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Photosynthetic Responses of *Smilax goyazana* (*Smilacaceae*)

By

Dario PALHARES*)**)***), Augusto César FRANCO**)

and Lílian Beatriz PENTEADO ZAIDAN***)

With 4 Figures

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Summary

PALHARES D., FRANCO A. C. & PENTEADO ZAIDAN L. B. 2011. Photosynthetic responses of *Smilax goyazana* (*Smilacaceae*). – Phyton (Horn, Austria) 51 (1): 115–132, with 4 figures.

Smilax goyazana A. DC. (*Smilacaceae*) is a herbaceous species very common in the Cerrado. The gas exchange, the fluorescence of chlorophyll, the leaf water potential, the titratable acidity and the chlorophyll and carotenoid contents were measured in order to disclose its photosynthetic plasticity of responses to field conditions. The female plants tended to a higher degree of electron transport rate than the male plants. The maximum value of net CO_2 assimilation and the transpiration rates were similar between male and female plants. The rates of CO_2 assimilation, transpiration and electron transport on sunny days were stable throughout the day and also during the rainy and dry seasons. The leaf water potential is isohydric and constant even during the dry season. There was no difference in titrable acidity between dawn and dusk. The chlorophyll contents are typical of plants adapted to high levels of sunlight.

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*) D. PALHARES, SQS 408 bl. C ap. 307. 70257-030 Brasília, DF, Brazil. Corresponding author: e-mail: dariompm@unb.br

**) D. PALHARES, A. C. FRANCO, Department of Botany, University of Brasilia.

***) D. PALHARES, L.B. PENTEADO ZAIDAN, Laboratory of Plant Physiology and Biochemistry, Botanical Institute of Sąo Paulo.

Zusammenfassung

PALHARES D., FRANCO A. C. & PENTEADO ZAIDAN L. B. 2011. Photosynthetic responses of *Smilax goyazana* (*Smilacaceae*). [Photosynthetische Reaktionen von *Smilax goyazana* (*Smilacaceae*)]. – Phyton (Horn, Austria) 51 (1): 115–132, 4 Abbildungen.

Smilax goyazana A.DC. (*Smilacaceae*) ist eine in Cerrados (Savannen Zentral-Brasiliens) sehr häufig vorkommende krautige Pflanze. Gaswechsel, Chlorophyllfluoreszenz, Wasserpotential des Blattes, der titrierbare Säuregehalt, sowie Chlorophyll- und Carotinoidgehalt wurden gemessen, um den Verlauf der Photosynthese unter Freilandbedingungen bestimmen zu können. Die weiblichen Pflanzen hatten eine leicht höhere Elektronenntransportrate als die männlichen. Die Höchstwerte der Netto-CO_2-Assimilation und der Transpirationsrate waren bei männlichen und weiblichen Pflanzen annähernd gleich. Keine Unterschiede der CO_2 Assimilation, der Transpirationsrate und des Elektronentransport wurden zwischen Sonnen- und Regentagen gefunden. Selbst während Trockenperioden waren diese Parameter stabil. Das Wasserpotential der Blätter ist auch während Trockenperioden konstant. Im titrierbaren Säuregehalt gab es ebenfalls keine Unterschiede während eines Tagesgangs. Die Chlorophyllgehalte sind typisch für an Starklicht angepasste Pflanzen.

Introduction

The Cerrado is a savannic formation subject to an annual dry season (from April to August) and a high solar irradiance (FRANCO 1998). The combination of high luminosity with water restriction is potentially harmful to the photosystems (DEMMIG-ADAMS & ADAMS 1992). Therefore, the plants adapted to this environment show photosynthetic strategies to overcome such situation (PRADO & al. 2004).

Among the dominant plant species of the Cerrado, there is a great variability in the photosynthetic responses (PALHARES & al. 2010). In general, the plants tolerate the high solar incidence, but the biochemical mechanisms for that vary from photoinhibition to a great efficiency in the photorespiration cycle (FRANCO & al. 2007), which is reflected, for example, in the great variability in the carbon assimilation rate at the dry season and/or the period of the day: some species keep the carbon assimilation rate per leaf area throughout all day long even during the dry season, while others reduce it at midday even during the rainy season (PRADO & MORAES 1997, PRADO & al. 2004, FRANCO & al. 2005).

