Oecologia (1993) 95:393–400

Oecologia
© Springer-Verlag 1993

Photosynthetic and structural acclimation to light direction in vertical leaves of *Silphium terebinthinaceum*

Mary E. Poulson, Evan H. DeLucia

Department of Plant Biology, University of Illinois, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, USA

Received: 9 December 1992 / Accepted: 23 April 1993

Abstract. The azimuth of vertical leaves of *Silphium terebinthinaceum* profoundly influenced total daily irradiance as well as the proportion of direct versus diffuse light incident on the adaxial and abaxial leaf surface. These differences caused structural and physiological adjustments in leaves that affected photosynthetic performance. Leaves with the adaxial surface facing East received equal daily integrated irradiance on each surface, and these leaves had similar photosynthetic rates when irradiated on either the adaxial or abaxial surface. The adaxial surface of East-facing leaves was also the only surface to receive more direct than diffuse irradiance and this was the only leaf side which had a clearly defined columnar palisade layer. A potential cost of constructing East-facing leaves with symmetrical photosynthetic capcity was a 25% higher specific leaf mass and increased leaf thickness in comparison to asymmetrical South-facing leaves. The adaxial surface of South-facing leaves received approximately three times more daily integrated irradiance than the abaxial surface. When measured at saturating CO_2 and irradiance, these leaves had 42% higher photosynthetic rates when irradiated on the adaxial surface than when irradiated on the abaxial surface. However, there was no difference in photosynthesis for these leaves when irradiated on either surface when measurements were made at ambient CO_2. Stomatal distribution (mean adaxial/abaxial stomatal density = 0.61) was unaffected by leaf orientation. Thus, the potential for high photosynthetic rates of adaxial palisade cells in South-facing leaves at ambient CO_2 concentrations may have been constrained by stomatal limitations to gas exchange. The distribution of soluble protein and chlorophyll within leaves suggests that palisade and spongy mesophyll cells acclimated to their local light environment. The protein/chlorophyll ratio was high in the palisade layers and decreased in the spongy mesophyll cells, presumably corresponding to the attentuation of light as it penetrates leaves. Unlike some species, the chlorophyll a/b ratio and the degree of thylakoid stacking was uniform throughout the thickness of the leaf. It appears that sun-shade acclimation among cell layers of *Silphium terebinthinaceum* leaves is accomplished without adjustment to the chlorophyll a/b ratio or to thylakoid membrane structure.

Key words: Functional symmetry – Leaf anatomy – Leaf orientation – Light environment – Photosynthesis

Correspondence to: E.H. DeLucia

The ability of leaves to harvest light incident on different surfaces or from different directions (i.e. direct vs. diffuse rays) is determined by the interplay of structural, biochemical, and physiological properties of component cell layers. Depending on azimuth, vertical leaves may receive similar daily irradiance on the adaxial and abaxial surfaces and typically have similar photosynthetic rates when irradiated on either surface (Moss 1964; Václavík 1984; DeLucia et al. 1991). These functionally symmetrical leaves are amphistomatous and have unifacial leaf anatomy, with palisade cells beneath the adaxial and abaxial epidermis. In contrast, the light environment of horizontal leaves is highly asymmetrical, and these leaves typically have photosynthetic rates that are 30–50% lower when irradiated on the abaxial surface (Moss 1964; Syvertsen and Cunningham 1979; Terashima 1989; DeLucia et al. 1991). These functionally asymmetrical leaves are anatomically bifacial, with one or more layers of palisade mesophyll beneath the adaxial epidermis and spongy mesophyll beneath the abaxial epidermis. An exception is the near-vertical leaves (mean leaf angle 71.3°) of *Rumex densiflorus*. These leaves have unifacial leaf anatomy, yet they have significantly lower rates of photosynthesis when irradiated on the abaxial surface (Day et al. 1990). *Rumex densiflorus* leaves are primarily South-facing and thus receive more light on the adaxial than the abaxial surface (Geller and Smith 1982). This suggests that the local light environment on each surface may override, to some extent, the influence of leaf orientation and structure on photosynthetic symmetry.

Differential responses of adaxial and abaxial leaf surfaces to light have been attributed to differences in the biochemical characteristics of palisade and spongy mesophyll cells. These differences are similar to the characteristics of sun and shade leaves described by Boardman (1977) and Bjorkman (1981). Horizontal leaves irradiated predominantly on the adaxial side during development have chloroplasts in the palisade cells with sun-type properties including higher chlorophyll a/b ratio, a lower ratio of appressed to non-appressed thylakoid membranes, and higher electron transport and CO_2 fixation rates than found in spongy mesophyll cells (Schreiber et al. 1977; Kulandaivelu et al. 1983; Terashima and Inoue 1984, 1985; Terashima and Takenaka 1986). When horizontal leaves are irradiated on the abaxial side during expansion, sun- and shade-type properties become reversed; the palisade cells are populated with shade-type chloroplasts and the spongy mesophyll cells are populated with sun-type chloroplasts (Schreiber et al. 1977; Terashima 1986).

