}$  is gross photosynthesis at light saturation (asymptote).

Leaf optical properties and quantitative aspects of anatomy were measured on 5 leaves per species. Reflectance and transmittance of the ad- and abaxial leaf surfaces were measured with a Taylor-type integrating sphere and a spectroradiometer (LI-COR 1800). Three scans between 400 and 800 nm were averaged for each leaf surface. Absorbance was calculated as  $1 - \text{transmittance} - \text{reflectance}$ . Leaf thickness and the thickness of component cell layers were measured on fresh-frozen tissue. Sections (ca.  $2 \times 2$  mm) were cut from between the midrib and leaf margin and were rapidly frozen in Tissue-Tek O.C.T. compound. Transverse sections, 8 to



**Fig. 1.** Leaf angles from horizontal for *Asarum canadense* (ASCA), *Hydrophyllum canadense* (HYCA), *Ambrosia trifida* (AMTR), *Solidago canadensis* (SOCA), *Eryngium yuccifolium* (ERYU), and *Silphium terebinthinaceum* (SITE).  $N = 100$  leaves from 5 to 40 plants for each species. Predominant leaf orientations and light environments are indicated on the right

10- $\mu\text{m}$  thick, were cut in a cryotome (model CTI, International Cryostat, MA) at ca.  $-25^\circ\text{C}$  and measured with a transmitted-light microscope and calibrated ocular micrometer at 100x. Two leaf disks ( $0.85\text{ cm}^2$ ) were sampled from 10 leaves per species for determination of chlorophyll content as in Arnon (1949).

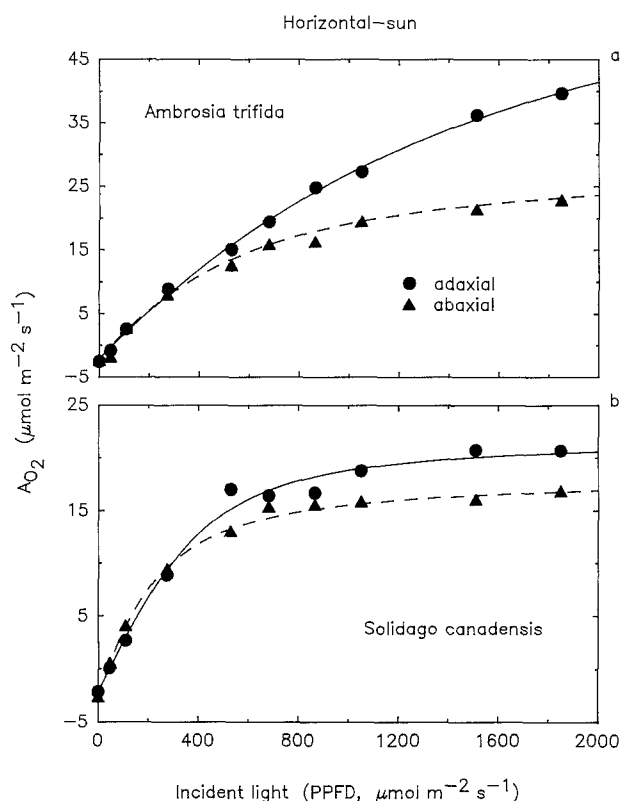
Where appropriate, mean values were compared among species with a one-way ANOVA and LSD multiple range test ( $p \leq 0.05$ ). A t-test was used for intraspecific comparisons of the optical properties of adaxial and abaxial leaf surfaces.