Also, the ability to overcome water restriction is an important feature for surviving in the Cerrado. In this way, the dominant evergreen species present different responses during the dry season: compared to the rainy season, some species show lower water potential at midday and at predawn with enhanced transpiration rate and massive leaf shedding, while others maintain the leaf water potentials and some others even renew the leaves during the dry season (BUCCI & al. 2005, 2006). In general, all year along, during the night many species present a considerable transpiration rate, while others show a negligible transpiration (LABOURIAU & al. 1964, NAVES-BARBIERO & al. 2000).

With such variability of strategies, it is not surprising that photosynthetic parameters such as light saturation point, water use efficieny, carbon assimilation rate, daily course of transpiration, etc. have shown a large variation among species and between dry and rainy seasons. These values can be checked in BUCCI & al. 2005, 2006, FRANCO 1998, FRANCO & LÜTTGE 2002, FRANCO & al. 2005, 2007, PRADO & MORAES 1997, PRADO & al. 2004, PALHARES & al. 2010.

In this ecological context, in the herbaceous layer of the Cerrado of Central Brazil, *Smilax goyazana*, a dioecious evergreen monocotyledon, is present as a dominant plant. In South America there are about 50 species of *Smilax* and around seven species have been occasionaly found in the Cerrado (ANDREATA 1997), but only *S. goyazana* has been systematically found in phytosociological studies (MUNHOZ & FELFILI 2005, 2006, 2007). Further information regarding habit and general aspect can be found in PALHARES & SILVEIRA 2005, PALHARES & al. 2009a, 2009b.

Also, in spite of the ecological importance of the herbaceous layer in the Cerrado, photosynthetic data has come mostly from trees or shrubs. The case of *S. goyazana* is even particular since female plants of dioecious species are submitted to higher metabolic demands than the male plants, due to the necessity for formation of fruits and seeds.

The objective of this study is to know about the plasticity of photosynthetic responses of *Smilax goyazana*, comparing male and female plants.

Material and Methods

The study site was the Cerrado area of the Olimpic Center of University of Brasília, a place with an area of 160 ha located between the co-ordinates of 15°46'S – 47°50'W and 15°45'S – 47°51'W that provides a representative flora of cerrado sensu stricto, according to the phytosociological study of ASSUNÇÃO & FELFILI 2004. Voucher specimens were deposited at the herbarium of the University of Brasília (UB) under the registration numbers of UB 15269 and UB 15270. The local climate is Aw type according to Koeppen's classification, with a rainy season from October to April and a dry season from May to early September. The soil is oxisol with a high percentage of clay and a deep water table. For details of climate and soil, consult JACKSON & al. 1999, FRANCO & al. 2005 and BUCCI & al. 2006.

In this place, flowering plants of *S. goyazana* were identified and marked. The fully developed and apparently healthy leaves were selected for the study. The measurements during the dry season were carried out from July to September, after at least 80 days without rains. The measurements in the rainy season were carried out from November to April. The conditions of the field experiments are summarized in table 2.

Gas Exchanges

The net CO_2 assimilation rate (A, $\mu mol.m^{-2}.s^{-1}$), the net transpiration rate (E, $mmol.m^{-2}.s^{-1}$) and the conditions of the field experiments were measured in a portable infrared gas analyser (IRGA) model LCA_4 from ADC BioScientific (Hoddesdon, UK), as follows:

Light Response Curve

The sample was made up of three male and three female plants, with two leaves per plant, in field conditions, during the rainy season, between 09:00 and 11:00 (see Table 2). A light source PLU-002 (ADC BioScientific) was connected to the foliar chamber of the IRGA. The maximum photosynthetically active radiation (PAR) inside the leaf chamber was 1250 μmol quanta.m^{-2}.s^{-1}. The various light intensities were obtained with the placement of hand-made paper cards between the light source and the acrylic coverage of the foliar chamber. The light intensities were determined with a quantum sensor Li-Cor 190 (Li-Cor, Lincoln, USA) placed inside the leaf chamber, as described by MARIMON & al. 2008. The measurements were carried out in both an ascendant and descendant way (which means, from darkness to maximum light intensity and again from maximum intensity to darkness). The values of gas exchanges were saved after stabilisation that took about 2 to 5 minutes at each new light level. The sigmoidal equation of dose-response of Boltzmann was used for the non-linear adjustment of the curve, with the help of the free software Grapes 6.71.

Daily Course of A and E

The sample was made up of two male and two female plants, with two leaves per plant. On sunny days in the dry and the rainy seasons, hourly measurements of the ambiental conditions, A and E with the IRGA were carried out from 07:00 to 17:00.

