

Assessing the role of vertical leaves within the photosynthetic function of *Styrax camporum* under drought conditions

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Abstract

Previous evidence has demonstrated that vertical leaves of *Styrax camporum*, a woody shrub from the Brazilian savanna, have a higher net photosynthetic rate (P_N) compared with horizontal leaves, and that it is detected only if gas exchange is measured with light interception by both leaf surfaces. In the present study, leaf temperature (T_{leaf}), gas exchange and chlorophyll (Chl) *a* fluorescence with light interception on adaxial and also on abaxial surfaces of vertical and horizontal mature fully-expanded leaves subjected to water deficit (WD) were measured. Similar gas-exchange and fluorescence values were found when the leaves were measured with light interception on the respective surfaces of horizontal and vertical leaves. WD reduced P_N values measured with light interception on leaf surfaces of both leaf types, but the effective quantum yield of PSII (Φ_{PSII}) and the apparent electron transport rate (ETR) were reduced only when the leaves were measured with light interception on the adaxial surface. WD did not decrease the maximum quantum yield of PSII (F_v/F_m) or increase T_{leaf} , even at the peak of WD stress. Vertical leaf orientation in *S. camporum* is not related to leaf heat avoidance. In addition, the similar P_N values and the lack of higher values of Φ_{PSII} and ETR in vertical compared with horizontal leaves measured with light interception by each of the leaf surfaces suggests that the vertical leaf position is not related to photoprotection in this species, even when subjected to drought conditions. The exclusion of this photoprotective role could raise the alternative hypothesis that diverse leaf angles sustain whole plant light interception efficiency increased in this species.

Additional key words: Brazilian savanna; chlorophyll fluorescence; gas exchange; leaf surface; paraheliotropism; Styracaceae.

Introduction

High-irradiance stress commonly occurs in the nature, producing oxidative damage and photoinhibition, and plants have evolved physiological, anatomical, and morphological strategies to deal with this stress (Takahashi and Badger 2011).

Vertical leaf orientation is thought to be a photoprotective strategy (Ehleringer and Forseth 1980). It reduces excessive sunlight interception (Liu *et al.* 2003) and avoids photodamages caused by reactive oxygen species (ROS), which can impair (Edreva 2005) or even prevent

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Abbreviations: Chl – chlorophyll; E – transpiration rate; ETR – apparent electron transport rate; F_0 – minimal fluorescence level in the dark-adapted state; F_m – maximal fluorescence level in the dark-adapted state; F_s – steady-state fluorescence in the light-adapted state; F_m' – maximal fluorescence level in the light-adapted state; F_v/F_m – maximum quantum yield of PSII; Φ_{PSII} – effective quantum yield of PSII; g_s – stomatal conductance; P_N – net photosynthetic rate; ROS – reactive oxygen species; T_{leaf} – leaf temperature; VPD – vapor pressure deficit; WD – water deficit; WW – well watered; WUE – water-use efficiency (P_N/E); Ψ_{pd} – predawn leaf water potential, measured before sunrise; Ψ_{md} – midday leaf water potential, measured when VPD was maximal.

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the photosynthetic apparatus from being repaired (Takahashi and Badger 2011). In contrast to horizontal leaves, vertical leaves are less likely to overheat and to suffer photoinhibition (Bielenberg *et al.* 2003, Liu *et al.* 2003). In addition, they exhibit reduced costs of biochemical photoprotective processes, such as the xanthophyll cycle (Liu *et al.* 2003).

The steepest leaf angles are commonly observed in plants from dry environments (Ehleringer 1988, Smith *et al.* 1998), suggesting that minimizing sunlight interception might be advantageous to plants in such environments. In the Cerrado biome or Brazilian savanna, the drought season (April to September) is accompanied by high irradiance load (Franco and Lüttge 2002; Habermann *et al.* 2011a), which would be a typical environment for species with vertical leaves.

S. camporum, a Cerrado woody shrub, possesses static mature leaves at vertical, horizontal and intermediate angles in relation to the horizon. Its horizontal and vertical mature leaves have been referred to as dia- and paraheliotropic leaves, respectively (Habermann *et al.* 2008, 2011b), but heliotropic movements are only observed in immature leaves of *S. camporum*. In one of these studies (Habermann *et al.* 2008), higher P_N , stomatal conductance values (g_s), and transpiration rates (E) were found in vertical leaves compared with horizontal leaves of nonstressed plants that were measured with a transparent leaf chamber in which sunlight was intercepted by both leaf surfaces. However, the higher g_s of the vertical leaves did not explain the elevated P_N or its

lack of influence on the intercellular CO_2 (C_i). In addition, vertical leaf orientation was not related to lower leaf temperatures. Even though low interception of excessive irradiance that prevented photoinhibition could have accounted for the higher P_N values of vertical compared with horizontal leaves in *S. camporum*, Habermann *et al.* (2008) argued that the compact spongy parenchyma seen in both mature leaf types could have allowed the abaxial surface to significantly cooperate with P_N in vertical leaves, which may intercept direct and diffuse sunlight during most of the day. Differences in gas-exchange rates between horizontal and vertical leaves of *S. camporum* have been mainly observed when natural irradiance is intercepted by both leaf surfaces (Habermann *et al.* 2011b). An alternative hypothesis for the benefit of vertical leaves states that they improve the daily carbon gain by increasing the total sunlight interception (Falster and Westoby 2003).

To further investigate the role of vertical leaves in *S. camporum*, two main questions were addressed: (1) do vertical leaves of *S. camporum* show increased photochemical or gas-exchange performances under drought conditions, and (2) do adaxial and abaxial leaf surfaces of both leaf types demonstrate distinct P_N values under well watered conditions? It is discussed whether vertical leaf orientation represents a strategy to deal with drought and excess sunlight in this species, or if vertical leaf orientation could be related to the optimization of P_N by enhancing the photosynthetic light-use efficiency.

