Leaf paraheliotropism in *Styrax camporum* confers increased light use efficiency and advantageous photosynthetic responses rather than photoprotection

Gustavo Habermann [a,*], Patricia F.V. Ellsworth [b], Juliana L. Cazoto [c], Aline M. Feistler [a], Leandro da Silva [d], Dario A. Donatti [e,1], Silvia R. Machado [f,1]

[a] Univ Estadual Paulista, Unesp, Departamento de Botânica, IB, Rio Claro-SP, 13506-900, Brazil
[b] University of Miami, Department of Biology, Coral Gables, FL, 33124, USA
[c] Universidade Estadual de Campinas, Unicamp, Departamento de BIOLOGIA Vegetal, IB, Campinas-SP, 13083-970, Brazil
[d] Instituto Agronômico de Campinas, Centro de Ecofisiologia e Biofísica, Campinas-SP, 13001-970, Brazil
[e] Univ Estadual Paulista, Unesp, Departamento de Física, IGCE, Rio Claro-SP, 13506-900, Brazil
[f] Univ Estadual Paulista, Unesp, Departamento de Botânica, IB, Botucatu-SP, 18618-000, Brazil

ABSTRACT

Styrax caporum is a native shrub from the Brazilian savanna. Most of its leaves are diaheliotropic, whereas some are paraheliotropic, mainly at noon. A previous study of this species revealed higher stomatal conductance (gs) and transpiration rates (E) in para- compared to diaheliotropic leaves, and a rise in CO_2 assimilation rates (A) with an increase of irradiance for paraheliotropic leaves. We hypothesized that this species exploits the paraheliotropism to enhance the light use efficiency, and that it is detected only if gas exchange is measured with light interception by both leaf surfaces. Gas exchange was measured with devices that enabled light interception on only one of the leaf surfaces and with devices that enabled light interception by both leaf surfaces. Water relations, relative reflected light intensity, leaf temperature (T_1), and leaf anatomical analyses were also performed. When both leaf surfaces were illuminated, a higher A, E, and gs were observed in para- compared to diaheliotropic leaves; however, A did not depend on gs, which did not influence CO_2 accumulation in the stomatal cavity (Ci). When only the adaxial leaf surface was illuminated, a greater A was detected for para- than for diaheliotropic leaves only at 11:00 h; no differences in T_1 were observed between leaf types. Light curves revealed that under non-saturating light the adaxial side of paraheliotropic leaves had higher A than the abaxial side, but they showed similar values under saturating light. Although the abaxial leaf side was highly reflective, both surfaces presented the same response pattern for green light reflection, which can be explained by the compact spongy parenchyma observed in the leaves, increasing light use efficiency in terms of CO_2 consumption for paraheliotropic leaves. We propose that paraheliotropism in *S. camporum* is not related to leaf heat avoidance or photoprotection.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Trees and shrubs growing in the cerrado, or the Brazilian savanna, which is characterized by wet (October–March) and dry (April–September) seasons, have to adjust their morpho-physiological traits seasonally to successfully cope with the soil water availability. The cerrado vegetation is a vertically structured mosaic of grassland, scrubland, and dense woodland physiognomies (Haridasan, 2008). The soils of these areas are deep, acidic, sandy, contain low levels of organic matter and phosphorus, and are rich in aluminum (Haridasan, 2008).

Concomitant to seasonal water deficit, the cerrado environment experiences a high irradiance load and elevated vapor pressure deficits (VPD). Under such conditions, paraheliotropic leaf movement is one of the strategies used by many plants. Diaheliotropic leaves, which are oriented at an angle perpendicular to incoming light, maximize light interception, while paraheliotropic leaves, which orient parallel to the light, minimize it (Koller, 1986, 1990; Bielenberg et al., 2003; Pastenes et al., 2005; Arena et al., 2008).

In leguminous species, paraheliotropic leaf movements are rapidly induced by unfavorable conditions. In beans, leaf paraheliotropism is induced by water deficit (Pastenes et al., 2005), leaf heat and excess sunlight interception (Bielenberg et al., 2003). In the soybean, leaf paraheliotropism is induced with increasing irradiance (Jiang et al., 2006). For leguminous cerrado species, paraheliotropism may be a strategy to avoid excess sunlight interception at noon (Caldas et al., 1997; Rodrigues and Machado, 2006),

which reinforces the photo-protective role of paraheliotropism. Even the leaf wilting movement of non-leguminous species, such as cotton plants, confers photo-protection and maintains carbon assimilation (Zhang et al., 2010).

Another leguminous species, *Robinia pseudoacacia* exhibits paraheliotropic leaf movement at high irradiance levels (Liu et al., 2007), reducing light interception and leaf temperature (Arena et al., 2008). However, paraheliotropic leaves of *R. pseudoacacia* show higher stomatal conductance (gs) and CO_2 assimilation rates (A), which are attributed to a higher photochemical performance of unrestrained paraheliotropic leaves, compared to restrained leaves (Arena et al., 2008).

However, the physiological role of paraheliotropism may not be universal. *Styrax camporum*, a non-leguminous species from the cerrado, possesses leaves that are always diaheliotropic as well as other leaves that assume a paraheliotropic position from 10:00 h to 17:00 h (Habermann et al., 2008). Paraheliotropic leaves of this species have higher A and transpiration rates (E) compared to the diaheliotropic leaves, although this high E has a weak relationship with the reduction in leaf temperature (Habermann et al., 2008). This study also demonstrated compact spongy parenchyma in both leaf types, and a rise in A with the increase of irradiance for paraheliotropic leaves. Thus, this species might exploit the paraheliotropic leaf movement to enhance light use efficiency.

In the present study, we hypothesized that, compared to diaheliotropic leaves, the paraheliotropic leaves of *S. camporum* would show higher values of gas exchange rates if measured with light intercepted by adaxial and abaxial leaf surfaces. Measurements of gas exchange with devices that enabled artificial light interception only by adaxial or abaxial leaf surfaces (LI-6400 Irga with a 6400-02B red/blue LED light source) and with devices that enabled sunlight interception by both leaf sides (LI-6200 Irga with a transparent 6000-12 one liter chamber) were performed in April 2006, in Botucatu-SP and Corumbataí-SP, Brazil. The leaf water potential, the leaf temperature of para- and diaheliotropic leaves, and the relative reflected light intensity of adaxial and abaxial leaf surfaces of *S. camporum* were also measured. Anatomical and ultra-structural leaf analyses were performed as a framework for the functional studies (see online supplementary information, Fig. S1).

2. Materials and methods

2.1. Site description

This study was conducted using adult plants of *S. camporum* Pohl. from cerrado fragments characterized by scattered trees and shrubs and a large proportion of grassland ("closed field") in the municipalities of Botucatu, São Paulo (SP) state, Brazil (22° 51′ S, 48° 26′ W) and Corumbataí, SP state, Brazil (22° 13′ S, 47° 37′ W).