Comparisons of A and E Between Male/Female Plants, Dry/Rainy Seasons and Morning/Early Afternoon

The sample was 10 male and 10 female plants, with two leaves per plant. Measurements of A and E on days of full sun were carried out at 09:00 and at 13:00 in both the dry and the rainy seasons. The statistical test was ANOVA followed by Tukey's test at 0.05.

CO_2 Response Curve

The sample was three male and three female plants, with two leaves per plant. In the rainy season, on a clear day, between 09:00 and 11:00 in field conditions (see Table 2), a response of A regarding artificial reduction of CO_2 by the IRGA to the levels of 320, 300, 250, 200 and 150 ppm of CO_2 was obtained. After measurement at the lowest level, ambiental air was again offered to the leaf and after stabilisation of the reading, the new value was recorded. The sigmoidal equation of dose-response of Boltzmann was used for the non-linear adjustment of the curve. Student's T-test was used to compare the values of A before and after the experiment.

Nocturnal Gas Exchange Behaviour

The sample was three male and three female plants, with two leaves per plant. In the rainy season, the values of A and E were measured at 20:00, thus being after at least one hour of complete darkness. The light fountain PLU-002 (ADC BioScientific) at the maximum intensity was then connected to the leaf chamber for 10 minutes and new measurements were taken. To test the effect of duration of light necessary for the stomata to re-open, these leaves were then covered with aluminum foil to keep them in darkness after the sunrise. On the following sunny morning, the aluminum foil covers were removed and new measurements of A and E were taken after 3, 5 and 10 minutes of solar irradiance.

Dawn and Dusk Leaf Titratable Acidity

The sample was five male and five female plants (with two leaves per plant and per time), in the dry season. The predawn samples were carried out at 05:00 (dawn being at 05:40) and the dusk samples at 17:30 (dusk being at 18:30). The collected leaves were kept in a dry cooled bag for about 30 minutes before being processed at the laboratory. The organic acids of 1g of fresh limb were extracted in 30 mL of distilled boiling water for a period of 30 minutes. As the time of acid extraction in the literature varied from 5 minutes (NIEVOLA & al. 2005) to two hours (LIEBIG & al. 2001), previous tests were carried out to determine the time of 30 minutes of extraction. The solution was filtered, the volume completed with distilled water to 40 mL and the required volume of NaOH 0.01N to bring the pH of the solution to 7.0 was used to calculate the titratable acidity, expressed in μEq of H^+ per gram of fresh leaf mass.

Chlorophyll a Fluorescence

The fluorescence of chlorophyll a of the photosystem II was obtained with a portable fluorometer PAM-2100 (Heinz Walz GmbH, Effeltrich, Germany). The frequency of the light pulses is at 600 Hz for detection of F0 or at 20 000 Hz for the recording of light-induced kinetics. The fluorescence is excited by a very weak (< 0.1 μmol quanta $m^{-2}s^{-1}$) red pulsed measuring light (655 nm) and the saturation pulse (700 ms of about 10 000 μmol quanta $m^{-2}s^{-1}$) is provided by halogen lamps. The fiber optic probe was kept at constant distance (1 cm) and angle (60°) to the leaf. Care was taken to not shade the leaf. The maximal quantum yield of PSII photochemistry was considered as F_V/Fm and apparent electron transport rate (ETR) was estimated as being $0.84 \times 0.5 \times \text{PAR} \times (\text{Fm}'\text{-F})/\text{Fm}'$. Such nomenclature of the fluorescence parameters follows GENTY & al. 1989 and KOOTEN & SNEL 1990.

Light Response Curve/Quantum Yield

The sample was three male and three female plants, with two leaves per plant and in field conditions. The maximal quantum yield was measured at 21:00, thus, after more than two hours under night darkness. During the day, previous to the light measurements, the leaves were fully covered with aluminum foil for 40 minutes and, as the quantum yields were the same as the ones obtained at night, the various light intensities of the halogenous lamp of the fluorometer started to be given. At each new light level, the stabilisation of the basal fluorescence (Ft) was waited for, which took from 20 to 40 seconds, then a saturating pulse was given for the estimation of quantum yield and electron transport rate (ETR). The sigmoidal equation of dose-response of Boltzmann was used for the non-linear adjustment of the curve.

Comparison of ETR Between Male/Female Plants, Dry/Rainy Season and Morning/Early Afternoon

The sample was ten male and ten female plants (two leaves per plant) in field conditions. The leaf clip of the fluorometer was kept in order to maximize the solar irradiation, according to BILGER & al. 1995. The statistical test was ANOVA followed by Tukey's test at 0.05.