The purpose of this study was to determine the capacity of adaxial and abaxial sides of *Silphium terebinthinaceum* Jacq. (Asteraceae) leaves to acclimate to the quantity of incident light as determined by leaf orientation. *S. terebinthinaceum* is a taprooted dicotyledonous perennial common to tallgrass prairies. The leaves grow vertically (more than 75% at angles $>70°$ from horizontal) from a basal rosette, and are large (typically 70-cm long and 35-cm wide) and unlobed. After emergence most leaves orient with the adaxial laminar surface facing East or West (leaf edges oriented North or South; Smith and Ullberg 1989). However, some leaves (ca. 30%) orient with the adaxial laminar surface facing North or South (edges East or West). Fewer than 10% of leaves orient in any azimuth other than one of the four cardinal directions. We hypothesize that leaf orientation, and consequently the light environment of each leaf surface, determine the degree of photosynthetic (functional) symmetry in *S. terebinthinaceum*. Using leaves which had developed in the field with the adaxial surface facing either East or South, photosynthetic symmetry was determined by measuring gas-exchange characteristics while leaves were irradiated on the ad- or abaxial surface. To gain a better understanding of the mechanisms responsible for functional symmetry and leaf acclimation to light direction, anatomical and biochemical properties of leaves from the two orientations were also examined.

Materials and methods

Plant material

Measurements were made on leaves of *Silphium terebinthinaceum* growing in a restored tallgrass prairie in the University of Illinois Ecological Research Area, 6 km northeast of Urbana, Illinois. Mature leaves $>80°$ from horizontal were chosen for measurements. Leaf azimuth was defined as the angle between magnetic North and the plane normal to the adaxial surface of the leaf. The azimuths of East- and South-oriented leaves were 85–95° and 175–185°, respectively. To confirm that leaves were not heliotropic, the orientation of 10 leaves on 5 plants were measured hourly throughout a single clear day. Other data were collected between 20 June and 20 August, 1991.

Measurement of photosynthetic photon flux density (PPFD)

Irradiance were measured with gallium arsenide-phosphide photodiodes (6-mm diameter, G1118, Hamamatsu Photonics, K.K., Japan; Gutschick et al. 1985; Pearcy 1989). Photodiodes were fitted with teflon diffusion disks as in Ögren and Sjöström (1990) to improve the cosine response to within 5% error from 0 to 85°. Voltage from each photodiode was measured across a 500-Ω resistor by a datalogger (21×, Campbell Scientific, Logan UT, USA). Photodiodes were calibrated individually against a quantum sensor (LI-190SB LI-Cor Inc., Lincoln, Neb., USA) under sunlight. Photodiodes were mounted on bases and placed in configurations to represent 6 different leaf orientations: Horizontal-up, Horizontal-down,East, West, North and South. Photodiodes were placed within the canopy at a depth equal to that of the upper (leaf tip region) one-third of *S. terebinthinaceum* leaves, where photosynthetic, biochemical and anatomical measurements were also made. Values therefore represent incident irradiance similar to that received by *S. terebinthinaceum* leaves within the canopy. Two photodiodes were mounted on each base. At sixty-second intervals, one photodiode measured total photosynthetic photon flux density (PPFD) while the other photodiode, which was equipped with a shadow band, measured the diffuse component of PPFD. Shadow bands were constructed by attaching a hemipherical plastic band (4.5 × 22 cm) across the base such that one photodiode would remain shaded throughout the day. The bands could be rotated and moved laterally on the base and were adjusted periodically to ensure that sensors remained shaded. Bases and shadow bands were painted black to minimize reflection. Diffuse light measurements were corrected for diffuse radiation blocked by the shadow bands as in Iqbal (1983). Direct radiation was calculated by subtracting the corrected diffuse component from total PPFD.

Leaf and chloroplast anatomy

Interveinal regions from *S. terebinthinaceum* leaves were cut in the field, immersed in 4% aqueous glutaraldehyde buffered with 0.2 M Sorenson's phosphate (pH 6.8), and fixed for 12 h. Material was then washed in buffer three times and post-fixed in 2% osmium tetroxide for 3 h. Following fixation the tissue was dehydrated in a graded ethanol series and embedded in a 1:1 mixture of Spurr's and Epon 812 resin. Leaf samples were sectioned on a microtome into 3 μm and 900 Å transverse sections for light microscopy and transmission electron microscopy (TEM), respectively. Using a light microscope and calibrated ocular micrometer, the thickness of adaxial and abaxial palisade mesophyll, spongy mesophyll, and total leaf thickness were measured. Ultrathin sections for TEM were stained with uranyl acetate followed by lead citrate (Reynolds 1963). Photomicrographs of chloroplasts were taken at 100-μm increments from the adaxial to the abaxial surfaces of leaf cross-sections with a Hitachi H-600 electron microscope operated at 100 kV. The total length of appressed and of non-appressed thylakoid membranes were measured to the nearest mm on micrographs (25000× prior to printing) and the ratio of the total length of appressed to non-appressed thylakoids was calculated.

Biochemical properties of leaves

Silphium terebinthinaceum leaves were collected from the field by cutting and then promptly re-cutting the petioles while they were immersed in water to ensure that continuity in the xylem was maintained. Five leaves from each orientation were transported to the

laboratory where measurements of soluble protein and chlorophyll were made in paradermal leaf sections. Leaves were kept under diffuse light in a cold room (10° C) and measurements were completed within 3 h of collection. Leaf disks (22 mm^2) were cut from the upper one-third of the leaf, frozen on the stage of a microtome (Model 880, American Optical Company, Buffalo NY, USA), and cut paradermally into 30 μm-thick sections. Every three successive 30 μm-sections were pooled (a total of 90 μm) and placed into microfuge tubes containing either protein extraction solution (50 mM tris hydrochloride, pH 8.0, 2.5 mM EDTA) or 80% acetone (v/v) for chlorophyll determination. Sections were made using four disks from each leaf, alternatively beginning with the adaxial or abaxial surface. For the soluble protein determination, a small amount (<1 mg) of polyvinylpolypyrrolidone (PVPP) was added to each tube and the tissue was homogenized with a teflon pestle. Samples were centrifuged to remove cellular debris and the supernatant was added to 4 times the sample volume of acetone. Samples were re-spun and pellets suspended in 0.1 N NaOH. Soluble protein was determined spectrophotometrically (4052 TDS, LKB Biochrom Ltd. Cambridge, England) according to Lowry et al. (1954). Chlorophyll content and chlorophyll a/b ratios were determined from paradermal sections homogenized in 80% acetone using extinction coefficients determined by Porra et al. (1989).