Materials and methods

Plants: *S. camporum* is a woody shrub from the Cerrado and it is considered to be a light-demanding plant, which occurs at the edge of Cerrado vegetation fragments. Young plants (10 months old and 0.8 m high) of *S. camporum* Pohl. were cultivated in pots (100 L) containing a 3:2:1 mixture of oxisoil:sand:organic substrate (*Plantmax*, *Eucatex Inc.*, Paulínia, São Paulo, Brazil). Before starting the water-deficit experiment, a nutritive solution containing 20 mg L⁻¹ of Al³⁺ was applied monthly to induce aluminum accumulation, which is common in Cerrado plants (Haridasan 2008) and it is also observed in *S. camporum* (unpublished observation).

Experimental description and study site: Five plants were subjected to water deficit (WD), which was applied by withholding water, while five other plants were well watered (WW). To prevent rain from entering the pots of WD plants, a circular piece of plastic, which was cut from the edge to the center, was placed around the main stem and over the soil and affixed with rubber bands around the pot opening.

Using one leaf per each one of the five replicates (plants), the predawn (ψ_{pd}) and midday (ψ_{md}) water

potential, temperature (T_{leaf}), gas exchange and Chl *a* fluorescence were measured in vertical and horizontal leaves of *S. camporum* during 35 days (August 29th to October 2nd, 2009) of water deficit, followed by 2 d of re-irrigation. The photosynthetic active radiation coming from different positions was also measured at the experimental site (Centro Experimental de Campinas, 22°54'S, 47°05'W), in Campinas, SP, Brazil. The maximum, medium, and minimum air temperatures, obtained from a local weather station during the experimental period were 25.9 ± 3.7, 20.4 ± 2, and 16 ± 1.6°C, respectively. The air humidity oscillated between 70.1 ± 14.1% and 88 ± 10.3%. In addition, to further investigate P_N of vertical leaves, T_{leaf} , gas-exchange and fluorescence parameters were measured in leaves intercepting light by the adaxial leaf surface and in leaves intercepting light by the abaxial surface. For this investigation a saturating light was used, since disturbances on P_N , rather than the assumption of different light absorption by vertical and horizontal leaves, were of main interest.

ψ_{pd} and ψ_{md} were measured on days 1, 6, 19, and 35 after WD was imposed. Day 35 was considered to be the day of maximum stress because g_s and ψ_w values were the lowest observed. WD plants were re-irrigated on the

35th d, and ψ_w was measured after 2 d. Measurements of ψ_w were made on vertical and horizontal mature leaves of WW and WD plants.

Gas-exchange and fluorescence measurements were performed on day 1 after WD was imposed, on the 35th d (maximum stress), and 2 d after re-irrigation (on day 37). These measurements were made on the adaxial and abaxial surfaces of vertical and horizontal mature leaves in WW and WD plants.

Both leaf types, of approx. 4–6 months old, occurred on woody stems, and had mature fully-expanded leaf blades. Very young and very old leaves were avoided.

Gas exchange and fluorescence measurements: Measurements were performed on clear days from 9:00 to 11:00 h, as recommended by Prado *et al.* (2004).

Gas exchange was measured with an open-gas portable infrared analyzer (*LI-6400*, *LI-COR Inc.*, Lincoln, NE, USA). P_N , E and g_s were calculated by the *LI-6400* data analysis program. The photosynthetic photon flux density (PPFD) was provided with an artificial red–blue LED light source (*6400-02B*, *LI-COR*, USA) held on the opaque top of the leaf cuvette. The PPFD was set to 1,500 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, which corresponds to the saturating PPFD for *S. camporum* P_N (Habermann *et al.* 2011b), and the CO_2 concentration inside the leaf cuvette was maintained at 390 $\mu\text{mol mol}^{-1}$ by using a CO_2 injector (*6400-01*, *LI-COR*, USA). However, other cuvette variables, such as air temperature and vapor pressure deficit (VPD), were allowed to vary with the external environment. Therefore, gas exchange was measured by clamping onto leaves in their normal position, which were exposing the adaxial surface to the artificial light; and also by clamping onto leaves in their twisted position, which were exposing the abaxial surface to the artificial light.

T_{leaf} was assessed with a thermocouple (Bielenberg *et al.* 2003) held on the opaque bottom of the 2×3 *LI-COR* leaf cuvette and it touched the abaxial or the adaxial leaf surface that was opposite the artificially illuminated surface. Water-use efficiency (WUE) was calculated as P_N/E (Berry and Downton 1982).

Considering that PPFD was artificially provided for gas-exchange measurements and that vertical leaves were not totally flat on both sides of the midvein, the planes of vertical leaves were forced into a flat position when the leaf cuvette was closed. However, the petiole angle was not disturbed. Horizontal leaves exhibited a flat position, following the petiole angle, which was not disturbed.

Chl *a* fluorescence was measured with a modulated fluorometer (*FMS 1*, *Hansatech Inc.*, Norfolk, UK). Leaves were dark-adapted for 30 min using leaf clips. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulate pulse [PPFD < 0.05 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ with a waveband of 594 nm, for 1.8 μs]. Maximal fluorescence in this state (F_m) was obtained with a saturating actinic light pulse of 18,000

$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 0.7 s. The maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) was calculated as $[(F_m - F_0)/F_m]$ (Bolh ar-Nordenkampf and  quist 1993).