## Results

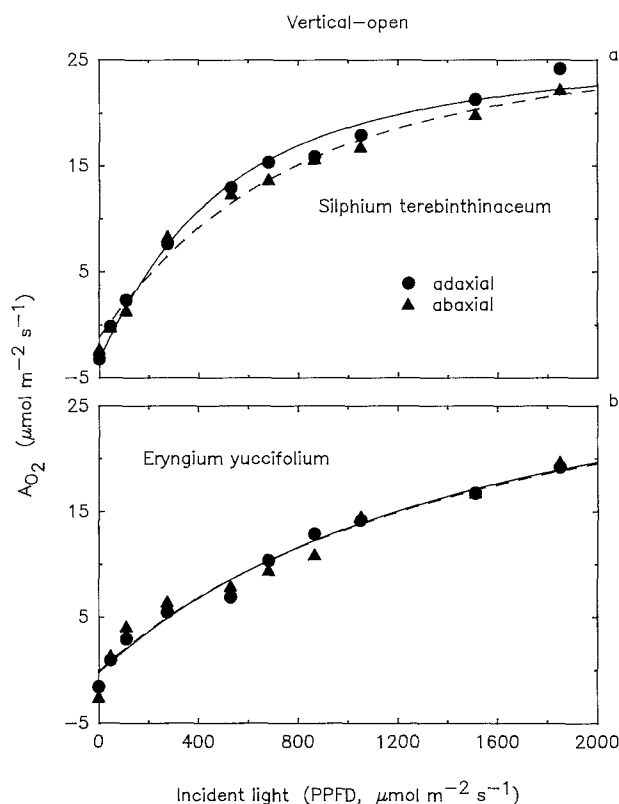
The opposite branching pattern of *Ambrosia* and *Solidago* (data not shown) resulted in a slight clustering of leaf azimuths in the four cardinal directions. Leaves of *Silphium* were primarily east-west facing, and leaf azimuths of the other three species were randomly distributed. Despite the appearance of strongly horizontal or vertical leaf orientation, there was considerable variation in leaf angle within each species (Fig. 1). This variation was largely attributable to leaf-age effects for individual plants. Recently expanded foliage had greater leaf angles than older foliage. The horizontal-leaf species, *Asarum*,

*Hydrophyllum*, *Ambrosia*, and *Solidago*, had median leaf angles of 22, 12, 8, and  $5^\circ$ , respectively. The vertical-leaf species, *Eryngium* and *Silphium*, had median leaf angles of 76 and  $80^\circ$ , respectively.

Rates of net photosynthesis for the horizontal-sun species, *Ambrosia* and *Solidago*, were higher when leaves were irradiated on the adaxial compared to the abaxial leaf surface (Fig. 2a, b). Although variation in the absolute rates of photosynthesis among leaves was high (the coefficient of variation for light-saturated photosynthetic rates for a given leaf surface among replicate leaves was 17–22%), differences between leaf surfaces were consistent within a leaf. The residuals between photosynthetic-light response data and the fitted functions were less than 5% of the data values for all curves. Differences in the photosynthetic response to light for *Ambrosia* and *Solidago* became evident only at irradiances greater than ca.  $600\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ; apparent quantum yields were similar regardless of direction (adaxial or abaxial) of irradiation for leaves of these species. Values for apparent quantum yield ( $\alpha$ ) presented in the figure legends were estimated from fitting the model (equation 1). Only two photosynthetic measurements were made below  $100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$