Five individual plants between 1.5 and 2-m tall from each of these sites were used. Plants were completely leafy at the beginning of the fall season in April 2006, when the measurements were obtained.

2.2. Leaf angle measurement

In order to classify leaves as para- or diaheliotropic, the petiole angle formed with the horizon was measured. A fine wire was positioned between the petiole and a ruler with a water level, which represented the horizon. The curvature radius formed by the wire mirrored the petiole angle. Then, the angle defined by the wire was reproduced on paper and determined with a goniometer, similar to the method presented by Arena et al. (2008).

Leaves showing petiole angle greater than 50° were classified as paraheliotropic leaves, which were marked on each plant at noon

on the day before the measurements were performed. Leaves displaying a petiole angle between 0° and 10° were considered as diaheliotropic. Both leaf types occurred on woody stems, and had mature fully expanded leaf blades. Very young and very old leaves were avoided.

2.3. Leaf gas exchange measurements

Gas exchange was measured using an infrared gas analyzer (LI-6200, LI-Cor, USA) with a 6000-12 one liter chamber, which is made of Lexan® and Margard™ transparent materials. These materials have a transmittance of 90% in the visible and near infra-red spectra, but bellow 450 nm it falls markedly to 60% at 400 nm (Li-Cor, 1990). Therefore, this leaf cuvette enables sunlight absorptance from 300 to 1100 nm (Li-Cor, 1990). This leaf cuvette has inserts which were used to fix leaf area to 6 cm² (a predetermined size), allowing faster measurements and exposure of adaxial and abaxial leaf surfaces to direct sunlight or diffuse irradiance (soil reflection and scattered irradiance). Additionally, gas exchange was also measured with an infra-red gas analyzer (LI-6400, Li-Cor, USA) using a leaf cuvette that enables artificial red and blue LED light (6400-02B, Li-Cor, USA) interception by only one of the leaf surfaces. The 6400-02B red blue light source spectral output has one peak centered at about 670 nm and a secondary peak at about 465 nm (Li-Cor, 2004).

Because paraheliotropic leaves were not flat on both sides of the midrib, and showed different inclination planes, it was not possible to measure just one of the leaf sides of the midrib without disturbing the leaf planes. Otherwise, it would not match the minimum area necessary to make measurements in gas exchange chambers. Thus, the leaf planes of paraheliotropic leaves became entirely flat when leaf cuvettes of both equipments were closed. However, the petiole angle was not disturbed. Diaheliotropic leaves were naturally completely flat, following the petiole angle, which was not disturbed as well.

CO_2 assimilation (A) and transpiration (E) rates, stomatal conductance (gs), and intercellular CO_2 (Ci) were determined by the Irgas' data analysis programs, which employ the Von Caemmerer and Farquhar (1981) general gas exchange equations for both equipments. The photosynthetic radiation use efficiency (PhRUE), was also calculated (See online supplementary data for more details about the method for calculating PhRUE, Table S1). Both leaf cuvettes had external quantum sensors, which were used to measure the incoming sunlight. In the case of the leaf cuvette that enabled light interception by both leaf sides, the quantum sensor measured ambient PPFD intercepted by leaves. The leaf cuvette that enabled artificial light interception by only one of the leaf sides was set to provide 1800 µmol photons m⁻² s⁻¹, as ambient PPFD varied from 1000 to 1600 µmol photons m⁻² s⁻¹. The leaf temperature (T_l) was obtained using a small thermocouple within the leaf cuvettes of both systems, according to Bielenberg et al. (2003).

Curves of A, gs, and Ci as a function of the PPFD values established in the leaf cuvette that enables artificial light interception by only one of the leaf sides were also constructed to detect the sole responses of the adaxial and abaxial leaf surfaces of para- and diaheliotropic leaves. These curves were generated at a controlled leaf temperature (25 ± 1 °C).

2.4. Estimation of leaf reflectance

An estimation of the relative intensity of reflected light from both leaf sides was performed using a fluorometer (Cary Eclipse, Varian, USA), which detects scattered light reflected within the same spectrum (λ) of incident light. Five young one-year-old *S. camporum* potted plants were maintained under natural sunlight. As no previous differences were detected from the same leaf surface between para- or diaheliotropic leaves, one randomly selected leaf

from each of the five plants was detached and immediately inserted into the equipment. The angle between the leaf and light beam was close (but not exactly) to 45°, so that reflected light intensity could not saturate the detector.

2.5. Leaf water potential

The leaf water potential at predawn (Ψ_{pd}) and midday (when the vapor pressure deficit, VPD, was maximum) (Ψ_{md}) were measured by the pressure chamber method (Turner, 1981) using a DIK-7000 (Daiki Kogyo, Japan) pressure chamber.

2.6. Data analysis

Statistical analysis was carried out using two leaves randomly selected from five plants (replicates) within an area of five hectares (ha) in Botucatu and 37 ha in Corumbataí. Gas exchange variables (A, E, gs, and Ci), T_l, Ψ_{pd}, and Ψ_{md} were determined (mean and standard deviation), and they were then subjected to one-way analysis of variance (comparisons between para- and diaheliotropic leaves), followed by the Tukey's test ($P < 0.05$).

2.7. Light and electron microscopy

See online supplementary data for more details about the methods used for obtaining and analyzing light and electron microscopic images (Fig. S1).

3. Results

3.1. Morpho-anatomical traits

Leaves of *S. camporum* are almost all diaheliotropic, but some mature fully expanded leaves assume paraheliotropic position from 10:00 h to 17:00 h. These mature completely expanded para- and diaheliotropic leaves are observed on woody stems, and also on primary branches of adult plants (Fig. S1a).

Young leaves of *S. camporum* presented the trichome indumentum on both surfaces of the leaf blade (Fig. S1b), while mature fully expanded leaves displayed a glabrous adaxial surface, regardless of its heliotropic position (Fig. S1d).

The mesophyll of both young (Fig. S1c) and mature (Figs. S1d and e) leaves was differentiated into a unistratified palisade and a two- to three-layered spongy chlorenchyma. Spongy parenchyma cells showed numerous wall ingrowths (Figs. S1d and e), which developed into slit-like gas spaces. Such cells presented thin walls and a peripheral cytoplasm with numerous chloroplasts (Fig. S1e) and a single central vacuole that could be translucent or dense if filled with phenolic substances. See online supplementary data (Fig. S1) for details.

3.2. Gas exchange variables

When the leaves were measured with light interception by both leaf sides, the CO_2 assimilation rate (A) was significantly higher for para- than for diaheliotropic leaves at 9:00 h, 11:00 h, and 14:00 h, but A was similar between leaf types at the end of the day (Fig. 1a). The stomatal conductance (gs) and transpiration rates (E) followed almost the same response pattern (Fig. 1c and e), with paraheliotropic leaves demonstrating higher values than diaheliotropic leaves during most parts of the day. Paraheliotropic leaves exhibited a significant lower value of internal CO_2 (Ci) compared to diaheliotropic leaves, but only at 9:00 h (Fig. 1g).