Nitrogen analysis, specific-leaf-mass, and leaf optical properties

Leaf disks (10 cm^2) were taken from opposite sides of the midrib on the upper one-third of five *S. terebinthinaceum* leaves, oven dried (70° C) to constant mass, and used for gravimetric determination of specific-leaf-mass (SLM: leaf mass/leaf area). The same disks were ground to 40 mesh in a Wiley mill, digested with a Kjeldahl catalyst (Lowther 1980) and analyzed for nitrogen concentration in an ammonium analyzer (Model 360, Wescan Inst., Santa Clara, CA, USA). Reflectance and transmittance of leaves from 400–800 nm were measured in the laboratory using a Taylor-type integrating sphere and a spectroradiometer (LI-1800, LI-Cor) on the adaxial and abaxial surfaces of field-cut leaves. Absorptance was calculated as 1 – transmittance – reflectance.

Photosynthetic response to light

The photosynthetic response of *Silphium terebinthinacium* leaves to adaxial or abaxial irradiance at saturating CO_2 was measured with a leak-disk oxygen electrode (LD2, Hansatech Ltd., Kings Lynn, England) as described by Delieu and Walker (1981). A fixed output metal halogen lamp (LS2H, Hansatech) was used in conjunction with neutral-density filters to generate a range of irradiances. Photosynthetically active radiation (PAR) transmitted through the filters was measured with a quantum sensor (LI-190SB LI-Cor Inc.). Leaf disks (10 cm^2) were collected from the upper one-third of mature leaves on cloudless or partly-clear days between mid-morning and early-afternoon. This ensured that plants had been exposed to relatively high irradiances before measurements were taken, while minimizing afternoon water-stress. Photosynthesis was measured during adaxial irradiation using a disk taken from one side of the leaf midrib and during abaxial irradiation using a disk from the opposite side of the midrib on the same leaf. Measurements were made in a field laboratory and were initiated with 5 min of tissue collection. Leaf disks were exposed to an initial induction period consisting of repeated cycles of moderate irradiance (ca. 500 μmol m^{-2} s^{-1}) and darkness. The photosynthetic response to light was measured from lower to higher irradiances. The sample chamber was flushed with hydrated 5% CO_2 (v/v) prior to measurement at each irradiance. Doubling the concentration of CO_2 in the chamber did not increase photosynthetic rates indicating that 5% CO_2 was saturating for this species.

Photosynthesis of *S. terebinthinaceum* leaves in ambient CO_2 was measured with a closed infra-red gas analysis system (LI-6200, LI-Cor Inc.). A portion (35 cm^2) of the upper one-third to one-half of a leaf was enclosed in a one-liter chamber. Gas exchange from both leaf surfaces was measured but the cuvette was modified so that only the upper half of the chamber permitted the entry of light. This allowed whole-leaf photosynthesis to be measured on leaves irradiated on only the adaxial and abaxial surface. Measurements were completed in 1 min, and chamber temperature remained within 3° C of ambient air temperature during this time. Humidity in the chamber was maintained within 5% of ambient humidity by passing a portion of the air entering the cuvette through a desiccant. Irradiance was measured with a cosine-corrected photodiode mounted at leaf-level inside the chamber. Assimilation of CO_2 was measured on leaves in their natural orientation on 4 clear days from mid-morning to early afternoon. Assimilation data presented are the maximum assimilation rate measured at each irradiance and therefore represent the boundary condition.

Results

Daily integrated PPFD and the ratio of adaxial/abaxial and direct/diffuse irradiation incident on photodiodes and placed in the position of leaves was strongly influenced by orientation (Table 1). Total daily irradiance (sum of adaxial and abaxial irradiance) was greatest for horizontal leaves followed by vertical East-facing and South-facing leaves. Horizontal leaves received a much greater percentage (>95%) of daily integrated irradiance on the adaxial than on the abaxial surface. Vertical East-facing leaves received the same integrated irradiance on each surface, resulting in an adaxial/abaxial ratio close to one. However, as a result of afternoon cloud cover, abaxial irradiance on East-facing leaves was composed of more diffuse light than was the irradiance incident on the adaxial leaf surface. South-facing vertical leaves received nearly 3 times as much daily integrated irradiance on the adaxial surface than on the abaxial surface. Light striking both surfaces of these leaves was predominantly diffuse.

There were no statistically significant differences in the ratio of the total length of appressed to non-appressed thylakoid membranes at different depths within or among leaves of different orientations (Table 2). Leaves from both orientations had unifacial anatomy with 2–3 layers of palisade mesophyll cells beneath the adaxial and abaxial epidermis (Fig. 1a and b). Only the palisade cells beneath the adaxial surface of East-facing leaves were densely packed and thus formed distinct cell layers. Cell layers throughout South-facing leaves and near the adaxial side of East-facing leaves contained large intercellular air spaces that made palisade cells difficult to distinguish from spongy mesophyll. These air spaces were still conspicuous when cross-section thickness was increased from 3 to 7 μm (data not shown). Leaves of both orientations contained prominent bundle sheath extensions composed of spherical cells lacking chloroplasts (Fig. 1a and b).