Measurement of Chlorophyll a, Chlorophyll b and Carotenoid Contents

The sample was five female and five male plants (two leaves per plant). From the medium third of the limb, a rectangle of 0.5 cm^2 was cut and immediately immersed in 2 mL of dimethylformamide in a dark cooled box and then kept in darkness at 4 °C for 48 hours (MORAN 1982, INSKEEP & BLOOM 1985, GARCÍA & NICOLÁS 1998). The chlorophyll and carotenoid contents were read and calculated according to the equations of WELLBURN 1994 for extraction in dimethylformamide and read on a spectrophotometer of 0.1 nm of precision and 2 mm of spectral slit width (UV-3600, Shimadzu of Brazil).

Diurnal Variation of Leaf Water Potential

The sample was three male and three female plants, with one leaf per plant, the most apical one. The measurements were taken in a pressure bomb of SCHOLANDER & al. 1964, model 1000 from PMS Instruments. The measurements were taken in the dry and the rainy seasons at 06:00, 13:00 and 17:00. The leaf water content (percentage of water mass regarding total leaf mass was estimated in five male and five female plants (two leaves per plant), at 06:00 and at 17:00, in the dry and rainy seasons, by lyophilization of 1g of fresh limb.

Results

Gas Exchanges/Titratable Acidity

In LCA_4, the stomatal conductance is indirectly calculated and based on mathematical models for hypostomatous leaves. As some values presented as negative, these data could not be taken into consideration.

The table 1 shows the comparison of A and E of male and female plants, in the dry and the rainy seasons and in the morning and the beginning of the afternoon. There were no statistically significant differences regarding any of these parameters. At 13:00, some plants presented lower values of A, which lowered the media, however, these individuals were not enough to influence the statistical test used.

Table 1. Net assimilation rate of CO_2 (A, μmol m^{-2} s^{-1}, mean ± standard deviation), transpiration rate (E, mmol m^{-2} s^{-1}, mean ± standard deviation) and water use efficiency (WUE, μmol of assimilated CO_2 /mmol transpirated H_2O) in male (n =10) and female (n = 10) plants of *Smilax goyazana* in the dry and the rainy seasons, at 09:00 and at 13:00 in days of plentiful sunlight.

	Dry season		Rainy season	
	09:00	13:00	09:00	13:00
Female Plants				
A:	8.98 ± 1.90	7.07 ± 1.34	8.87 ± 1.71	7.22 ± 1.62
E:	3.08 ± 0.71	3.79 ± 0.97	3.02 ± 1.15	2.50 ± 0.58
WUE:	2.92 ± 1.31	1.86 ± 1.16	2.94 ± 1.43	2.89 ± 1.10
Male Plants				
A:	9.64 ± 1.96	7.74 ± 1.60	8.31 ± 1.67	7.24 ± 2.00
E:	3.89 ± 1.73	4.03 ± 1.73	3.29 ± 0.73	3.09 ± 1.39
WUE:	2.48 ± 1.85	1.92 ± 1.67	2.53 ± 1.20	2.34 ± 1.70

There were no statistically significant differences among male or female plants or at dry and rainy seasons or at 09:00 and 13:00