Leaves measured with light interception by only the adaxial leaf surface demonstrated similar A values for para- and diaheliotropic leaves throughout the day, except at 11:00 h, when paraheliotropic

leaves exhibited a higher A compared to diaheliotropic leaves (Fig. 1b). For gs and E, differences were detected only at 9:00 h and 14:00 h, respectively (Fig. 1d and f). Internal CO_2 concentrations were similar in para- and diaheliotropic leaves, with the latter showing greater values at 14:00 h, although this increase was not significantly higher than the CO_2 concentrations in paraheliotropic leaves (Fig. 1h).

Variations in gs did not seem to influence the A of leaves measured with sunlight interception by both leaf sides; daily results revealed a conserved low gs range with respective low carbon assimilation rates for diaheliotropic leaves, while most of the data for paraheliotropic leaves demonstrated a higher gs range with respective higher CO_2 assimilation rates (Fig. 2a). In contrast, when leaves were measured with light interception by only the adaxial leaf surface, increases in gs resulted in greater CO_2 assimilation rates, regardless of the leaf type (Fig. 2b).

The Ci values of leaves measured with sunlight interception by both leaf sides were not dependent on gs (Fig. 3a). However, when leaves were measured with light interception by only the adaxial leaf surface, Ci fluctuated between 200 and 300 μmol mol^{-1}, and this effect was dependent on gs (Fig. 3b).

Similarly, A was not influenced by Ci in either paraheliotropic or diaheliotropic leaves intercepting light by both leaf sides (Fig. 4a); but when measuring gas exchange with light interception by only the adaxial leaf side, increases in Ci resulted in greater carbon assimilation rates, regardless of the leaf type (Fig. 4b).

Both surfaces of para- and diaheliotropic leaves displayed a similar ($P < 0.05$) A from 0 to 100 PPFD; but from 200 to 1800 μmol photons m^{-2} s^{-1}, diaheliotropic leaves showed significantly higher ($P < 0.05$) A for the adaxial than for the abaxial side (Fig. 5a). The adaxial surface, compared to the abaxial surface of paraheliotropic leaves, showed a higher ($P < 0.05$) A within the range of 200 and 800 μmol photons m^{-2} s^{-1}; however, from 1000 to 1800 PPFD, adaxial and abaxial sides of the paraheliotropic leaves exhibited a similar ($P < 0.05$) A (Fig. 5a). Regarding the gs/PPFD curves, adaxial and abaxial sides of both leaf types presented similar ($P < 0.05$) values at each PPFD (Fig. 5b). For Ci, the abaxial side presented greater values than the adaxial side for only diaheliotropic leaves, considering a range between 200 and 800 μmol photons m^{-2} s^{-1}; from 1000 to 1800 PPFD, both surfaces of the two leaf types had similar ($P < 0.05$) Ci values (Fig. 5c).

The photosynthetic radiation use efficiency (PhRUE) was higher in paraheliotropic than in diaheliotropic leaves at every time of day considered, when the leaves were measured with sunlight interception by both leaf sides. However, when leaves were artificially illuminated only on the adaxial leaf surface, PhRUE was the same between para- and diaheliotropic leaves, except at 11:00 h, when paraheliotropic leaves presented increased PhRUE in relation to diaheliotropic leaves. See online supplementary data (Table S1) for details.

3.3. Leaf temperature

The leaf temperature remained between 30 °C and 38 °C for leaves measured when light intercepted both leaf sides, and paraheliotropic leaves demonstrated lower values than diaheliotropic leaves only at 11:00 h and 16:00 h (Fig. 6a). When leaves were measured with light interception by only the adaxial leaf side, the leaf temperature fluctuated between 25 °C and 32 °C, although no significant differences between leaf types were detected throughout the day (Fig. 6b).

3.4. Water relations

The leaf water potentials at predawn (Ψ_{pd}) or midday (Ψ_{md}) were statistically similar among para- and diaheliotropic
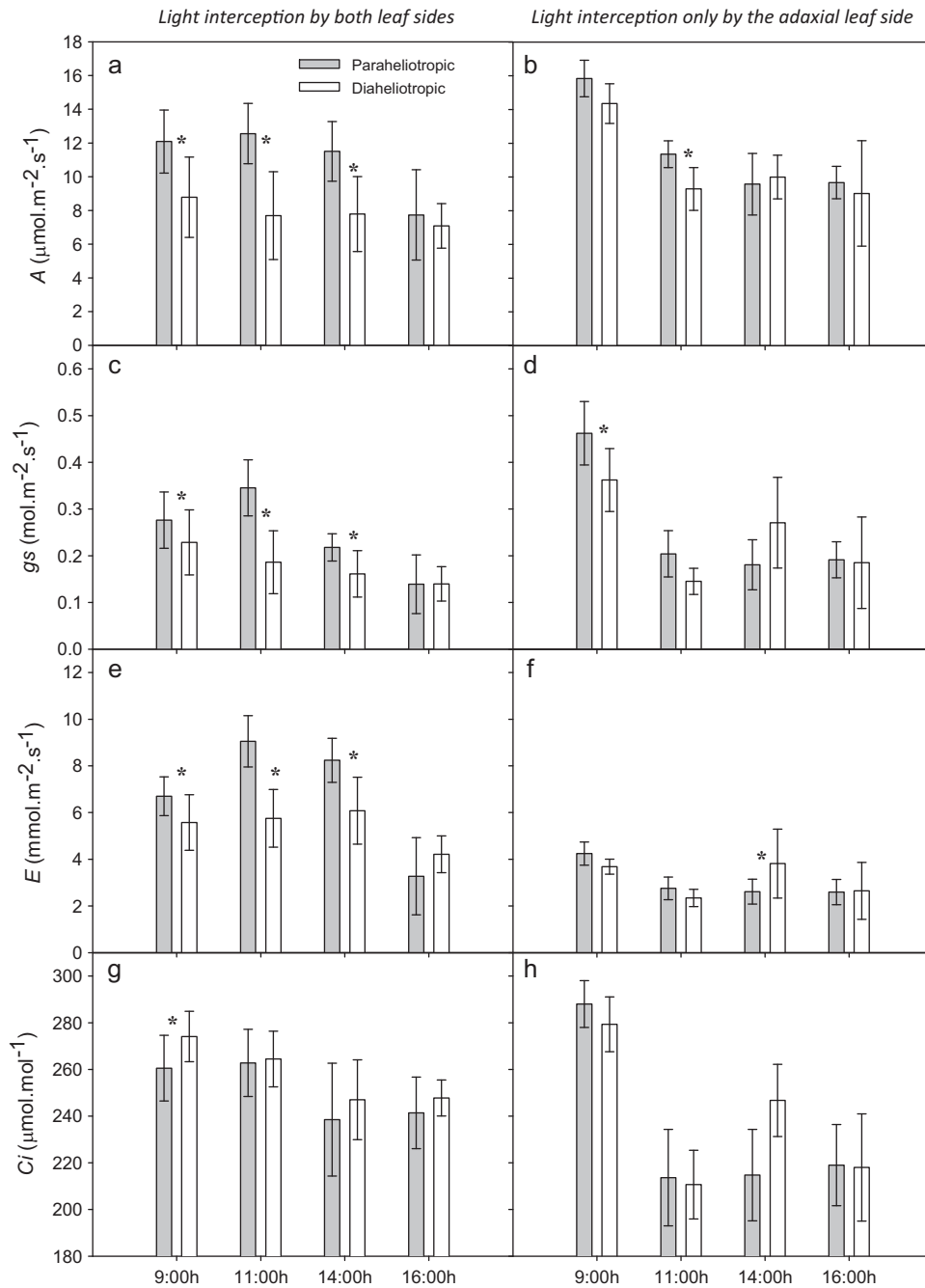
Fig. 1. Daily variations in CO_2 assimilation rates (a and b), stomatal conductance (c and d), transpiration rates (e and f), and intercellular CO_2 (g and h) for para- and diaheliotropic leaves of *S. camporum* ($n = 5$) in the field, measured using natural (direct and diffuse) irradiance interception by both leaf surfaces (a, c, e and g) as well as light interception by only the adaxial leaf surface (b, d, f and h). Asterisks between columns indicate significant difference between para- and diaheliotropic leaves ($P < 0.05$). (Vertical bars = SD).