East-facing leaves were significantly thicker than South-facing leaves (Table 2). This difference was due mostly to a thicker layer of spongy mesophyll cells, although the adaxial and abaxial palisade layers were also

Table 1. Direct and diffuse irradiance (mol m^{-2} d^{-1}, 400–700 nm) incident on photodiodes in the position of leaves of different orientations. East-West vertical leaves had the adaxial surface facing East. South-North vertical leaves had the adaxial surface facing South. Two photodiodes were placed within the canopy of a tallgrass prairie to measure light at each orientation. One photodiode in each orientation measured total irradiance (direct + diffuse) and the other was equipped with a shadow band to measure diffuse irradiance. Direct irradiance was calculated as total irradiance – diffuse irradiance. Diffuse and Direct is the sum of these values for each leaf surface, the Total Irradiance is the grand total of all irradiance incident on a leaf. Adaxial/Abaxial and Direct/Diffuse are ratios of these values for each surface. Values are means (±1 SD) for 5 relatively clear days in a tallgrass prairie in central Illinois

Leaf angle	Leaf azimuth		Direct irradiance	Diffuse irradiance	Diffuse and direct	Total irradiance	Adaxial / Abaxial	Direct / Diffuse
Horiz.		Ad	21.51 (8.07)	16.78 (1.39)	38.29 (8.05)	40.59 (8.57)	16.18 (0.52)	1.13 (0.44)
		Ab	* *	2.30 (0.49)	2.30 (0.52)			
Vert.	E-W	Ad	8.35 (4.42)	7.84 (1.17)	16.19 (5.49)	32.13 (6.28)	1.03 (0.34)	0.73 (0.27)
		Ab	5.35 (2.39)	10.59 (0.76)	15.94 (2.60)			
Vert.	S-N	Ad	3.20 (2.53)	13.03 (1.52)	16.23 (3.72)	22.08 (4.11)	2.75 (0.51)	0.24 (0.14)
	Ab	1.19	4.66 (0.15)	5.85 (0.43)	(0.47)			

* <0.01

Table 2. Anatomical and biochemical characteristics of *Silphium terebinthinaceum* leaves with adaxial lamina oriented East or South. Mean values (±1 SD) designated with a single asterisk (*) are significantly different at $p \leq 0.05$; double asterisks (**) are significantly different at $p \leq 0.01$; $N = 5$ for all values except nitrogen and specific leaf mass (SLM) in which $N = 10$

	East orientation	South orientation
Leaf thickness (μm)	586 (47.7)*	490 (55.7)*
Adaxial epidermis (μm)	31 (1.7)	30 (2.5)
Adaxial palisade (μm)	200 (14.4)*	168 (25.9)*
Spongy mesophyll (μm)	142 (14.8)**	96 (18.2)**
Abaxial palisade (μm)	194 (8.9)	166 (24.1)
Abaxial epidermis (μm)	28 (2.5)	29 (2.4)
Stomatal density (mm^{-2})		
Adaxial	161 (18.9)	175 (24.6)
Abaxial	274 (39.2)	274 (31.6)
Ttl Chl (μg cm^{-2})	392 (70.4)	363 (58.80)
Ttl Sol protein (μm cm^{-2})	2.16 (0.428)	1.82 (0.490)
Chl a/b	3.12 (0.269)	3.22 (0.211)
Appressed/Non-appressed thylakoid membranes	2.46 (0.930)	2.44 (0.891)
% Nitrogen (dry weight)	1.75 (0.272)	1.99 (0.411)
N/area (g cm^{-2})	218 (39.0)	207 (68.0)
SLM (mg cm^{-2})	12.50 (1.23)**	9.36 (2.03)**

thicker. Stomatal density was independent of leaf orientation (Table 2) but differed between adaxial and abaxial surfaces of leaves; significantly more stomates per unit area were present on abaxial surfaces. Total chlorophyll and soluble protein content expressed per unit leaf area and the chlorphyll a/b ratio were similar in leaves of both orientations. There was no difference in nitrogen content when calculated on a dry weight or leaf area basis in leaves of either orientation. Specific-leaf-mass was greater in East-facing leaves than in South-facing leaves (Table 2).

The highest amount of soluble protein for East-facing leaves was in the second (65–130 μm) and eighth (520–555 μm) layers of the leaves (Fig. 2a). These layers correspond to the adaxial and abaxial palisade mesophyll, respectively. For South-facing leaves more protein was observed in the second (61–122 μm) and seventh (366–427 μm) layers, also corresponding to the adaxial and abaxial palisade mesophyll (Fig. 2b). Overall, South-facing leaves contained a more uniform distribution of soluble protein than East-facing leaves. The chlorophyll content of East-facing leaves was highest in the third through seventh layers (130–451 μm; Fig. 2c) and the chlorophyll content of South-facing leaves was highest in the third through sixth layer (122–366 μm; Fig. 2d). In both East- and South-facing leaves chlorophyll-enriched layers were composed predominantly of spongy mesophyll cells. For leaves of both orientations, the ratio of protein/chlorophyll was more than 3 times higher in layers of the leaf that contained mostly palisade mesophyll compared to layers containing mostly spongy mesophyll.