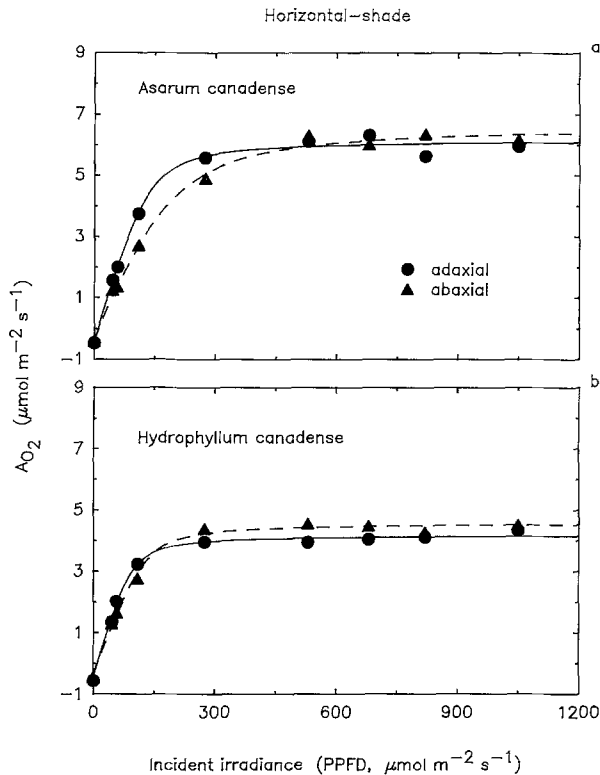
**Fig. 2.** The response of net photosynthesis ( $A_{\text{O}_2}$ : net photosynthesis measured as  $\text{O}_2$  evolution) to irradiance incident on the adaxial (solid line and filled circles) or abaxial (dashed line and triangles) leaf surface for *Ambrosia trifida* (a) and *Solidago canadensis* (b). Curves were fit by least-square regression (see Methods). The fitting parameters for each curve were: *Ambrosia*-adaxial:  $\alpha$ -0.0395,  $\theta$ -0.50,  $A_{\text{max}}$ -70.7, dark respiration ( $R_d$ )-2.12; *Ambrosia*-abaxial:  $\alpha$ -0.0448,  $\theta$ -0.50,  $A_{\text{max}}$ -30.6,  $R_d$ -2.51; *Solidago*-adaxial:  $\alpha$ -0.0503,  $\theta$ -0.79,  $A_{\text{max}}$ -24.3,  $R_d$ -3.93; *Solidago*-abaxial:  $\alpha$ -0.0705,  $\theta$ -0.56,  $A_{\text{max}}$ -20.8,  $R_d$ -2.53. Each symbol shows the mean of 3 or 4 measurements but curves were fit to the scatter of all points



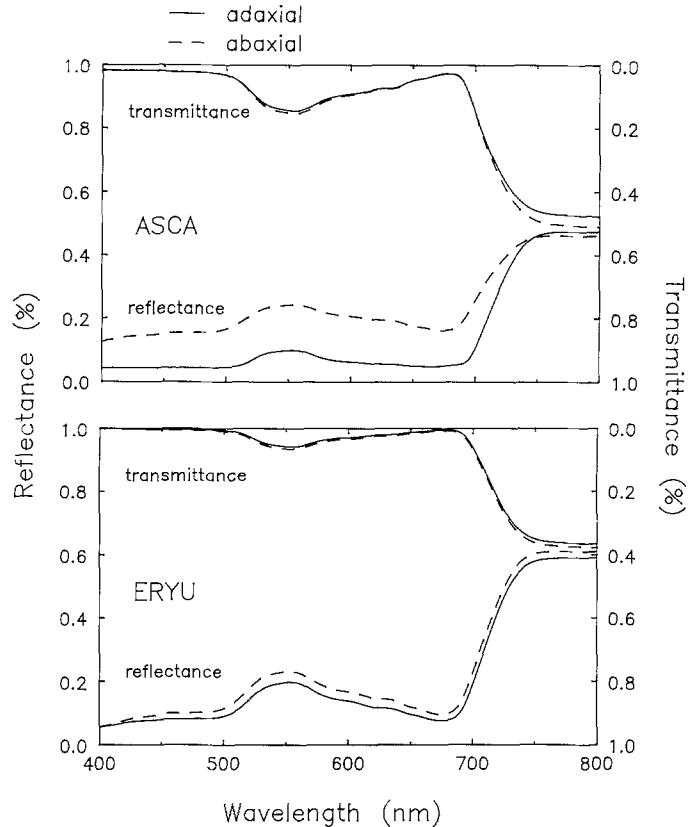
**Fig. 3.** The response of net photosynthesis ( $A_{\text{O}_2}$ : net photosynthesis measured as  $\text{O}_2$  evolution) to irradiance incident on the adaxial (solid line and dots) or abaxial (dashed line and triangles) leaf surface for *Silphium terebinthinaceum* (a) and *Eryngium yuccifolium* (b). The fitting parameters for each curve were: *Silphium*-adaxial:  $\alpha$ -0.0499,  $\theta$ -0.52,  $A_{\text{max}}$ -30.2, dark respiration ( $R_d$ )-3.32; *Silphium*-abaxial:  $\alpha$ -0.0331,  $\theta$ -0.48,  $A_{\text{max}}$ -30.1,  $R_d$ -1.21; *Eryngium*-adaxial:  $\alpha$ -0.0216,  $\theta$ -0.1,  $A_{\text{max}}$ -35.3,  $R_d$ -0.23; *Eryngium*-abaxial:  $\alpha$ -0.0217,  $\theta$ -0.01,  $A_{\text{max}}$ -35.8,  $R_d$ -0.13. Each symbol shows the mean of 3 or 4 measurements but curves were fit to the scatter of all points