leaves measured in both locations (Botucatu and Corumbataí) (Fig. 7).

3.5. Leaf reflectance

Reflectance from both leaf sides showed a similar response pattern, showing the peak of reflectance in the green spectrum (550 nm). The relative light intensity reflected from the abaxial leaf surface was significantly higher than light reflected from the adaxial leaf surface within the visible light (400–700 nm). However, considering wavelengths of 200–400 nm and 700–800 nm, reflectance was similar between both leaf surfaces (Fig. 8).

4. Discussion

4.1. Water relations

Plants from Botucatu and Corumbataí displayed a similar Ψ_w, indicating no differences in night rehydration (Ψ_{pd}) or in water uptake capacity under the highest VPD (Ψ_{md}) (Fig. 7); these results assure comparable physiological conditions between both populations. It is unlikely that these plants were experiencing a soil water deficit, as the relative water content of both leaf types remained around 80% (data not shown). Moreover, *S. camporum* adult plants evaluated in the field during the dry season (July to September)
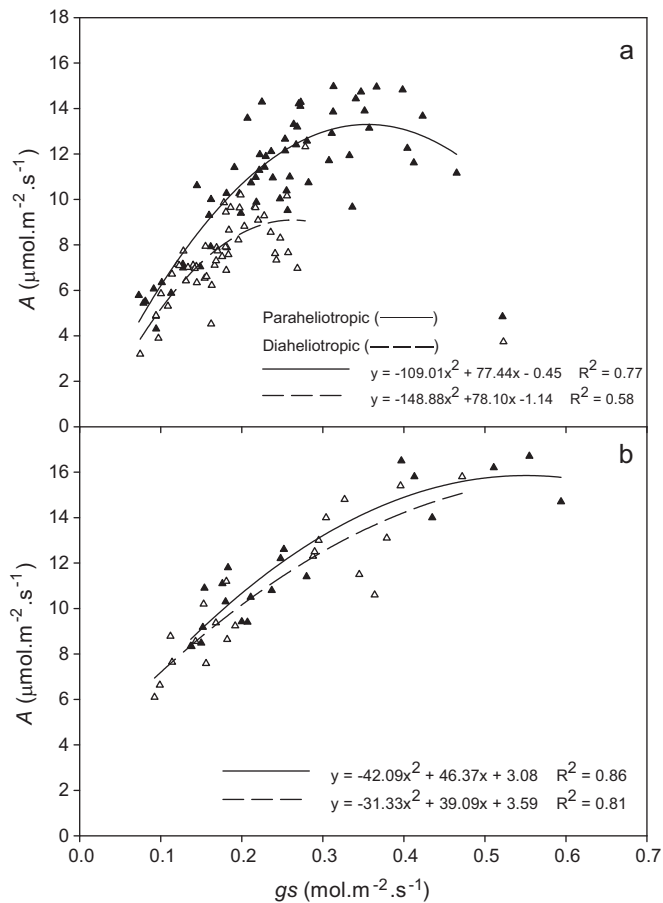
Fig. 2. Individual daily readings (replicates) for CO_2 assimilation rates in relation to the stomatal conductance for para- and diaheliotropic leaves of *S. camporum* ($n = 5$) in the field, measured using natural (direct and diffuse) irradiance interception by both leaf surfaces (a) as well as light interception by only the adaxial leaf surface (b). Only regression equations with $R^2 > 0.5$ are shown.

Fig. 3. Individual daily readings (replicates) of intercellular CO_2 in relation to the stomatal conductance for para- and diaheliotropic leaves of *S. camporum* ($n = 5$) in the field, measured using natural (direct and diffuse) irradiance interception by both leaf surfaces (a) as well as light interception by only the adaxial leaf surface (b). Only regression equations with $R^2 > 0.5$ are shown.

showed mean values of -0.5 MPa Ψ_{pd} and -1.6 MPa Ψ_{md} with low gs compared to the wet season (January to March) (data not shown).