There was no significant difference in photosynthetic rates measured at saturating CO_2 for East-facing leaves irradiated on the adaxial vs. abaxial surface (Fig. 3a). Photosynthetic rates were also similar for ad- and abaxial irradiation of South-facing leaves measured at irradiances below 700 μmol m^{-2} s^{-1}. However, at irradiances greater than 1000 μmol m^{-2} s^{-1}, photosynthetic rates were 42% higher for adaxial than for abaxial irradiation (Fig. 3b). There were no significant differences in leaf optical properties among surfaces or orientations. The average leaf absorptance was 0.81 (±0.03 SD), reflectance was 0.11 (±0.02 SD) and transmittance was

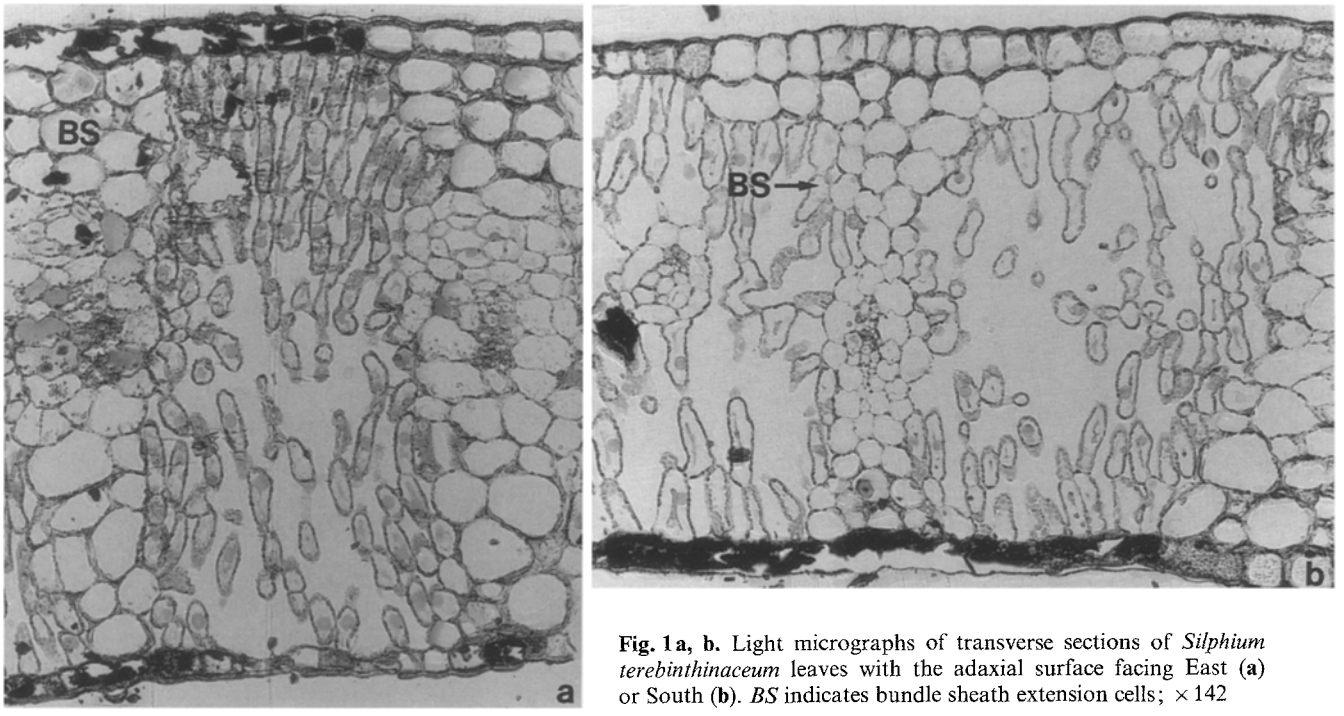

Fig. 1a, b. Light micrographs of transverse sections of *Silphium terebinthinaceum* leaves with the adaxial surface facing East (**a**) or South (**b**). *BS* indicates bundle sheath extension cells; ×142