(PPFD) in the linear region of the photosynthetic-light response, thus differences in estimated  $\alpha$  among leaf sides should be interpreted conservatively. There were no differences in net photosynthesis associated with direction of irradiation for species with vertical-sun leaves, *Silp-*

*hium* and *Eryngium*, or horizontal-shade leaves, *Asarum* and *Hydrophyllum* (Figs. 3 and 4). Presentation of the photosynthetic responses to absorbed light (Table 1) did not alter the relative response among leaf surfaces (data not shown).



**Fig. 4.** The response of net photosynthesis ( $A_{O_2}$ : net photosynthesis measured as  $O_2$  evolution) to irradiance incident on the adaxial (solid line and dots) or abaxial (dashed line and triangles) leaf surface for *Asarum canadense* (a) and *Hydrophyllum canadense* (b). The fitting parameters for each curve were: *Asarum*-adaxial:  $\alpha$ -0.0445,  $\theta$ -0.91,  $A_{\max}$ -6.6, dark respiration ( $R_d$ )-0.45; *Asarum*-abaxial:  $\alpha$ -0.0313,  $\theta$ -0.83,  $A_{\max}$ -7.0,  $R_d$ -0.36; *Hydrophyllum*-adaxial:  $\alpha$ -0.0457,  $\theta$ -0.91,  $A_{\max}$ -4.7,  $R_d$ -0.50; *Hydrophyllum*-abaxial:  $\alpha$ -0.0351,  $\theta$ -0.92,  $A_{\max}$ -4.9,  $R_d$ -0.38. Each symbol shows the mean of 3 or 4 measurements but curves were fit to the scatter of all points



**Fig. 5.** Reflectance and transmittance spectra (400–800 nm) for leaves irradiated on the adaxial (solid line) or abaxial (dashed line) leaf surfaces of *Asarum canadense* (upper panel) and *Eryngium yuccifolium* (lower panel). These species represent the extremes of greatest and least difference for reflectance and calculated absorptance among leaf surfaces

**Table 1.** Mean absorptance, reflectance, and transmittance (400–700 nm) for leaves irradiated on the adaxial or abaxial surfaces for six species with different leaf orientations and from different light environments. Absorptance was calculated as  $1 - \text{reflectance} - \text{transmittance}$ . The standard deviations for means calculated for

different leaves ( $n = 5$ ) for each species are in parentheses. Values with different superscripts among species (within rows) are significantly different, and values within a species but for different leaf surfaces (within columns) designated with an asterisk are significantly different at  $p \leq 0.05$

	Horizontal-shade		Horizontal-sun		Vertical-sun	
	<i>Asarum canadense</i>	<i>Hydrophyllum canadense</i>	<i>Ambrosia trifida</i>	<i>Solidago canadensis</i>	<i>Eryngium yuccifolium</i>	<i>Silphium terebinthinaceum</i>
<i>Adaxial surface</i>						
Absorptance	0.858(0.017)a*	0.885(0.016)c*	0.820(0.005)b*	0.824(0.004)b*	0.848(0.014)a	0.851(0.013)a
Reflectance	0.065(0.004)a*	0.060(0.003)b*	0.080(0.005)c*	0.088(0.003)d*	0.131(0.014)e	0.094(0.004)f*
Transmittance	0.077(0.014)b	0.054(0.012)a*	0.097(0.004)c	0.088(0.003)bc	0.021(0.002)d	0.055(0.009)a
<i>Abaxial surface</i>						
Absorptance	0.732(0.020)c*	0.767(0.020)b*	0.754(0.014)b*	0.747(0.017)bc*	0.837(0.007)a	0.830(0.007)a
Reflectance	0.184(0.006)c*	0.158(0.012)a*	0.150(0.011)a*	0.146(0.004)b*	0.140(0.006)b*	0.124(0.031)d*
Transmittance	0.080(0.022)bc	0.075(0.014)ab*	0.097(0.002)cd	0.107(0.018)d	0.022(0.003)e	0.058(0.008)a