4.2. Leaf temperature

Forseth and Teramura (1986) estimated that naturally orienting leaves of *Pueraria lobata* would maintain temperatures up to 7 °C lower than horizontally restrained leaves. However, when *S. camporum* leaves were illuminated on both sides, the low leaf temperature (T_l) observed in paraheliotropic leaves at 16:00 h seemed to have a weak relationship with E or gs, which were similar between the leaf types (Fig. 1c and e). Habermann et al. (2008) noted that *S. camporum* paraheliotropic leaves illuminated on both leaf sides had lower T_l and higher E, gs, and A during part of the day compared to diaheliotropic leaves, but neither E nor gs was responsible for the low T_l of paraheliotropic leaves, and there was no indication that the low T_l explained their high A. When leaves were measured with light interception only on the adaxial leaf sides, no differences in T_l were noted between para- and diaheliotropic leaves (Fig. 6b), even when evaluated for an entire year (March, April, June, August, and October) (data not shown). T_l for the vertical leaflets of *P. pubescens* at midday was only 1.5 °C lower compared to horizontal leaflets (Caldas et al., 1997). For Arena et al. (2008), the reduction in T_l caused by paraheliotropism may be relevant only if the air temperature reaches values higher than the optimum temperature for photosynthesis. Therefore, paraheliotropism in *S. camporum* does not seem to be related to leaf heat avoidance. More-
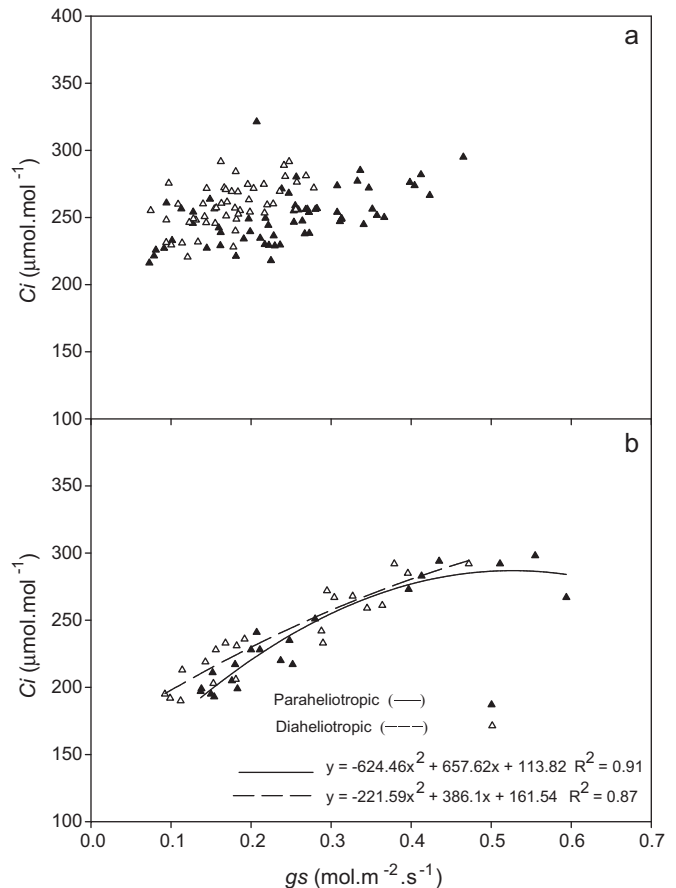
over, cerrado species seem to be well adapted to high temperatures (Franco et al., 2007; Simon et al., 2009).

4.3. Leaf gas exchange and light interception

S. camporum paraheliotropic leaves that intercepted light on both leaf sides displayed significantly higher A, E, and gs than diaheliotropic leaves during most times of the day (Fig. 1a, c and e). This high A for paraheliotropic leaves could not be explained by the higher gs of para- compared to diaheliotropic leaves (Fig. 1c). When leaves were illuminated on both leaf sides, it was clear that A did not depend on gs (Fig. 2a). Separate groups of data demonstrate the greater response ranges for A and gs in paraheliotropic compared to diaheliotropic leaves (Fig. 2a). For both leaf types, the increased stomatal aperture did not result in enhanced intercellular CO_2 (Fig. 3a), the variation of which clearly did not affect the carbon assimilation rates (Fig. 4a). Furthermore, in nature, sunlight interception by the abaxial leaf side certainly does not explain the greater gs found in para- compared to diaheliotropic leaves (Fig. 1c), because adaxial and abaxial sides of both leaf types when illuminated by the artificial red/blue light demonstrated the same values of gs at each PPFD of the gs/PPFD curves (Fig. 5b).

Leaves measured with light interception by only the adaxial leaf surface showed significantly higher A in para- than in diaheliotropic leaves only at 11:00 h (Fig. 1b). However, leaves displayed daily Ci values that were clearly influenced by the opening of the stomatal pores (Fig. 3b). Consequently, higher carbon assimilation
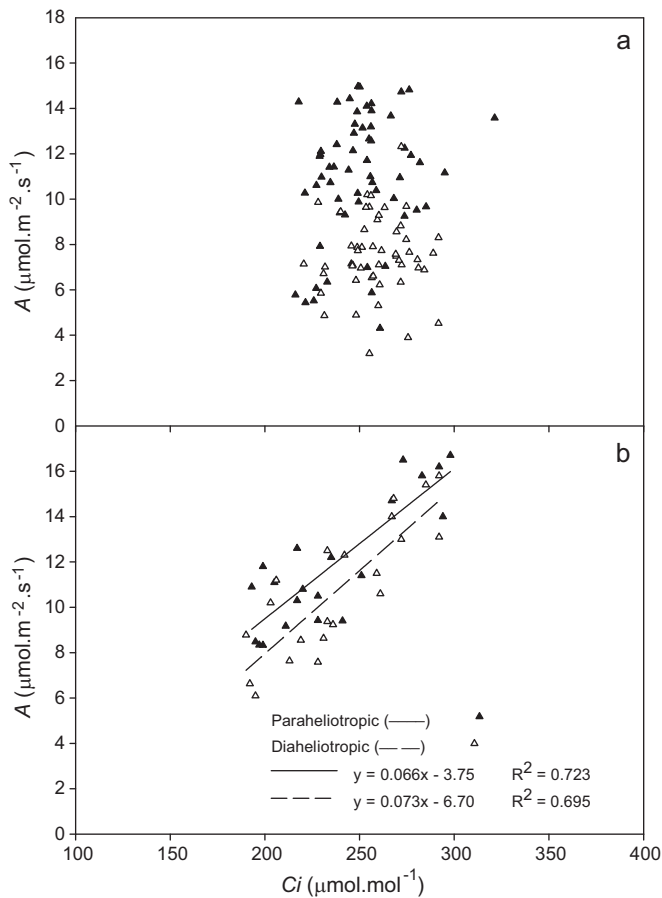
Fig. 4. Individual daily readings (replicates) of CO_2 assimilation in relation to intercellular CO_2 for para- and dia- heliotropic leaves of *S. camporum* ($n = 5$) in the field, measured using natural (direct and diffuse) irradiance interception by both leaf surfaces (a) as well as light interception by only the adaxial leaf surface (b). Only regression equations with $R^2 > 0.5$ are shown.
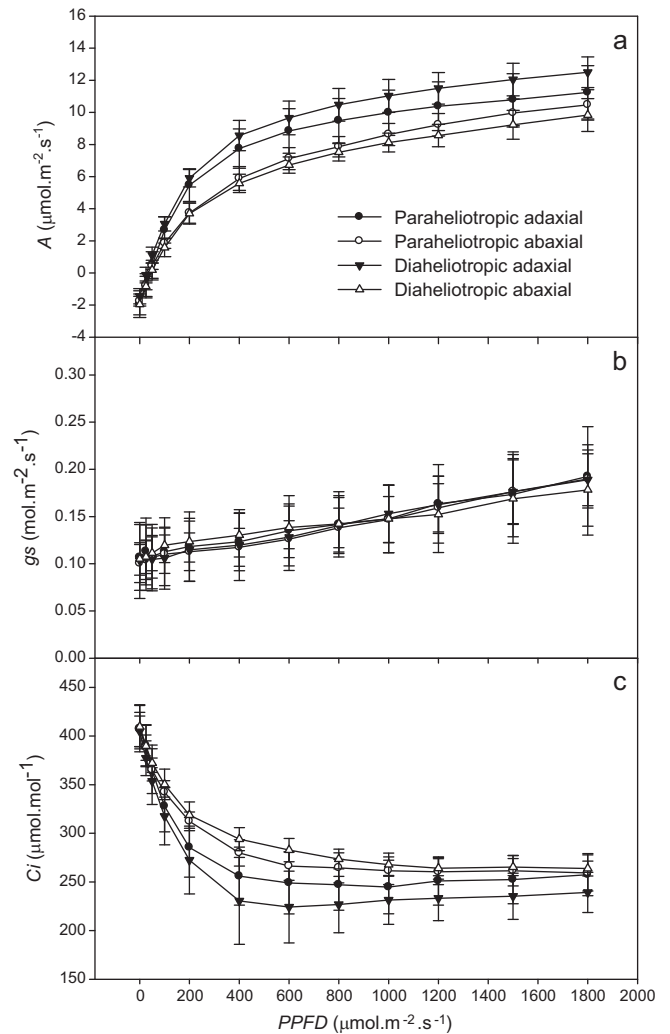