The same leaf section of each plant was used to measure light-adapted variables. Steady-state fluorescence (F_s) was registered after plants were adapted to ambient light conditions, and the leaf to be measured was held by the fluorometer leaf clip holder in a position that enabled the leaf surface to perpendicularly intercept the incoming sunlight [$1,400 \leq \text{PPFD} \leq 1,800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] between 9:00 and 11:00 h (Fig. 1). A saturating actinic light pulse of 18,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 0.7 s was used to return the maximum fluorescence (F_m'). With fluorescence variables obtained in both light- and dark-adapted states, the effective quantum yield of PSII [$\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$], and the apparent electron transport rate [ETR = $\Phi_{\text{PSII}} \text{PPFD} \times 0.5 \times 0.84$] were calculated. The relative excessive PPFD was calculated as $[(F_v/F_m - \Phi_{\text{PSII}})/(F_v/F_m)]$ (Bilger *et al.* 1995).

To calculate the ETR, 0.5 was used for the fraction of excitation energy distributed between the two photosystems. Although the relative light reflectance of abaxial leaf surfaces is twice as high as adaxial surfaces of *S. camporum* leaves (Habermann *et al.* 2011b), we used 0.84 as the fraction of light that was absorbed by the leaves (Baker 2008), regardless of the leaf surface. A fraction of light absorbance lower than 0.84 for the abaxial leaf surface would probably correct an overestimate of ETR on the abaxial surface. On the other hand, it would be considered an empirical fraction value and it would not change the analysis because ETR measured with light interception by either one of the two leaf surfaces would continue to be different, changing only the amplitude of values.

Under natural conditions, vertical leaves intercept sunlight on distinct planes and surfaces, mainly from low angles (when the sun is low in the sky), and horizontal leaves intercept the highest irradiance on the adaxial surface at noon and the lowest (scattered) light on the abaxial surface throughout the day; therefore, the natural PPFD from multiple directions was measured throughout the day with a quantum sensor (*LI-190*, *LI-COR*, USA) that was pointed to the north, south, east, and west and upwards and downwards at 7:00, 9:00, 11:00, 13:00, 15:00, and 17:00 h (Fig. 1). Because of these complex sunlight interception possibilities for vertical leaves, the differences in sunlight absorption by vertical and horizontal leaves observed at noon were not considered, since the saturating photochemical and P_N response capacities were of main interest. Therefore, gas exchange was measured with a saturating artificial PPFD intercepted by the adaxial or by the abaxial leaf surface. When measuring fluorescence, the leaf surface to be measured was directly pointed at the incoming sunlight and held in a position that enabled the leaf surface to perpendicularly intercept the incoming sunlight. Thus, the PPFD values used for the calculation of the ETR ranged

between 1,400 and 1,800 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, which were the PPFD values that were observed between 9:00 and 11:00 h (Fig. 1).

ψ_w : Measurements of the ψ_w before sunrise (predawn, ψ_{pd}) and when VPD was maximal (midday, ψ_{md}) were obtained with a *Scholander* pressure chamber (*DIK-7000*, *Daiki Rika Kogyo Inc.*, Tokyo, Japan).

Data analyses: A two-way analysis of variance (*ANOVA*) was carried out, using ‘leaf factor’ (horizontal adaxial, horizontal abaxial, vertical adaxial, and vertical abaxial) and water deficit (WW and WD plants) as statistical factors in order to test each variable separately, for each evaluation day. *Tukey’s* test ($\alpha = 0.05$) was used as a multiple comparison procedure. It should be noted that a three-way *ANOVA* could not have been employed, that is,

Results

Although the amplitude of daily oscillations in the external PPFD detected from the north, south, east, west, above, and below were somewhat expected, the PPFD between 9:00 and 11:00 h (mostly coming from the east and above), used for the calculation of the ETR ranged from 1,400 to 1,800 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ (Fig. 1).

Significant decreases in both ψ_{pd} and ψ_{md} in plants that were submitted to water deficit (WD) were observed 6 d after the beginning of WD (Fig. 2*A,B*). Throughout the experiment, ψ_{pd} and ψ_{md} observed in the horizontal and vertical leaves were the same ($P > 0.05$) in well watered (WW) and WD plants. The lowest ψ_{pd} and ψ_{md} values observed in WD plants, compared with WW plants, occurred 35 d after WD was imposed. Two days

the following experimental design could not have been used in this paper: water deficit (two levels of water supply, WW and WD), leaf orientation (horizontal and vertical leaves), and leaf surface (adaxial and abaxial surfaces). This is because leaf orientation and leaf surface are nondissociated factors from both biological and ecological standpoints (*i.e.*, both horizontal and vertical leaves are composed of adaxial and abaxial surfaces). Moreover, using leaf orientation as a separate statistical factor would be meaningless. For example, with respect to P_N (the most important variable in the current paper), if a three-way *ANOVA* had been used, this multiway factorial analysis of variance would have revealed that leaf orientation would not be a significant factor, since the mean P_N values would be the same for horizontal and vertical leaves: $F = 0.002$, $P = 0.96$ for day 1; $F = 0.30$, $P = 0.59$ for day 35, and $F = 0.09$, $P = 0.76$ for day 37.

after re-irrigation of the WD plants (37 d), ψ_{pd} and ψ_{md} were the same in the WD and WW plants (Fig. 2*A,B*).

On the 1th d after WD was imposed, horizontal and vertical leaves had the same P_N values ($P > 0.05$) when the leaves were measured with light interception either by the adaxial or by the abaxial leaf surfaces in both WW and WD plants (Fig. 3*A,B*). On the day at which maximum stress occurred (35 d), WW plants showed higher P_N values compared with those of WD plants when the leaves were measured with light interception on the same surface of horizontal (Fig. 3*A*) and vertical (Fig. 3*B*) leaves.