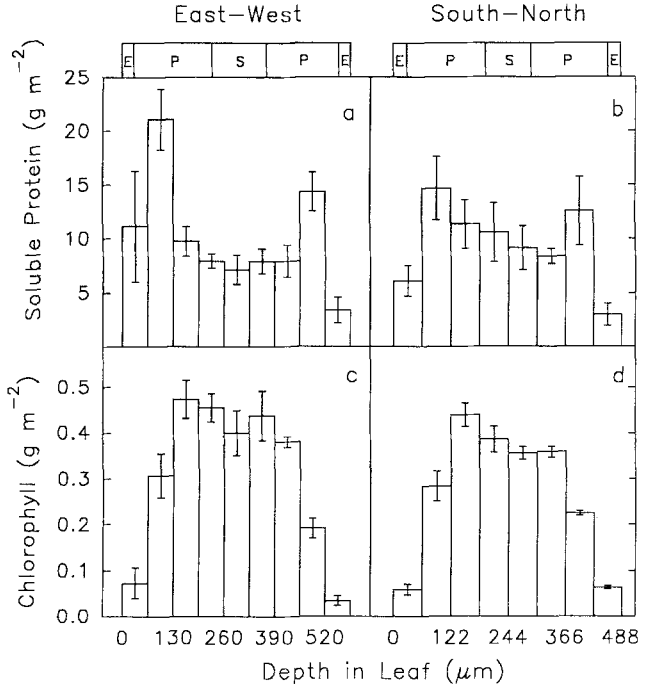

Fig. 2a–d. The distribution of soluble protein (**a** and **b**) and total chlorophyll (**c** and **d**) across vertical *Silphium terebinthinaceum* leaves from two orientations. East-West leaves were those leaves oriented with the adaxial surface facing East and South-North were those with the adaxial surface facing South. Protein and chlorophyll determinations were made on paradermal sections pooled from four leaf disks within a leaf. Bars represent averages and 1 SE of measurements made on 3–5 individual leaves. The thicknesses of the epidermis (*E*, adaxial is at left, abaxial is at right), palisade mesophyll (*P*), and spongy mesophyll (*S*) are indicated by the bars above the graphs

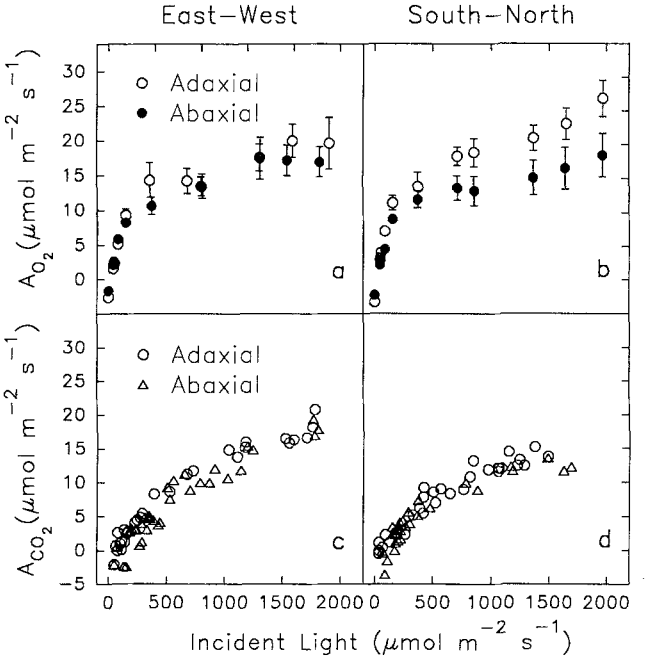

Fig. 3a–d. The response of net photosynthesis to irradiance incident on the adaxial (*open circles*) or abaxial (*filled circles and open triangles*) surfaces of vertical *Silphium terebinthinaceum* leaves from two orientations. East-West leaves were oriented with the adaxial surface facing East (**a** and **c**) and South-North leaves were oriented with the adaxial surface facing South (**b** and **d**). Measurements were made in saturating (A_{O2}: net photosynthesis measured as O_2 evolution; **a** and **b**) or ambient (A_{CO_2}: net photosynthesis measured as CO_2 flux; **c** and **d**) CO_2. For O_2 evolution each symbol shows the mean of 3 or 4 measurements and vertical lines represent 1 SE. For CO_2 flux measurements each symbol represents one measurement on a leaf in its natural orientation. These data represent the maximum assimilation rate at each irradiance (see methods)

0.07 (±0.02 SD). Thus, presentation of the photosynthetic data on an absorbed light basis did not alter the relationships illustrated in Fig. 3. Under ambient CO_2, no differences were observed for photosynthetic-light response curves measured under adaxial or abaxial irradiation for East- or South-facing leaves (Fig. 3c and d).

Discussion

Leaf orientation influenced total irradiance and the ratio of diffuse to direct irradiance incident on each leaf surface (Table 1). The penetration of direct and diffuse irradiance into leaves differs (Donahue 1991). Thus, the potential effect of the distribution of total irradiance on the photosynthetic function of each leaf surface may be confounded by differences in the ratio of direct to diffuse irradiance. The penetration of direct irradiance into leaves is facilitated by dense columnar palisade cells which minimize light scattering (McClendon 1984; Sharkey 1985; Knapp et al. 1988; Vogelmann et al. 1989). Photosynthetic rates of sun-acclimated leaves of *Thermopsis montana*, which have densely packed adaxial palisade cells, are consistently higher under direct than under diffuse irradiance (Donahue 1991). For *S. terebinthinaceum*, a clearly defined densely-packed palisade layer was observed only under the adaxial epidermis of East-facing leaves. This surface received a higher proportion of direct than diffuse irradiance (Table 1), which may have promoted the formation of the well-defined palisade layer. The irregularly shaped palisade cells and abundant air spaces of other leaf surfaces increase light scattering and therefore light absorption (McClendon 1984; Knapp et al. 1988).

The distribution of soluble protein across *S. terebinthinaceum* leaves was non-uniform, and was highest in the ad- and abaxial palisade layers and lowest in the spongy mesophyll. Soluble protein content is highly correlated with the concentration of ribulose bisphosphate carboxylase-oxygenase (RuBisCO; Terashima and Evans 1988) and electron transport capacity (Evans 1989). Thus, higher soluble protein content in the palisade layers of *S. terebinthinaceum* may indicate higher photosynthetic capacity in this region of the leaf. These results are consistent with greater electron transport capacity in palisade than spongy mesophyll observed in leaves of *Camellia japonica* and spinach (Terashima and Inoue 1984; 1985). The distribution of chlorophyll through vertical leaves of *S. terebinthinaceum* was inverse of the pattern for soluble protein, i.e. chlorophyll content increased toward the middle in all leaves. Light attenuates dramatically as it penetrates leaves (Vogelmann et al. 1989; Donahue 1991) and higher chlorophyll content at increased leaf depth may enhance absorbance by cells located in the region of lowest light (Knapp et al. 1988; Bornman et al. 1991; Cui et al. 1991). The distribution of chlorophyll and soluble protein suggests that light gradients within leaves drive cellular acclimation to the local light environment. A high protein/chlorophyll ratio is typical of sun leaves and represents a greater partitioning of nitrogen to RuBisCO relative to electron transport components (Evans 1988, 1989). The protein/chlorophyll ratio is lower in shade leaves.