Fig. 5. Mean values ($n = 5$ plants) for the CO_2 assimilation rates (a), stomatal conductance (b), and intercellular CO_2 (c) in response to the photosynthetic photon flux density (PPFD) in para- and diaheliotropic leaves of *S. camporum* ($n = 5$) measured using light interception by only adaxial or abaxial leaf sides. (Vertical bars = SD).

rates followed from increased C_i values, regardless of the leaf type (Fig. 4b).

The adaxial leaf surface of *S. camporum* seems to be specialized in direct light interception and absorptance, because it had a significantly lower light reflectance compared to the abaxial leaf side (Fig. 8). For *Helianthus annus* leaves, direct light reflectance was only slightly higher in the abaxial compared to the adaxial leaf surface, but diffuse light reflectance was significantly higher in the abaxial compared to the adaxial side (Gorton et al., 2010). The stellate pubescence, which covers the abaxial leaf surface and is absent on the adaxial side of *S. camporum* leaves (Habermann et al., 2008) is, then, responsible for this increased light reflectance. In fact, the effective quantum yield of photosystem II (Φ PSII) and the electron transport rate (ETR) for both leaf sides of one-year-old *S. camporum* plants were similar between leaf types, but higher in adaxial compared to abaxial sides (data not shown).

However, when carbon assimilation was measured with light interception by only one of the leaf sides using the artificial red/blue light, which precisely emits the photosynthetic active waveband, it seems that the vertical leaf position of *S. camporum* enables increased light use efficiency. When PPFD varied from 200 to 1800 μmol photons m^{-2} s^{-1}, the adaxial sides of diaheliotropic leaves displayed maximum CO_2 assimilation rates (A_{max} under saturating light, but non-saturating CO_2, for photosynthesis responses) that were significantly higher than those of their abaxial sides (Fig. 5a). Interestingly, non-saturating light for photosynthesis responses (200-800 μmol photons m^{-2} s^{-1}) did not induce CO_2

consumption on the abaxial sides of diaheliotropic leaves, which exhibited significantly higher C_i than those of their adaxial surfaces. When using more than 1000 μmol photons m^{-2} s^{-1} (saturating light), C_i was similar between both leaf sides of the two leaf types (Fig. 5c), but the adaxial leaf sides of diaheliotropic leaves maintained a higher A compared to their abaxial sides (Fig. 5a). This suggests that increased light interception by the abaxial sides of diaheliotropic leaves does not improve their light use efficiency; therefore, some *innate* low A_{max} values are present for the abaxial sides of diaheliotropic leaves. Adaxial surfaces of para- and diaheliotropic leaves presented the same ($P < 0.05$) A as the PPFD varied from 200 to 1800 μmol photons m^{-2} s^{-1} (Fig. 5a). Leaves illuminated only on the adaxial side, which did not render any conspicuous differences in A between the two leaf types (Fig. 1 b, d and f) were measured using a PPFD of 1800 μmol photons m^{-2} s^{-1}. Moreover, adaxial and abaxial sides of paraheliotropic leaves displayed a distinct A_{max} when PPFD was below 800 μmol photons m^{-2} s^{-1}, but similar values of A_{max} when PPFD was between 800 and 1800 μmol photons m^{-2} s^{-1} (Fig. 5a), indicating that paraheliotropic leaves somehow integrate the photosynthetic capacities of both leaf sides under elevated irradiances. In fact, the leaves of *Olea europaea*, when intercepting light on both sides, showed higher apparent quantum yield [mol (CO_2 assimilated) mol^{-1} (incident
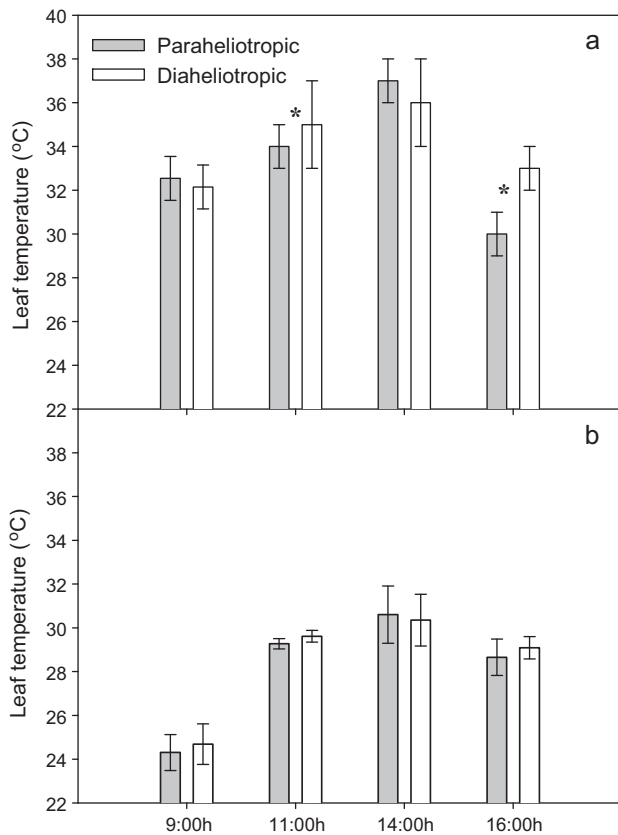
Fig. 6. Daily variations in leaf temperature for para- and diaheliotropic leaves of *S. camporum* ($n = 5$) in the field, measured using natural (direct and diffuse) irradiance interception by both leaf surfaces (a) as well as light interception by only the adaxial leaf surfaces (b). Asterisks between columns indicate significant difference between para- and diaheliotropic leaves ($P < 0.05$). (Vertical bars = SD).