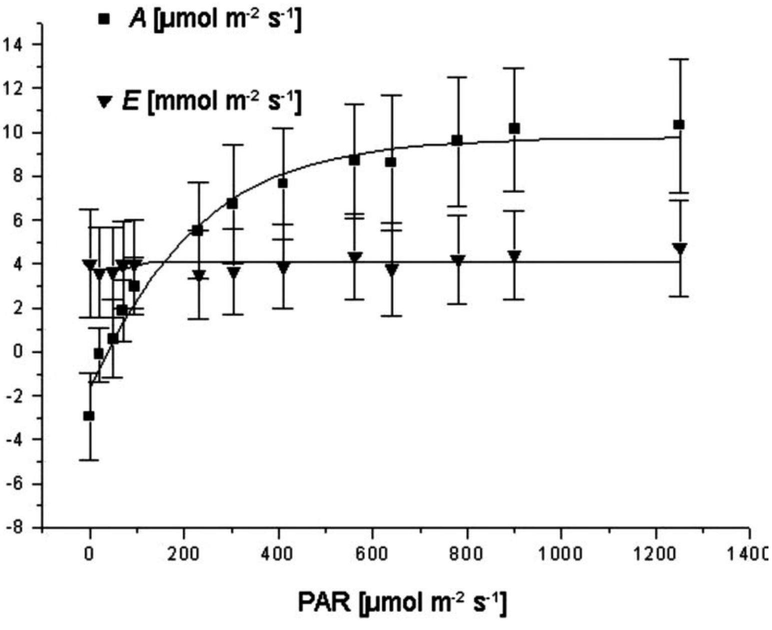

Fig. 1. Light-response curve of A (net carbon assimilation rate, μmol m^{-2} s^{-1}) and E (transpiration rate, mmol m^{-2} s^{-1}) of leaves (n = 12) of *Smilax goyazana* in field conditions during the rainy season under different values of photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}). Non-linear adjustment by the sigmoidal dose-response Boltzmann's equation.

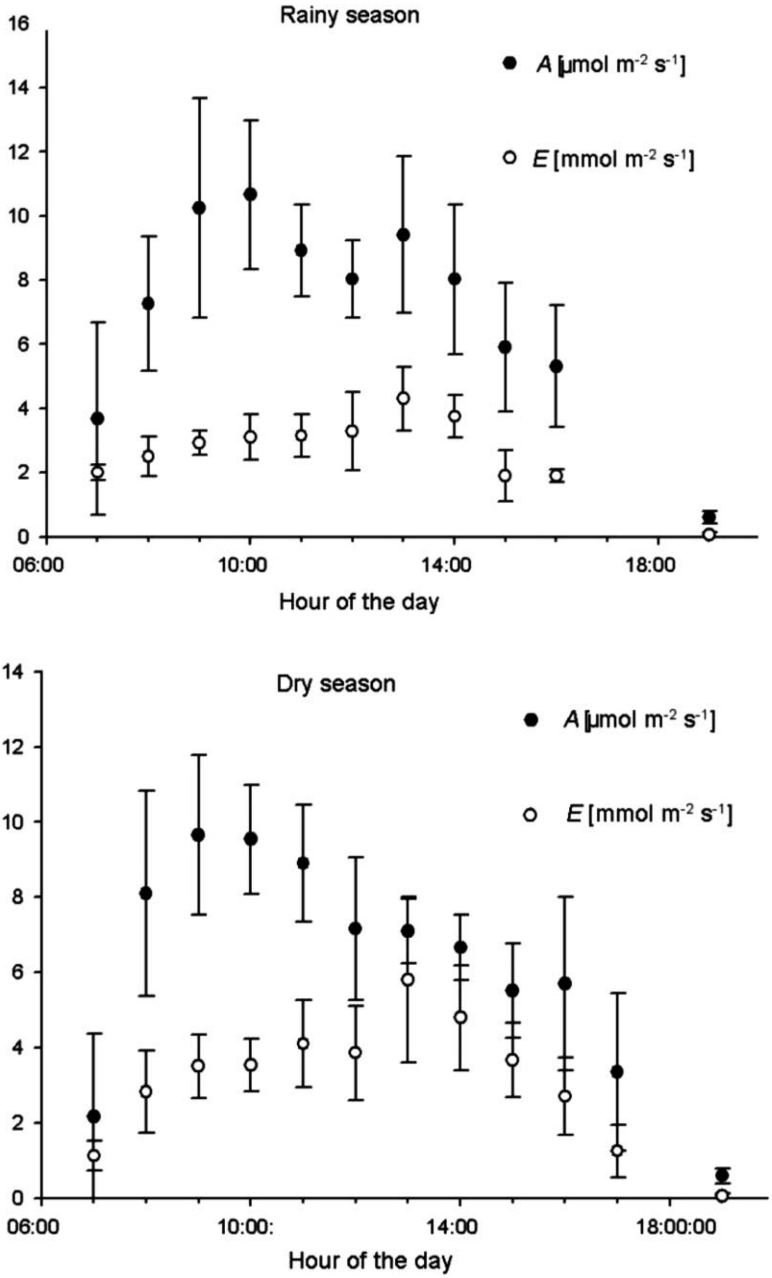

Fig. 2. Daily course of A (net carbon assimilation rate, µmol m^{-2} s^{-1}) and E (transpiration rate, mmol m^{-2} s^{-1}) of leaves (n=8) of *Smilax goyazana* in the dry and the rainy seasons in sunny days.