Other aspects of the photosynthetic response to irradiance were consistent with the sun and shade habitats of these species. The light compensation point ( $I$ ) and light-saturated photosynthetic rate ( $A_{\max}$ ) were substantially lower for the shade species than for the sun species (*Hydrophyllum*: 14  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD and 4.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , for  $I$  and  $A_{\max}$ , respectively (adaxial irradiation); *Asarum*: 12 and 5.9; *Eryngium* 39 and 9.2; *Silphium*: 65 and 24.2; *Solidago*: 49 and 20.7; *Ambrosia*: 54 and 39.7). Values for  $\theta$  in equation 1 may vary from 0 to 1 and provide an estimate of the convexity of the light response curve. The relationship between  $\theta$  and convexity is not linear, however, and small changes in  $\theta$  between 1 and 0.5 represent large changes in convexity. For horizontal and vertical-sun leaves  $\theta$  was less than 0.79 but for horizontal-shade leaves it was greater than 0.83. Thus, there was a more rapid transition from light-limited to light-saturated portions of the light response curve for shade leaves than for sun leaves.

Reflectance and transmittance (from 400 to 800 nm) when leaves were irradiated on the adaxial or abaxial leaf surfaces are shown for *Asarum* and *Eryngium* (Fig. 5). These species represent the extremes of greatest and least differences among leaf surfaces. Transmittance for adaxial- and abaxial-irradiated leaf surfaces was similar for both species. Reflectance, however, was substantially greater when leaves were irradiated on the abaxial compared to the adaxial surface of *Asarum* (Fig. 5). The data

for all species were summarized by calculating the mean reflectance and transmittance from 400 to 700 nm and calculating absorptance for each surface (Table 1). Absorptance was consistently greater when leaves were irradiated on adaxial compared to the abaxial leaf surface for both horizontal-sun and horizontal-shade leaves. This difference was attributable primarily to differences in reflectance among leaf surfaces. Absorptances were similar between leaf surfaces of vertical-sun species.

Compared to sun leaves, leaves of horizontal-shade species, *Asarum* and *Hydrophyllum*, were structurally simple (Table 2). The large proportion of leaf thickness occupied by spongy mesophyll was primarily air space with occasional widely separated mesophyll spongy cells. Thus, the dominant cell layer for photosynthesis in horizontal-shade species was the single layer of upper palisade cells. The palisade mesophyll and spongy layers of the sun species were densely packed with few air spaces. Transverse sections of *Ambrosia* were equally divided between palisade and spongy cells. *Solidago* had roughly twice the thickness of palisade as spongy mesophyll tissue, and also had a third layer of spherical cells below the spongy layer referred to as "lower palisade" (Table 2). Vertical-sun species, *Eryngium* and *Silphium*, were symmetrical in transverse section. Both had approximately equal upper and lower palisade layers, with a central spongy mesophyll layer in *Silphium*.