quanta of PPFD)] and greater *A* compared to cases in which their leaves intercepted light only on one leaf side under the same PPFD (Proietti and Palliotti, 1997). Similarly, leaves of *Eucalyptus maculata* and *E. pauciflora*, which had been horizontally or vertically restrained, exhibited higher *A* values when equally illuminated on both leaf sides compared to when abaxial or adaxial illumination
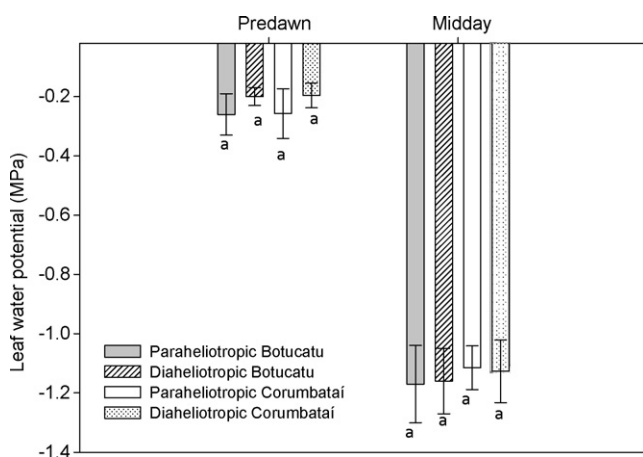

Fig. 7. Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential in para- and diaheliotropic leaves of *S. camporum* ($n = 5$) in the field, in Botucatu-SP, and in Corumbataí-SP, southeastern Brazil. For each evaluation time (predawn and midday), the same letters indicate the lack of statistical significance ($P < 0.05$) among para- and diaheliotropic leaves of plants from Botucatu and Corumbataí. (Vertical bars = SD).
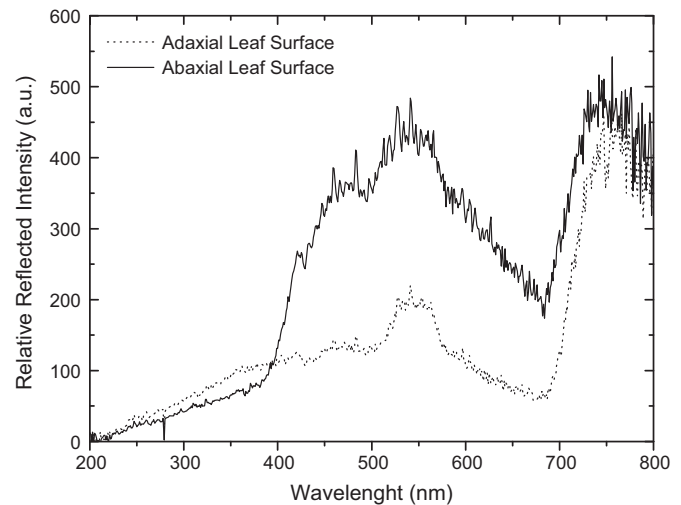

Fig. 8. Relative reflected light intensity within the 200–800 nm wavebands for leaves irradiated on the adaxial and abaxial leaf surfaces of *S. camporum*. Each curve represents data from leaves detached from five replicates (plants). (a.u. = arbitrary unit).

alone was applied (Evans and Jakobsen, 1993). These results support the integration of the photosynthetic capacities of adaxial and abaxial leaf sides, which may have occurred in *S. camporum* (Fig. 1a).

We have not measured the apparent quantum yield, but when leaves were illuminated on both surfaces, the photosynthetic radiation use efficiency (PhRUE) was higher in para- compared to diaheliotropic leaves throughout the day, and when the same range of PPFD intercepted adaxial leaf surfaces only, para- and diaheliotropic leaves showed similar PhRUE at 9:00 h, 14:00 h, and 16:00 h (See online supplementary data for details; Table S1). Thus, we propose that the leaves of *S. camporum* photosynthetically benefit from paraheliotropism, although there is no evidence that this great PhRUE increases plant ecological performance, for instance, by increasing plant biomass.

The vertical leaf position in *S. camporum* enables increased photosynthetic efficiency in terms of CO_2 consumption, but not in terms of light absorptance, especially when considering the contribution of the abaxial leaf surface, which is highly reflective. In fact, for some epiphytic fern leaves, the higher *A* values observed for the leaf side mostly exposed to direct sunlight was attributable to a greater CO_2 consumption (Martin et al., 2009). Notwithstanding, the reflectance pattern of the adaxial side is very similar to the abaxial leaf side, with both sides exhibiting great reflectance within the green spectrum (525–575 nm) (Fig. 8). This indicates that, although pubescence promotes light reflectance, the abaxial leaf surface certainly absorbs a small amount of light within the red spectrum, which is greater in direct sunlight than in scattered light. For *H. annus* leaves, there was almost the same direct light absorptance between the adaxial and abaxial leaf surfaces, but when diffuse light absorptance was measured, the abaxial side showed a slightly lower value (Gorton et al., 2010). Therefore, as supported by *A*/PPFD curves (Fig. 5a) abaxial leaf surfaces of *S. camporum* may not have light leaf absorptance significantly decreased under elevated irradiances. *S. camporum* leaves have compact spongy parenchyma and amplified palisade parenchyma (See online supplementary data for details; Figs. S1d, e and f), suggesting that these cells have high chlorophyll content. Features such as stomata limited to the abaxial surface, developed palisade parenchyma, and compactly arranged spongy parenchyma are constant in leaves of cerrado woody species (Bieras and Sajo, 2009). Indeed, compact leaf tissues have previously been suggested to enable greater light interception and high water use efficiency (Chaves et al., 2002).

One could still argue that different devices, rather than light interception, determined the differences in gas exchange, since natural sunlight was used for double sided illumination measurements and artificial red/blue light was used for single sided illumination measurements. However, regardless of the device/system used, the range of PPFD values reaching leaf surfaces was very similar. Moreover, results of A, gs, and Ci obtained from PPFD curves indicated that there are distinct responses in para- and diaheliotropic leaves when considering each leaf surface under saturating and non-saturating red/blue light (Fig. 5).

Finally, it is essential to emphasize that it was not the leaf age, rather than the leaf position, that determined our observations. Paraheliotropic leaves that were assessed were distinguishable from diaheliotropic leaves. These paraheliotropic leaves had mature fully expanded blades, and they were localized on woody stems and primary branches of adult plants. Additionally, for some stems, paraheliotropic and diaheliotropic leaves were adjacent to each other (See online supplementary data for details; Fig. S1a).