The figure 1 shows the light response curve of A and E. The data of male and female plants and of the ascendant and descendant measurements were grouped, since no differences between sex and direction of the curve were observed. The values of E stood around 4.0 mmol $H_2O.m^{-2}.s^{-1}$. The dark respiration was about 4.0 µmol $CO^2.m^{-2}.s^{-1}$. The light compensation point stood around 60 µmol quanta.$m^{-2}.s^{-1}$. Until a PAR of 300 µmol quanta.$m^{-2}.s^{-1}$ the values of A increased quite linearly, with a maximum photosynthetic efficiency of 0.03 µmol CO_2/µmol PAR. At about 500 µmol quanta.$m^{-2}.s^{-1}$, 95% of the maximum value of A was reached. The maximum value of A was obtained with light intensities of 800 to 1250 µmol quanta.$m^{-2}.s^{-1}$ and was about 10.5 (amplitude of 5.5 to 12.3) µmol $CO_2.m^{-2}.s^{-1}$ or, considering the leaf specific area of 5.5 m^2/kg (PALHARES & al. 2009a), varied from 30.2 to 67.6 µmol $CO_2.kg^{-1}.s^{-1}$.

Regarding to the daily course of A and E (Fig. 2), the table 2 shows the ambient conditions of temperature, CO_2, air water potential and PAR inside the leaf chamber (85% of the ambiental value). Except for the air water potential, the ambient conditions were not different between the dry and rainy seasons, so the data were grouped together. The atmospheric CO_2 varied throughout the day, being higher at night and at dawn, and lower at the beginning of the afternoon, with an amplitude of 40 ppm CO_2. The leaf chamber temperature was never overheated. One hour after dawn PAR stood over the light saturating point throughout the day, decreasing after 15:00. The decrease of light intensity was mostly caused due to shadowing by the surrounding vegetation, so that some plants received enough light until the end of the afternoon. After 18:00, all the plants were receiving light at an amount less than the light compensation point (data not shown). Once receiving enough light, the plants presented stable values of A and E throughout the day in the dry and rainy seasons. As they were shadowed, the values of A decreased one hour earlier than the values of E, indicating a slow stomata closure.

The reduction of atmospheric CO_2 in the leaf chamber provoked a reduction of A (Fig. 3), under a temperature that varied from 25.7 to 33.5 °C). The external CO_2 concentration of 250 ppm corresponded to the CO_2 compensation point. However, about 30% of the leaves presented a negative value of A at the first value of CO_2 reduction (320 ppm). In the same plant, could there be leaves that presented a negative A at 320 ppm CO2 and leaves that reduced A according to the reduction of CO_2. None of the leaves recovered the values of A when re-exposed to the normal levels of atmospheric CO_2.

At night, the values of A and E stood close to zero even after 10 minutes under artificial light. During the day, after three minutes of solar exposure, the covered leaves presented values of A (7.87 ± 1.53 µmol.$m^{-2}.s^{-1}$) and E (3.32 ± 0.93 mmol.$m^{-2}.s^{-1}$) similar to the other leaves of the same plants.

Table 2. Ambiental conditions of the daily course of photosynthetic responses of male and female plants of *Smilax goyazana*, in field conditions in sunny days, during the dry and rainy seasons. Tch: temperature of leaf chamber (amplitude of 1.5 °C related to the media). PAR: photosynthetically active radiation inside the leaf chamber (85% of the environmental value, in mean (min-max). C_{ref}: atmospheric CO_2 concentration (amplitude of 5 ppm related to the media). E_{ref}: partial water vapour pressure of the environment, in mean (min-max).

Hour	Tch °C	PAR $\mu mol.m^{-2}.s^{-1}$	C_{ref} ppm	E_{ref} (rainy season) Pa	E_{ref} (dry season) Pa
07:00	17.6	654 (380–840)	404.7	1.10 (1.08–1.12)	1.25 (1.17–1.49)
08:00	20.0	1215 (1121–1296)	397.5	1.41 (1.31–1.55)	1.25 (1.18–1.25)
09:00	23.0	1264 (954–1659)	395.8	1.63 (1.48–1.74)	1.27 (1.22–1.30)
10:00	25.6	1380 (1181–1461)	390.0	1.77 (1.76–1.78)	1.44 (1.41–1.55)
11:00	28.0	1614 (1205–1852)	385.6	1.65 (1.29–1.77)	1.50 (1.46–1.58)
12:00	30.7	1575 (1448–1763)	380.4	1.60 (1.55–1.63)	1.52 (1.37–1.70)
13:00	31.2	1481 (1419–1813)	375.7	1.56 (1.52–1.61)	1.44 (1.35–1.77)
14:00	31.3	1423 (983–1773)	374.9	1.46 (1.45–1.48)	1.36 (1.13–1.51)
15:00	28.7	1223 (185–1518)	390.3	1.45 (1.40–1.51)	1.26 (1.20–1.34)
16:00	28.9	834 (91–1408)	392.7	1.50 (1.49–1.52)	1.17 (1.09–1.24)
17:00	27.8	582 (31–1105)	393.6	1.49 (1.47–1.51)	1.08 (1.03–1.16)
20:00	22.0	0	414.3	1.42 (1.28–1.54)	Not measured