In WW plants evaluated 35 d after treatments were applied, the horizontal leaves that were measured with

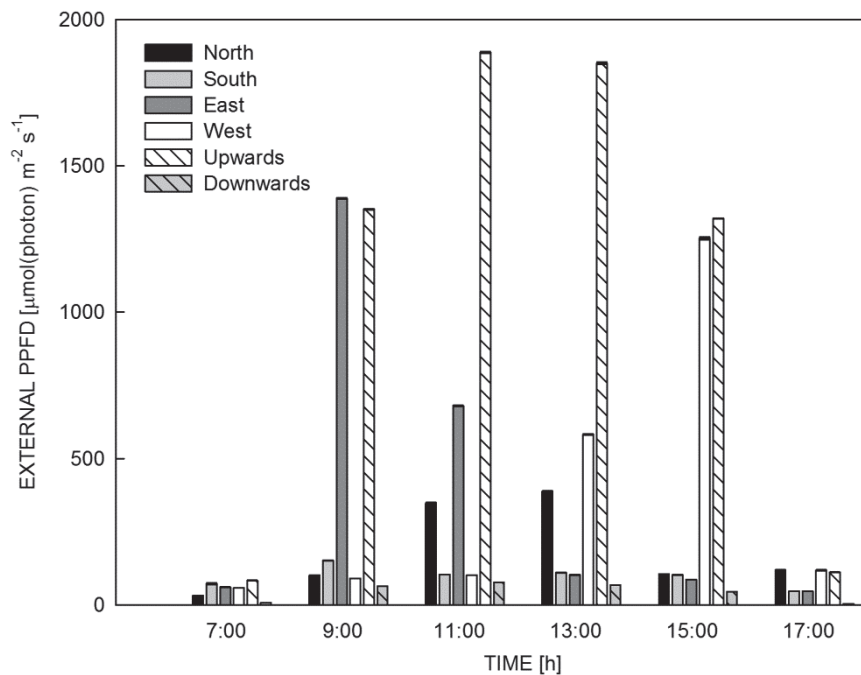


Fig. 1. Photosynthetic photon flux density (PPFD) measured with a quantum sensor pointed to the north, south, east, west, upwards, and downwards at the experimental site ($n = 10$). Each bar is the mean value of 10 replications (\pm SD).

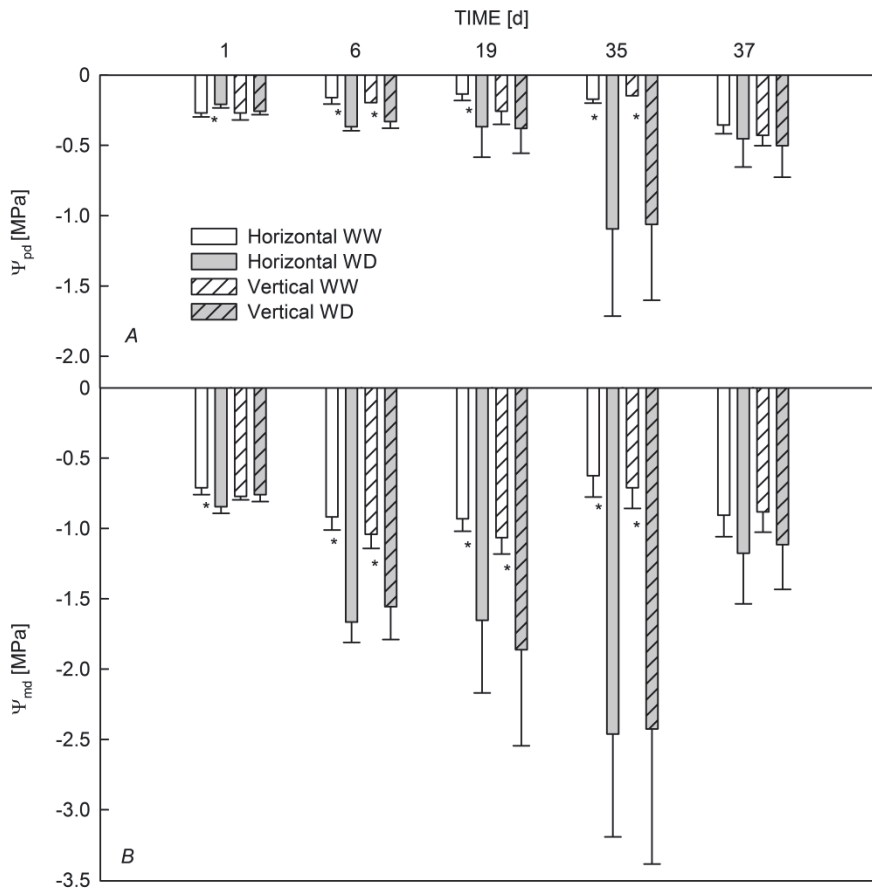


Fig. 2. Predawn (A) and midday (B) leaf water potentials in horizontal and vertical leaves of well watered (WW) and water-deficient (WD) *Styrax camporum* plants on different evaluation days. Each bar is the mean value of 5 replications (\pm SD). For each evaluation day and for the same leaf type, asterisks indicate significant differences ($P < 0.05$) between WW and WD plants.

light interception by the adaxial surface had increased P_N values compared with when the light was intercepted by the abaxial surface; however, this difference in P_N values obtained with distinct light interception between leaf surfaces was not observed in WD plants (Fig. 3A). On the other hand, vertical leaves measured with light interception by either of the leaf surfaces had similar P_N values in both WW and WD plants (Fig. 3B).