**Table 2.** Quantitative leaf anatomy for six species with different leaf orientations and from different light environments. Mean values ( $\pm 1$  SD) within rows designated with different superscripts were significantly different at  $p \leq 0.05$ ,  $N = 5$

	Horizontal-shade				Horizontal-sun				Vertical-sun			
	<i>Asarum canadense</i>		<i>Hydrophyllum canadense</i>		<i>Ambrosia trifida</i>		<i>Solidago canadensis</i>		<i>Eryngium yuccifolium</i>		<i>Silphium terebinthinaceum</i>	
Upper epidermis ( $\mu\text{m}$ )	35.3	(7.3) <sup>b</sup>	21.5	(3.5) <sup>a</sup>	12.7	(2.6) <sup>c</sup>	18.8	(3.8) <sup>d</sup>	22.5	(4.8) <sup>a</sup>	31.4	(4.3) <sup>b</sup>
Upper palisade ( $\mu\text{m}$ )	49.5	(6.9) <sup>a</sup>	35.8	(4.8) <sup>b</sup>	40.2	(8.9) <sup>ab</sup>	91.5	(5.9) <sup>c</sup>	132.5	(25.1) <sup>d</sup>	309.5	(35.9) <sup>e</sup>
Spongy mesophyll ( $\mu\text{m}$ )	144.0	(27.1) <sup>a</sup>	155.2	(30.1) <sup>a</sup>	40.9	(5.4) <sup>b</sup>	50.1	(7.9) <sup>b</sup>	153.3	(33.8) <sup>a</sup>	–	–
Lower palisade ( $\mu\text{m}$ )	–	–	–	–	–	–	19.4	(6.5) <sup>a</sup>	136.7	(22.6) <sup>b</sup>	292.0	(64.6) <sup>c</sup>
Lower epidermis ( $\mu\text{m}$ )	28.4	(5.2) <sup>a</sup>	18.1	(2.9) <sup>c</sup>	9.3	(2.6) <sup>d</sup>	17.9	(2.9) <sup>c</sup>	21.5	(4.0) <sup>b</sup>	27.9	(5.0) <sup>a</sup>
Total thickness ( $\mu\text{m}$ )	257.2	(29.4) <sup>a</sup>	238.1	(67.9) <sup>ab</sup>	128.7	(15.7) <sup>c</sup>	210.3	(60.7) <sup>b</sup>	466.3	(64.5) <sup>d</sup>	662.7	(54.7) <sup>e</sup>

**Table 3.** Chlorophyll content, chlorophyll a/b ratio, and specific leaf mass (SLM) for six species with different leaf orientations and from different light environments. Mean values ( $\pm 1$  SD) within rows

designated with different superscripts were significantly different at  $p \leq 0.05$ ,  $N = 10$

	Horizontal-shade				Horizontal-sun				Vertical-sun			
	<i>Asarum canadense</i>		<i>Hydrophyllum canadense</i>		<i>Ambrosia trifida</i>		<i>Solidago canadensis</i>		<i>Eryngium yuccifolium</i>		<i>Silphium terebinthinaceum</i>	
Chl a ( $\text{mg/m}^2$ )	318.6	(28.1) <sup>b</sup>	289.5	(35.3) <sup>b</sup>	251.6	(30.5) <sup>c</sup>	241.3	(29.8) <sup>c</sup>	370.8	(86.6) <sup>a</sup>	290.4	(45.1) <sup>b</sup>
Chl a ( $\text{mg/g}$ )	2.2	(0.3) <sup>b</sup>	2.5	(0.3) <sup>c</sup>	1.6	(0.4) <sup>d</sup>	1.2	(0.2) <sup>e</sup>	0.8	(0.1) <sup>a</sup>	0.8	(0.1) <sup>a</sup>
Chl b ( $\text{mg/m}^2$ )	142.8	(17.0) <sup>a</sup>	129.2	(18.9) <sup>a</sup>	82.3	(18.7) <sup>b</sup>	96.0	(23.5) <sup>b</sup>	139.5	(41.1) <sup>a</sup>	98.1	(21.2) <sup>b</sup>
Chl b ( $\text{mg/g}$ )	0.7	(0.2) <sup>c</sup>	1.1	(0.4) <sup>b</sup>	0.7	(0.2) <sup>a</sup>	0.5	(0.2) <sup>c</sup>	0.3	(0.6) <sup>d</sup>	0.3	(0.1) <sup>d</sup>
Chl a/b	2.2	(0.1) <sup>c</sup>	2.3	(0.9) <sup>c</sup>	3.1	(0.4) <sup>a</sup>	2.6	(0.4) <sup>b</sup>	2.7	(0.3) <sup>b</sup>	3.0	(0.4) <sup>a</sup>
SLM ( $\text{g/m}^2$ )	32.1	(3.6) <sup>a</sup>	23.0	(2.6) <sup>b</sup>	35.1	(4.4) <sup>a</sup>	66.6	(5.7) <sup>c</sup>	109.5	(20.6) <sup>d</sup>	83.7	(6.8) <sup>e</sup>