Shade leaves also typically exhibit lower chlorophyll a/b ratios and increased thylakoid staking, as indicated by a higher ratio of appressed to non-appressed thylakoid membranes, both of which are due to a higher amount of light harvesting chlorophyll a/b protein associated with Photosystem II (Boardman 1977; Bjorkman 1981; Aro et al. 1986). A decreased chlorophyll a/b ratio and higher radio of appressed/non-appressed thylakoid membranes has been observed with increasing depth in horizontal leaves (Terashima and Inoue 1984; Knapp et al. 1988; Bornman et al. 1991; Cui et al. 1991). This was not, however, observed for leaves of *S. terebinthinaceum* in which the degree of thylakoid stacking and the chlorophyll a/b ratio were constant throughout the depth of leaves. Moreover, the relatively high chlorophyll a/b ratio and low amount of thylakoid stacking suggest that palisade and spongy mesophyll cells from leaves of both orientations were populated with chloroplasts that had sun-type thylakoid structure. A recent study of light acclimation in *Tradescantia albiflora*, a shade-adapted understory species, also showed that the ratios of chlorophyll a/b and appressed/non-appressed thylakoid membranes were constant over a wide range of growth irradiances (Adamson et al. 1991). Acclimation of palisade and spongy mesophyll of *S. terebinthinaceum* to the local light environment seems to be accomplished by changes in soluble protein and chlorophyll content without accompanying changes in thylakoid structure.

Photosynthetic symmetry in *S. terebinthinaceum* was determined by the relative amount of irradiance incident on the adaxial versus the abaxial leaf surface. East-facing leaves received equal total irradiance on each surface and were functionally symmetrical. However, under saturating CO_2 and irradiance, South-facing leaves had 42% higher photosynthetic rates when irradiated on the adaxial compared to the abaxial surface (Fig. 3b). In their natural habitat these leaves received nearly 3 times more light on the adaxial surface. Thus, vertical leaves of *S. terebinthinaceum* had the ability to acclimate to light direction. The mechanism causing differential photosynthesis under CO_2-saturation is not clear. Both leaf surfaces had similar absorptances, and the distributions of soluble protein and chlorophyll through the leaf were not sufficiently different among leaf surfaces to explain the difference in photosynthetic performance. The ecological significance of acclimation to light direction in this species is also in question.

In contrast to measurements made at saturating CO_2, photosynthetic rates measured under ambient conditions were the same for adaxial and abaxial irradiation of South-facing leaves. This apparent loss of the acclimation to light direction may have resulted from stomatal limitations to gas exchange. The ratio of ad- to abaxial stomatal density for leaves of the two orientations were not significantly different (East-facing, 0.59; South-facing, 0.64). An increase in stomatal density and a tendency toward amphistomaty is often observed in response to increased irradiance (Boardman 1977; Bjork-

man 1981; Mott et al. 1982). However, there was no alteration in the distribution of stomata in response to differences in irradiance on each leaf surface of East- or South-facing *S. terebinthinaceum* leaves. As a result of low stomatal density, palisade cells on the adaxial side of South-facing leaves may have been unable to realize their maximum photosynthetic potential.

Vertical orientation of *S. terebinthinaceum* leaves increases carbon gain while minimizing water loss (Smith and Ullberg 1989; Jurik et al. 1990). However, these leaves receive less total irradiance for photosynthesis than horizontal leaves. Photosynthetic symmetry of vertical *S. terebinthinaceum* leaves increases total daily carbon gain, but high specific leaf mass and high nitrogen content at increasing depths within leaves may represent carbon and nitrogen costs of maintaining functional symmetry for this species.

Acknowledgements. We thank Bryan Siegfried for collection of oxygen electrode and leaf optical properties data and Scott Heckathorn for help with the nitrogen analyses. We gratefully acknowledge Dr. R.F.E. Crang for guidance with the electron microscopy and Dr. R.A. Donahue for stimulating discussions on leaf form and function. We thank the Center for Electron Microscopy at the University of Illinois for the use of microscopy equipment and Dr. Z.B. Carothers for the use of his microtome. This research was funded by a USDA CRGO grant (no. AG 89-37280-4817) to E.H. DeLucia.