Horizontal and vertical leaves had similar values ($P > 0.05$) of g_s , E , WUE (Fig. 3), F_v/F_m , Φ_{PSII} , ETR, relative excessive PPFD (Fig. 4), and T_{leaf} (Table 1) when leaves were measured with light interception on either the adaxial or the abaxial leaf surfaces in both WW and WD plants throughout the experiment.

After 35 d of drought, g_s (Fig. 3C,D) and E (Fig. 3E,F) were lower in WD than in WW plants, regardless of the leaf type and its surface that was illuminated when measuring leaves. Despite these responses to drought for g_s and E , for each evaluation day, WUE varied substantially among the leaf types and surfaces, and drought conditions did not cause any significant differences in the WUE measured in leaves intercepting light by either the adaxial or abaxial surfaces of horizontal or vertical leaves (Fig. 3G,H).

After 35 d, WD did not cause a significant increase in T_{leaf} (Table 1) or a decrease in F_v/F_m measured in leaves intercepting light either by the adaxial or abaxial surfaces

of horizontal or vertical leaves in the morning (Table 1, Fig. 4E,F) or even in the afternoon (data not shown). F_v/F_m was also the same when the leaves were measured with light interception either by the adaxial or abaxial surfaces of horizontal (Fig. 4E) and vertical (Fig. 4F) leaves of WW and WD plants.

Φ_{PSII} (Fig. 4A,B) and ETR (Fig. 4C,D) were higher in the leaves that were measured with light interception on the adaxial surface compared with leaves that were measured with light interception on the abaxial surface of both leaf types in WW plants. On day 35, WD reduced Φ_{PSII} (Fig. 4A,B) and ETR (Fig. 4C,D) values only in leaves that were measured with light interception on adaxial surfaces of both leaf types. WW and WD plants had the same Φ_{PSII} (Fig. 4A,B) and ETR (Fig. 4C,D) when leaves were measured with light interception on the abaxial surface of both leaf types on the 1st and 35th d.

On the 1th day, the relative excessive PPFD was higher in leaves that were measured with light interception on the abaxial surface compared with when leaves were measured with light interception on the adaxial leaf surface, regardless of the leaf type (Fig. 4G,H). Thirty-five days of WD significantly increased the relative excessive PPFD assessed in leaves intercepting light on the adaxial, but not on the abaxial surface of both leaf types (Fig. 4G,H).

Discussion

The present data demonstrate that even though vertical leaves can intercept less solar radiation at noon (Fig. 1), these leaves do not show higher photochemical or gas-exchange performances when intercepting saturating light in comparison with horizontal leaves, and their static vertical position is not related to photoprotection in *S. camporum*. These conclusions are supported by the similar P_N , T_{leaf} , and excessive PPFD values observed when the leaves were measured with light interception by the adaxial and by the abaxial surfaces of horizontal and vertical leaves, especially in WD plants; these conclusions are also supported by the lack of higher values of Φ_{PSII} , ETR, and F_v/F_m in vertical compared with horizontal leaves, responses which are frequently found by other authors. Thus, in opposition to our findings, previous analyses of vertically *vs.* horizontally oriented leaves (Falster and Westoby 2003, Liu *et al.* 2003) demonstrated that because horizontal leaves have increased excessive light interception, these leaves showed higher leaf temperature under stress conditions, which may contribute to photoinhibition, whereas vertical leaves avoid such effects. Even analyses of paraheliotropic leaves (Ehleringer and Forseth 1980, Bielenberg *et al.* 2003, Takahashi and Badger 2011) emphasize that the paraheliotropic movements help avoid the effects of excessive light interception. Considering that vertical and horizontal leaves were exposed to the same saturating PPFD on both surfaces, we conclude that both horizontal and vertical leaves can cope with saturating light, and none of the leaf types showed advantages or disadvantages over one another under saturating light and WD, as the literature suggests. Therefore, although sunlight interception at noon was avoided, like any other vertical or paraheliotropic leaf, the prevention of leaf heat or photoprotection under drought conditions is not applicable to static vertical leaves of *S. camporum*.

On the day of maximum water deficiency, reductions in P_N , g_s , and E were observed when the leaves were measured with light interception by the adaxial and abaxial surfaces of both leaf types (Fig. 3A–F). However, on the same day, there were no reductions in F_v/F_m when the leaves were measured with light interception on either surface of both leaf types (Fig. 4E,F). This suggests that both leaf types either have an efficient electron sink that prevents the formation of reactive oxygen species (ROS), such as superoxides and singlet oxygen, or they have an efficient antioxidant system to deal with these toxic compounds, which could damage (Edreva 2005) or prevent a repair of the photosynthetic apparatus (Takahashi and Badger 2011) and lead to photoinhibition. Nevertheless, when the leaves were measured with light interception by the adaxial surface of both leaf types, they exhibited higher susceptibility to WD compared with when leaves were measured with light interception by the abaxial leaf surface. When the leaves were measured with

light interception by the adaxial surface, WD caused significant reductions in Φ_{PSII} and ETR (Fig. 4A–D) and an increase in the relative excessive PPFD (Fig. 4G,H), which suggests that elevated thermal energy dissipation and down-regulation of PSII was employed to reduce the excessive light energy in the photosynthetic apparatus (Franco and Lüttge 2002) in the adaxial compared with the abaxial leaf surface.

P_N was reduced by WD in both leaf types, regardless of the leaf surface that was illuminated when leaves were measured (Fig. 3A,B). Biochemical damage is unlikely in this case because an intercellular/atmospheric CO_2 (C_i/C_a) increase was not observed, even at the peak of stress on day 35 (data not shown). When the leaves were measured with light interception by each of the leaf surfaces of WD plants, gas-exchange rates (P_N and E) appeared to be dependent on the low conductance of stomata that are present only on the abaxial leaf surface, since *S. camporum* leaves are hypostomatic (Habermann *et al.* 2008). In addition, in the vertical and horizontal leaves of *S. camporum*, P_N and E largely depend on g_s , but C_i is unresponsive to g_s changes (Habermann *et al.* 2008). Thus, under WD, the low P_N found when the leaves were measured with light interception by either of the leaf surfaces of both leaf types seemed to be caused by the low g_s .