4.4. Ecophysiological significance of heliotropism for S. camporum

It has been accepted that paraheliotropism is a mechanism for reducing transpiration rates, irradiance interception, consequently minimizing leaf heat and the potential for photoinhibition (Ehleringer and Forseth, 1980; Forseth and Ehleringer, 1982; Forseth and Teramura, 1986; Bielenberg et al., 2003; Pastenes et al., 2005; Liu et al., 2007; Arena et al., 2008), although paraheliotropism may also prevent optimum CO_2 assimilation rates (Pastenes et al., 2005). These traditional explanations for the significance of leaf heliotropism have been based on leguminous species, including *P. pubescens* from the cerrado (Caldas et al., 1997).

Nonetheless, our data strongly suggest that, although the abaxial leaf surface is highly reflective, it may absorb a small amount of natural direct light within the red spectrum, which photosynthetically increases the efficiency of paraheliotropic leaves in terms of CO_2 consumption, because adaxial and abaxial leaf sides seem to integrate their respective carbon assimilation rates. However, these observations are detected only if measured with devices that enable light interception by both leaf surfaces. Moreover, paraheliotropism in *S. camporum* does not seem to be related to leaf heat avoidance (Fig. 6), neither does it minimize the potential for photoinhibition (data not shown). Therefore, in contrast to the results for leguminous species, the paraheliotropism of only some leaves of *S. camporum*, which do not even show pulvinus in their petioles (Machado, 1991), could have novel significance for plant ecophysiology.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2010.10.012.

References

Arena, C., Vitale, L., De Santo, A.V., 2008. Paraheliotropism in *Robinia pseudocacia* L.: an efficient strategy to optimise photosynthetic performance under natural environmental conditions. Plant Biol. 10, 194–201.

Bielenberg, D.G., Miller, J.D., Berg, V.S., 2003. Paraheliotropism in two *Phaseolus* species: combined effects of photon flux density and pulvinus temperature, and consequences for leaf gas exchange. Environ. Exp. Bot. 49, 95–105.

Bieras, A.C., Sajo, M.G., 2009. Leaf structure of the cerrado (Brazilian savanna) woody plants. Trees 23, 451–471.

Caldas, L.S., Lüttge, U., Franco, A.C., Haridasan, M., 1997. Leaf heliotropism in *Pterodon pubescens*, a woody legume from the brasilian cerrado. Rev. Bras. Fisiol. Veg. 9, 1–7.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field, photosynthesis and growth. Ann. Bot. 89, 907–916.

Ehleringer, J.R., Forseth, I.N., 1980. Solar tracking by plants. Science 210, 1094–1098.

Evans, J.R., Jakobsen, I., Ögren, E., 1993. Photosynthetic light response curves 2. Gradients of light absorption and photosynthetic capacity. Planta 189, 191–200.

Forseth, I.N., Ehleringer, J.R., 1982. Ecophysiology of two solar tracking desert winter annuals II. Leaf movements, water relations and microclimate. Oecologia 54, 41–49.

Forseth, I.N., Teramura, A.H., 1986. Kudzu leaf energy budget and calculated transpiration: the influence of leaflet orientation. Ecology 67, 564–571.

Franco, A.C., Matsubara, S., Orthen, B., 2007. Photoinhibition, carotenoid composition and the co-regulation of photochemical and non-photochemical quenching in neotropical savanna trees. Tree Physiol. 27, 717–725.

Gorton, H.L., Brodersen, C.R., Williams, W.E., Vogelmann, T.C., 2010. Measurement of the optical properties of leaves under diffuse light. Photochem. Photobiol. 86, 1076–1083.

Habermann, G., Machado, S.R., Guimarães, V.F., Rodrigues, J.D., 2008. Leaf heliotropism in *Styrax camporum* Pohl from the Brazilian cerrado–distinct gas exchange and leaf structure, but similar leaf temperature and water relations. Braz. J. Plant Physiol. 20, 71–83.

Haridasan, M., 2008. Nutritional adaptations of native plants of the cerrado biome in acid soils. Braz. J. Plant Physiol. 20, 183–195.

Jiang, C.-D., Gao, H.-Y., Zou, Q., Jiang, G.-M., Li, L.-H., 2006. Leaf orientation, photorespiration and xanthophyll cycle protect young soybean leaves against high irradiance in field. Environ. Exp. Bot. 55, 87–96.

Koller, D., 1986. The control of leaf orientation by light. Photochem. Photobiol. 44, 819–826.

Koller, D., 1990. Light-driven leaf movement. Plant Cell Environ. 13, 615–632.

Li-Cor, 1990. LI-6200 Technical Reference. LI-COR Inc., Lincoln, 183p.

Li-Cor, 2004. Light sources and sensors. In: Li-Cor Using the LI-6400/LI-6400 XT Portable Photosynthesis System version 6. Part II: Useful Details. LI-COR Biosciences Inc., Lincoln, 8-1-8-26.

Liu, C.C., Welham, C.V.J., Zhang, X.Q., Wang, R.Q., 2007. Leaflet movement of *Robinia pseudoacacia* in response to a changing light environment. J. Integr. Plant Biol. 49, 419–424.

Machado, S.R., 1991. Morfologia e anatomia dos órgãos vegetativos de *Styrax camporum* Pohl. (Styracaceae) em desenvolvimento. PhD thesis. São Paulo, University of São Paulo.

Martin, C.E., Hsu, R.C.-C., Lin, T.-C., 2009. Comparative photosynthetic capacity of abaxial and adaxial leaf sides as related to exposure in two epiphytic ferns in a subtropical rainforest in Northeastern Taiwan. Am. Fern J. 99, 145–154.

Pastenes, C., Pimentel, P., Lillo, J., 2005. Leaf movements and photoinhibition in relation to water stress in field-grown beans. J. Exp. Bot. 56, 425–433.

Proietti, P., Palliotti, A., 1997. Contribution of the adaxial and abaxial surfaces of olive leaves to photosynthesis. Photosynthetica 33, 63–69.

Rodrigues, T.M., Machado, S.R., 2006. Anatomia comparada do pulvino primário de leguminosas com diferentes velocidades de movimento foliar. Rev. Bras. Bot. 29, 709–720.

Simon, M.F., Grether, R., Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E., 2009. Recent assembly of the cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proc. Nat. Acad. Sci. 106, 20359–20364.

Turner, N.C., 1981. Techniques and experimental approaches for the measurment of plant water status. Plant Soil 58, 339–366.

Von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.

Zhang, Y.-L., Zhang, H.-Z., Du, M.-W., Li, W., Luo, H.-H., Chow, W.-S., Zhang, W.-F., 2010. Leaf wilting movement can protect water-stressed cotton (*Gossypium hirsutum* L.) plants against photoinhibition of photosynthesis and maintain carbon assimilation in the field. J. Plant Biol. 53, 52–60.