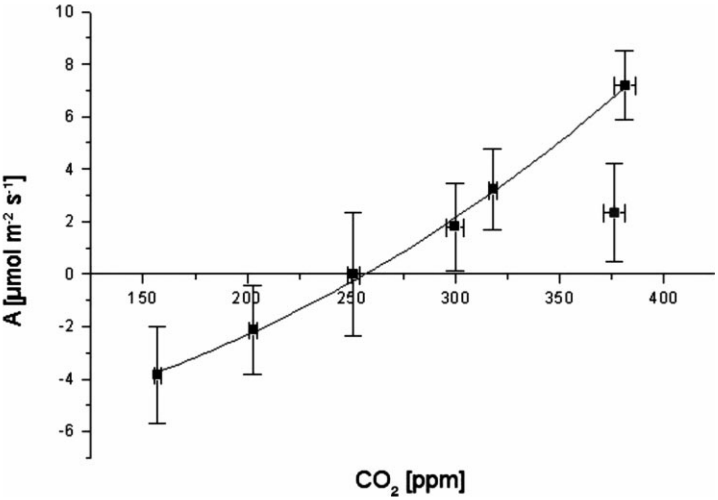

Fig. 3. Curve of net carbon assimilation rate (A, $\mu mol\ m^{-2}\ s^{-1}$) related to the external concentration of CO_2 in leaves (n = 12) of *Smilax goyazana* under field conditions, in the rainy season and in a day of plentiful sunlight. Non-linear adjustment of the curve by the sigmoidal dose-response equation of Boltzmann. The isolated point represents the value obtained after re-exposure to the ambiental CO_2. The leaf chamber temperature varied from 26.7 to 33.5 °C.

The titratable acidity did not significantly vary between dawn and dusk: at dawn, the obtained value was 43.9 ± 1.38 μEq $H^+.g^{-1}$ fresh leaf mass; at dusk, 37.6 ± 1.22 μEq H^+. g^{-1} fresh leaf mass.

Chlorophyll Fluorescence/Chlorophyll and Carotenoid Contents

The female plants tended to a higher ETR than the male ones (table 3), but the values did not reach the significance at 0.05 ($0.05 < P < 0.10$). Regarding the annual season and the time of the day, no statistically significant difference occurred ($P > 0.5$).

The maximal quantum yield was 0.787 ± 0.014. 90% of the maximum value of ETR was obtained at approximately 1000 μmol quanta.$m^{-2}.s^{-1}$ (figure 4) and even under artificial intensities higher than 2500 μmol quanta.$m^{-2}.s^{-1}$ the ETR values stood above 95% of the maximum value for both male and female plants, so the data were grouped together.