In contrast to gas exchange, photochemical efficiency was impaired by WD only when the leaves were measured with sunlight interception by the adaxial surface (Fig. 4A–D). Therefore, fluorescence responses measured on both leaf surfaces in WD plants exclusively depended on light absorption by the leaf surface that was illuminated. The adaxial surface of *S. camporum* leaves is glabrous, whereas the abaxial surface is pubescent (Habermann *et al.* 2008). For this reason, in *S. camporum* leaves, light reflectance within the photosynthetic spectrum (400–700 nm) is approximately two-times greater on the abaxial compared with the adaxial surface (Habermann *et al.* 2011b). Therefore, WD affected P_N when gas exchange was measured with light interception by either of the leaf surfaces, but the photochemical reactions were affected only when light was intercepted by the adaxial surface. However, this does not mean that the abaxial leaf surface does not absorb sunlight; otherwise, when the leaves were measured with light interception by the abaxial surface, they would have exhibited null values of P_N and ETR. Moreover, abaxial surfaces of horizontal and vertical leaves of *S. camporum* illuminated by artificial red/blue saturating light demonstrated P_N values between 8 and 10 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ (Habermann *et al.* 2011b).

The present study also aimed to verify whether the adaxial and abaxial surfaces of each leaf type have different photosynthetic capacities in WW plants. The photosynthetic capacity of a leaf surface is influenced by

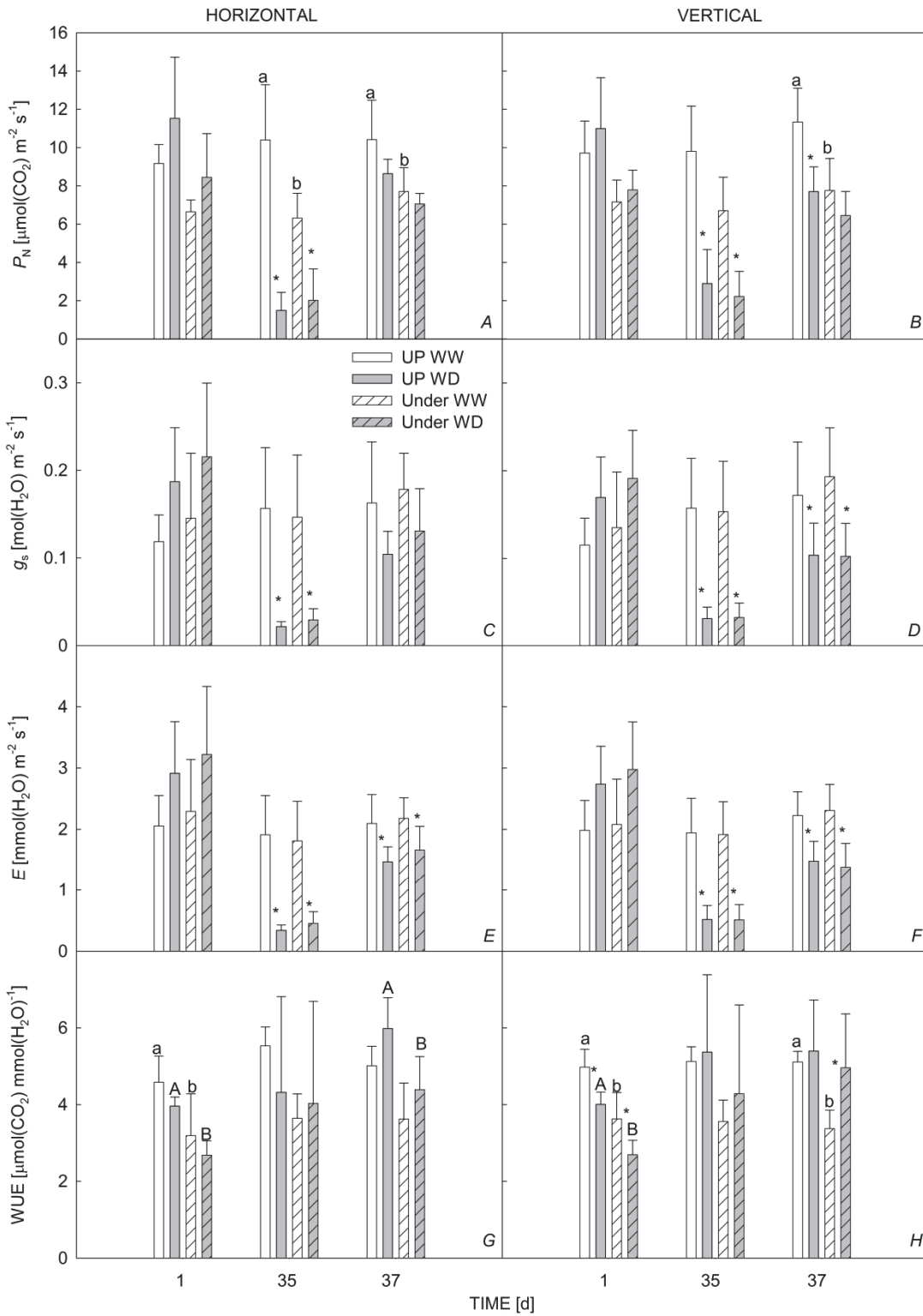


Fig. 3. Net photosynthetic rate (P_N) (A,B), stomatal conductance (g_s) (C,D) and transpiration (E) (E,F) rates, and water-use efficiency (WUE) values (G,H) measured with light interception by the upperside (UP) and underside (UNDER) surfaces of horizontal (A,C,E,G) and vertical (B,D,F,H) leaves of well-watered (WW) and water-deficient (WD) *Styrax camporum* plants. For each evaluation day and on the same surface of the same leaf type, asterisks indicate significant differences ($P < 0.05$) between WW and WD plants. For each evaluation day and on the same leaf type, letters indicate significant differences ($P < 0.05$) between leaf surfaces of WW (lowercase letters) or WD (uppercase letters) plants. Each bar is the mean value of 5 replications (\pm SD).