Chlorophyll a/b ratio and specific leaf mass were significantly greater for horizontal-sun and vertical-sun leaves than for horizontal-shade leaves (Table 3). Specific leaf mass (SLM, Table 3) and total thickness (Table 2) were greatest for vertical-sun leaves. Total leaf thickness was greater for horizontal-shade than horizontal-sun leaves, but the additional densely packed tissue layers contributed to greater SLM for horizontal-sun leaves (Table 3).

## Discussion

The results for vertical-sun and horizontal-sun leaves support our initial prediction that photosynthetic symmetry is related to leaf orientation. Vertically-oriented leaves of *Silphium* and *Eryngium* maintained equal rates of net photosynthesis when irradiated on the adaxial or abaxial leaf surfaces, while rates of photosynthesis were substantially greater for adaxial irradiation of horizontal leaves of *Solidago* and *Ambrosia* (Figs. 2 and 3). In contrast to previous studies (Syvertsen and Cunningham 1979; Moss 1964; Terashima 1986), photosynthetic measurements ( $O_2$  evolution) in this study were made at saturating  $CO_2$  concentration and presumably reflect the complex interactions of light with leaf optical properties, rather than direct stomatal effects (Smith 1981).

Structural asymmetry and biochemical differentiation among cell layers may have been the primary factors contributing to unilateral function of horizontal-sun leaves of *Solidago* and *Ambrosia*. Direct measurements using fiber optic microprobes indicate steep attenuation of light incident on the adaxial surface of unifacial horizontal leaves as it passes through the upper palisade cell layer (Knapp *et al.* 1988; Vogelmann 1989; Vogelmann *et al.* 1989). Chlorophyll content per unit area changes in a parallel manner, decreasing precipitously from adaxial palisade parenchyma to abaxial spongy mesophyll cells (Knapp *et al.* 1988). Absorbance of visible light, however, may be higher in the spongy cells than in the palisade layer despite lower chlorophyll content in the spongy mesophyll (Terashima and Saeki 1983, 1985). Numerous air spaces in the spongy parenchyma cause high levels of light scattering, and the longer path-length due to scattering increases absorbance (detour effect). Although steep intra-leaf light gradients from ad- to abaxial leaf surfaces are evident from these studies, it appears that light incident on the adaxial surface is more effectively transmitted to the spongy layer than light incident on the abaxial surface would be transmitted through the spongy layer to the palisade layer.

In addition to the potential contributions of leaf structure to functional symmetry, Terashima and Inoue (1984) have shown that chloroplasts in the palisade and spongy mesophyll layers acclimate to the localized light environment. Palisade chloroplasts have biochemical, structural, and functional attributes of "sun-type" chloroplasts, while "shade-type" chloroplasts are prevalent in the spongy tissue. Sun chloroplasts support higher maximum rates of electron transport, and presumably higher rates of carbon reduction, than shade chloroplasts, similar to responses of whole leaves from

sun and shade environments (Boardman 1977). Vertical leaves of *Eryngium* and *Silphium* were photosynthetically bilateral and showed a high degree of structural symmetry (Table 2). Both species had palisade cell layers adjacent to the adaxial and abaxial epidermis, and presumably these layers were populated with sun chloroplasts.