References

Adamson HY, Chow WS, Anderson JM, Vesk M, Sutherland MW (1991) Photosynthetic acclimation of *Trasescantia albiflora* to growth irradiance: morphological, ultrastructural and growth responses. Physiol Plant 82:353–359

Aro EM, Rintamäki E, Korhonen P, Mäenpää P (1986) Relationship between chloroplast structure and O_2 evolution rate of leaf discs in plants from different biotypes in South Finland. Plant Cell Environ 9:87–94

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Ann Rev Plant Physiol 28:355–377

Bjorkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Zeigler H (eds) Physiological plant ecology I. (Ency in plant physiology, NS, vol 12A). Springer, Berlin Heidelberg New York, pp 57–107

Bornman JF, Vogelmann TC, Martin G (1991) Measurement of chlorophyll fluorescence within leaves using a fibreoptic microprobe. Plant Cell Environ 14:719–725

Cui M, Vogelmann TC, Smith WK (1991) Chlorophyll and light gradients in sun and shade leaves of *Spinacia oleracea*. Plant Cell Environ 14:493–500

Day TA, DeLucia EH, Smith WK (1990) Dorsiventrality of the photosynthetic-light response in naturally occurring C_3 dicots. Proceedings of the VIIIth International Congress of Photosynthesis. IV:883–886

Delieu T, Walker DA (1981) Polarographic measurement of photosynthetic oxygen evolution by leaf discs. New Phytol 89:165–178

DeLucia EH, Shenoi HD, Naidu SL, Day TA (1991) Photosynthetic symmetry of sun and shade leaves of different orientations. Oecologia 87:51–57

Donahue RA (1991) Interactions of ambient light directionality with leaf anatomy and effects on photosynthesis of sun and shade leaves of *Thermopsis montana*. PhD. Dissertation, University of Wyoming USA

Evans JR (1988) Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Aust J Plant Physiol 15:93–106

Evans JR (1989) Partitioning of nitrogen between and within leaves grown under different irradiances. Aust J Plant Physiol 16:533–548

Geller GH, Smith WK (1982) Influence of leaf size, orientation and arrangement on temperature and transpiration in three high elevation large-leaved herbs. Oecologia 53:227–234

Gutschick VP, Barron MH, Waechter DA, Wolf MA (1985) Portable monitor for solar radiation that accumulates irradiance histograms for 32 leaf-mounted sensors. Agri For Meteor 33:281–290

Iqbal M (1983) An introduction to solar radiation. Academic press, Toronto New York London, pp. 362–365

Jurik TW, Zhang H, Pleasants JM (1990) Ecophysiological consequences of non-random leaf orientation in the prairie compass plant, *Silphium laciniatum* Oecologia 82:180–186

Knapp AK, Vogelmann TC, McClean TM, Smith WK (1988) Light and chlorophyll gradients within *Cucurbita* cotyledons. Plant Cell Environ 11:257–263

Kulandaivelu G, Noorudeen AM, Sampath P, Periyanan S, Raman K (1983) Assessment of the photosynthetic electron transport properties of upper and lower leaf sides in vivo by fluorometric method. Photosynthetica 17:204–209

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1954) Protein measurement with the folin phenol reagent. J Biol Chem 193:267–275

Lowther JR (1980) Use of a single sulphuric acid-hydrogen peroxide digest for the analysis of *Pinus radiata* needles. Commun Soil Sci Plant Anal 11:175–188

McClendon JH (1984) The micro-optics of leaves-I. Patterns of reflection from the epidermis. Am J Bot 71:1391–1397

Moss DN (1964) Optimum lighting of leaves. Crop Sci 4:131–136

Mott KA and Michaelson O (1991) Amphistomaty as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae) Am J Bot 78:76–79

Ögren E, Sjörström M (1990) Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. Planta 181:560–567

Pearcy RW (1989) Radiation and light measurements. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant Physiological Ecology: field methods and instrumentation. Chapman and Hall, NY pp. 97–116

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384–394

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Schreiber U, Fink R, Vidaver W (1977) Fluorescence induction in whole leaves: differentiation between the two leaf sides and adaptation to different light regimes. Planta 133:121–129

Sharkey TD (1985) Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Botanical Rev 51:53–105

Smith M, Ullberg D (1989) Effect of leaf angle and orientation on photosynthesis and water relations in *Silphium terebinthinaceum*. Am J Bot 76:1714–1719

Syvertsen JP, Cunningham GL (1979) The effects of irradiating adaxial or abaxial leaf surface on the rate of net photosynthesis of *Perezia nana* and *Helianthus annuus*. Photosynthetica 13:287–293

Terashima I (1986) Dorsiventrality in photosynthetic light response curves of a leaf. J Exp Bot 37:399–405

Terashima I (1989) Productive structure of a leaf. In: WR Briggs (ed) Plant biology vol 8, Photosynthesis. Alan R. Liss Inc. New York, pp 207–226

Terashima I, Evans JR (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. Plant Cell Physiol 29:143–155

Terashima I, Inoue Y (1984) Comparative photosynthetic properties of palisade tissue chloroplasts and spongy tissue chloroplasts of *Camellia japonica* L.: functional adjustment of the photosynthetic apparatus to light environment within a leaf. Plant Cell Physiol 25:555–563

Terashima I, Inoue Y (1985) Palisade tissue chloroplasts and spongy tissue chloroplasts in spinach: biochemical and ultrastructural differences. Plant Cell Physiol 26:63–75

Terashima I, Takenaka A (1986) Organization of photosynthetic system of dorsiventral leaves as adapted to the irradiation from the adaxial side. In: Marcell R, Clijsters H, Van Poucke M (eds) Biological control of photosynthesis. Martinus Nijhoff, Dordrecht pp. 219–230

Václavík J (1984) Photosynthetic CO_2 uptake by *Zea mays* leaves as influenced by unilateral irradiation of adaxial and abaxial leaf surfaces. Biol Plant 26:206–214

Vogelmann TC, Bornman JF, Josserand S (1989) Photosynthetic light gradients and spectral regime within leaves of *Medicago sativa*. Phil Trans R Soc Lond B 323:411–421