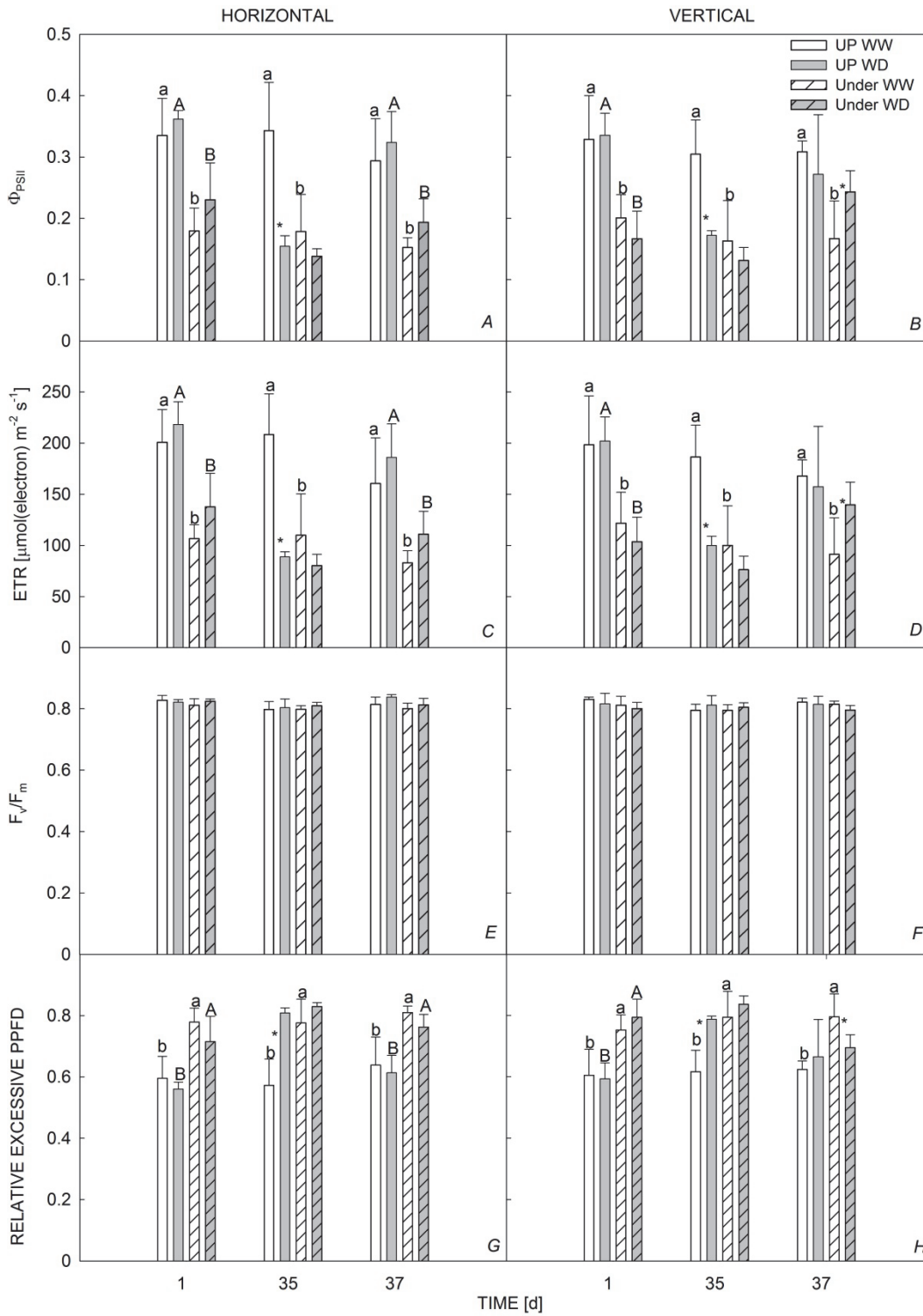


Fig. 4. Effective quantum yield of PSII (Φ_{PSII}) (A,B), apparent electron transport rate (ETR) (C,D), maximum quantum yield of PSII (F_v/F_m) (E,F), and relative excessive PPFDs [$(F_v/F_m - \Phi_{PSII})/(F_v/F_m)$] (G,H) measured with light interception by the upsides (UP) and undersides (UNDER) surfaces of horizontal (A,C,E,G) and vertical (B,D,F,H) leaves of well watered (WW) and water-deficient (WD) *Styrax camporum* plants. For each evaluation day and on the same surface of the same leaf type, asterisks indicate significant differences ($P < 0.05$) between WW and WD plants. For each evaluation day and on the same leaf type, letters indicate significant differences ($P < 0.05$) between leaf surfaces of WW (lowercase letters) or WD (uppercase letters) plants. Each bar is the mean value of 5 replications (\pm SD).