As a result of the structural and biochemical attributes of different cell layers, adaxial irradiation of horizontal leaves may cause palisade and spongy chloroplasts to operate at similar regions of their respective photosynthetic-light response curves (Terashima and Inoue 1984). Abaxial irradiation, however, may result in light saturation of chloroplasts in spongy mesophyll, while chloroplasts in palisade cells are light-limited; this may lead to overall lower leaf photosynthetic rates. Abaxial irradiation of horizontal leaves of soybean (Terashima 1986) and sunflower (Syvertsen and Cunningham 1979) resulted in lower photosynthetic rates at intermediate irradiances, as compared to adaxial irradiation, but rates at low and high irradiance were unchanged. These observations are consistent with the proposal that chloroplasts in spongy and palisade mesophyll become out of phase during abaxial irradiation (i.e., the spongy and palisade mesophyll are no longer operating on corresponding regions of their light-response curves). Abaxial irradiation of *Solidago* and *Ambrosia*, however, caused lower photosynthetic rates at irradiances above the linear region of the light-response, and the differences persisted as irradiance increased. A similar response was observed for lilac measured at saturating  $CO_2$  (Oya and Laisk 1976). Possibly, the shade chloroplasts in the spongy layer became photoinhibited with progressively higher levels of abaxial irradiation. Measurements of the induction kinetics of chlorophyll fluorescence from adaxial and abaxial leaf surfaces support this speculation (Schreiber *et al.* 1977).

In contrast to horizontal-sun leaves, horizontal-shade leaves of *Hydrophyllum* and *Asarum* were functionally bilateral (symmetrical) in their photosynthetic response to adaxial and abaxial irradiation. The leaves of these species had primarily one layer of photosynthetic cells, thus precluding the structural and biochemical differentiation among cell layers observed for horizontal-sun leaves. The simple structure of these leaves may minimize construction and maintenance costs which would be adaptive in light-limited understory habitats. Moreover, a single layer of photosynthetic cells reduces intra-leaf self-shading and causes most photosynthetic cells to receive similar irradiance and operate simultaneously with regard to the photosynthetic-light response. The simultaneous response of cells causes horizontal-shade leaves to have a high degree of convexity of the photosynthetic response to irradiance and maximizes carbon gain at intermediate to low light levels (Terashima and Takenaka 1986).

A striking feature of horizontal-shade leaves was significantly lower absorbance (400–700 nm) when leaves were irradiated on their abaxial surfaces (Table 1). Lighter abaxial leaf surfaces or "bicoloration" have been observed for other shade species (Lee 1986, Vogelmann unpublished data); such bicoloration is caused primarily

by greater reflectance from the abaxial surface. The large air spaces between the palisade cells and lower epidermis and the associated difference in refractive index between the lower epidermis and air space contribute to the high level of reflectance from this surface (Wooley 1971). Increased reflectance from the abaxial surface may also be manifested inside the leaf such that light penetrating the upper palisade layer is reflected back into the palisade layer and absorbed for photosynthesis (Lee 1986, Vogelmann unpublished data). This would substantially increase the light harvesting efficiency of shade leaves at intermediate levels of irradiance.

Vertical leaf orientation reduces leaf temperature and transpiration and increases water-use efficiency for leaves in open habitats (Jurik *et al.* 1990; Smith and Ullberg 1989). As compared to horizontal leaves, vertical leaves may receive substantially lower peak irradiance and lower total daily irradiance on the adaxial surface but higher totals on the abaxial surface. Bilateral symmetry of light processing for photosynthesis appears to be important in maximizing daily carbon gain for vertical leaf species. The nitrogen investment in supporting sun-type chloroplasts on both sides of the leaf may, however, be considerable (Evans 1989), and reduced photosynthetic nitrogen-use efficiency may be a cost of bilateral function. Horizontal-sun leaves maximize utilization of light incident on the adaxial surface, and because of the parallel gradients of light and physiological properties of chloroplasts within the leaf, horizontal-sun leaves may support higher photosynthetic rates with lower nitrogen content per unit leaf surface area. We speculate that vertical-sun leaves maintain high WUE and low PNUE integrated over the day, whereas horizontal-sun leaves maintain low WUE and high PNUE.

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