Table 1. Leaf temperature (T_{leaf}) of upperside (adaxial) and underside (abaxial) surfaces of horizontal and vertical leaves of well watered (WW) and water-deficient (WD) *S. camporum* plants (means \pm SD, $n = 5$). There were no significant differences in T_{leaf} among the comparable groups of leaf types, leaf surfaces, and water treatments observed.

Time of experiment [d]	T_{leaf} [%]		Vertical leaves					
	Horizontal leaves		WD plants		WW plants		WD plants	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	26.1 \pm 0.5	26.0 \pm 0.4	25.9 \pm 0.5	25.8 \pm 0.2	26.0 \pm 0.3	25.9 \pm 0.4	25.9 \pm 0.4	25.8 \pm 0.2
35	27.5 \pm 0.3	27.5 \pm 0.3	27.6 \pm 0.2	27.6 \pm 0.2	27.6 \pm 0.1	27.7 \pm 0.4	28.0 \pm 0.7	27.7 \pm 0.3
37	27.3 \pm 0.8	26.8 \pm 0.4	27.1 \pm 0.8	26.7 \pm 0.4	27.4 \pm 0.8	26.9 \pm 0.5	27.2 \pm 0.6	26.8 \pm 0.3

the light environment during development (Evans *et al.* 1993) and by the amount of sunlight which is exposed to after maturity (Terashima 1986). As a result, a higher photosynthetic capacity is observed in leaves of sun-adapted plants than in leaves of shade-adapted plants (DeLucia *et al.* 1991), in leaves that are mostly exposed to the sun in a particular canopy (Lichtenthaler *et al.* 2007), and in palisade rather than in spongy chloroplasts of horizontally oriented leaves (Terashima and Inoue 1985). Hence, considering that the surfaces of the horizontal and vertical leaves of *S. camporum* differ in their sunlight interception potentials (Fig. 1), when the leaves were measured with light interception by each of the leaf surfaces, we expected to find differences in P_N values between the surfaces of horizontal leaves and similarities between the surfaces of vertical leaves.

We did not obtain data that would conclusively confirmed either the expected similarity in P_N values between the adaxial and abaxial surfaces of vertical leaves or the expected difference in P_N values between the surfaces of horizontal leaves of WW plants (Fig. 3A,B), and this was mainly because of the great variability observed for the momentaneous responses. However, when such variability is drastically reduced, as when P_N /PPFD curves are evaluated, under a PPFD between 800 and 1,800 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ both surfaces of *S. camporum* vertical leaves present similar values of P_N , but the adaxial leaf sides of their horizontal leaves maintain significantly higher P_N values compared to their abaxial sides (Habermann *et al.* 2011b). Nevertheless, if *S. camporum* leaves are maintained at their natural orientation and measured with a leaf cuvette that allows sunlight to penetrate the top (*Sun + Sky Chamber*, LI-COR, USA) and the bottom (*Clear Bottom Chamber*, LI-COR, USA) of the leaf chamber, the P_N values measured with sunlight interception by only the adaxial leaf surface (by covering the bottom of the chamber) of both leaf types were significantly higher during most of the day, if compared with P_N values measured with sunlight interception by the abaxial leaf surface (by covering the top of the chamber). Under such conditions, the vertical leaves performed well at 15:00, when the sun was low in the sky (data not shown). This suggests that vertical leaves can potentially benefit from a direct

sunlight interception by both surfaces.

When both leaf types were measured with sunlight interception by the adaxial surface, higher Φ_{PSII} and ETR were observed compared with leaves measured with sunlight interception by the abaxial leaf surface (Fig. 4A–D). Greater adaxial photosynthetic capacity in relation to the abaxial surface has already been observed in horizontal sun leaves (DeLucia *et al.* 1991, Evans *et al.* 1993, Proietti and Palliotti 1997). In horizontally oriented leaves, chloroplasts from the palisade parenchyma have more electron carriers (Terashima and Inoue 1985) and higher amounts and activity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) (Nishio *et al.* 1993, Sun and Nishio 2001) than chloroplasts from the spongy parenchyma. The presence of trichomes on the abaxial surface of *S. camporum* leaves (Habermann *et al.* 2008) may have decreased the light absorption and the photochemical performance when the leaves were measured with sunlight interception by this surface, as leaf pubescence may decrease light absorption (Ehleringer *et al.* 1976). Nevertheless, the presence of compact spongy parenchyma on the abaxial surface of *S. camporum* leaves (Habermann *et al.* 2008) and in many Cerrado species (Bieras and Sajo 2009) could allow the vertical leaves to have carbon-gain benefits because in some species, irradiating both leaf surfaces (using a mirror) with sunlight enhances P_N values of the whole leaf (Proietti and Palliotti 1997).

One could still state that the present results are inconsistent with our earlier papers, since the present data do not confirm that vertical leaves exhibit higher P_N values than horizontal leaves, as shown by Habermann *et al.* (2008, 2011b). However, these authors were able to show a better photosynthetic performance of vertical, compared with horizontal leaves, because gas exchange was measured with light interception by both leaf surfaces, using a transparent leaf chamber. In the present study, gas exchange was not measured with light interception by both leaf surfaces, but rather, by one of the leaf surfaces at a time.

Therefore, vertical leaf orientation is not related to photoprotection or leaf heat avoidance in *S. camporum* plants under WD. Overall, WD reduced gas-exchange rates when the leaves were measured with light inter-

ception by either of the leaf surfaces of both leaf types, suggesting that low g_s may explain the gas exchange reduction. On the other hand, Φ_{PSII} and ETR were higher when the leaves were measured with sunlight interception by the adaxial leaf surface compared with the leaves measured with sunlight interception by the abaxial surface, even in WW plants, and water deficiency reduced the photochemical parameters only when the leaves were measured with light interception by the adaxial surface of both leaf types.

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