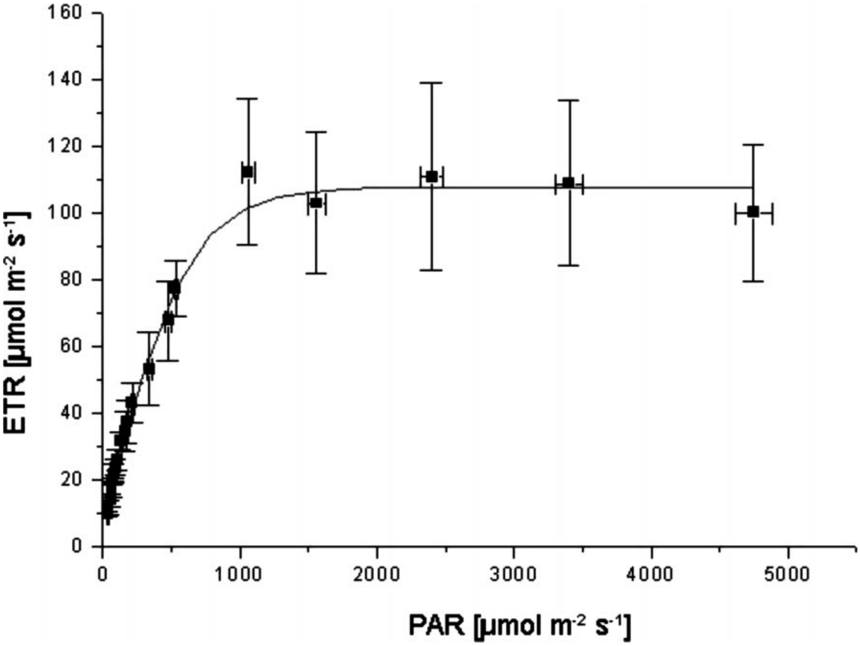

Fig. 4. Light response curve of apparent electron transport rate (ETR, μmol m^{-2} s^{-1}) through the photosystem II of leaves (n = 12) of *Smilax goyazana* under different photosynthetically active radiations (PAR, μmol m^{-2} s^{-1}) provided by the halogen lamps of PAM-2100 fluorometer during the rainy season, after non-linear adjustment by the sigmoidal dose-response Boltzmann's equation.

Table 3. Photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) over the leaves of *Smilax goyazana* (mean ± standard deviation) and the apparent electron transport rate (ETR, μmol m^{-2} s^{-1}) in the photosystems of male (n = 10) and female (n = 10) plants, in the dry and rainy seasons and in the morning and early afternoon.

		Dry season		Rainy season	
		09:00	13:00	09:00	13:00
Female	PAR*:	1395 ± 236	1355 ± 245	1396 ± 135	1376 ± 107
	ETR**:	104.7 ± 19.4	102.6 ± 32.0	122.1 ± 39.5	114.4 ± 33.9
Male	PAR*:	1394 ± 235	1309 ± 82	1356 ± 127	1388 ± 120
	ETR**:	74.8 ± 28.5	81.0 ± 35.2	94.5 ± 37.0	119.4 ± 32.5

* There were no statistically significant differences in the PAR measurements.

** There were no statistically significant differences in the ETR related to the season or the hour of the day (P ›50). Between male and female plants, $0.5 < P < 0.10$

Regarding chlorophyll and carotenoid contents, there were no differences between male and female plants. The chlorophyll a (59.29 ± 20.40 μg.cm^{-2}) presented a 3-fold concentration of the chlorophyll b (15.92 ± 5.88 μg.cm^{-2}). The carotenoid content reached an intermediary value (22.94 ± 8.25 μg.cm^{-2}) between the chlorophylls.

Diurnal Variation of Leaf Water Potential

The table 4 shows the water leaf potential and the water leaf content in the dry and the rainy seasons. There were no differences between male and female plants, so the data were pooled. The leaf water potential was isohydric and although there were differences in the medium values through the day and between dry and rainy seasons, there were no statistically significant differences (P=0.1) at Student's T-test. The leaf water content stood stable, at around 63% all the time.

Table 4. Leaf water potential (MPa, mean ± standard deviation) and leaf water content (%) in male and female plants of *Smilax goyazana* at the dry and the rainy seasons.

Hour	Leaf water potential (MPa)		Leaf water content (%)	
	Rainy season	Dry season	Rainy season	Dry season
06:00	– 0.32 ± 0.08	– 0.41 ± 0.12	65 ± 3.5	62 ± 3.3
13:00	– 1.10 ± 0.10	– 0.82 ± 0.22	–	–
17:00	– 0.48 ± 0.04	– 0.76 ± 0.25	66 ± 1.8	60 ± 3.5

Differences not statistically significant between dry and rainy seasons (P = 0.10)

Discussion

Regarding other Smilax Species

Although the genus *Smilax* occurs with a reasonable phytosociological dominance in tropical and temperate areas of all the continents (Chen & al. 2006), studies on the photosynthetic responses were carried out only in a few species, such as *S. australis*, occurrent in Australia (Adams & al. 1998, Adams & al. 1999), *S. rotundifolia*, from North America (Carter & Teramura 1998, Cobb & al. 2007) and *S. aspera*, from the Mediterranean region and species-type of the genus (Lillis & Fontanella 1992, Sack & al. 2003, Alessio & al. 2004). In general, these plants assimilate carbon under lower light intensities and at the same time present biochemical adaptations for dissipating the excess of energy of higher solar irradiances. Among the studied species in the literature cited, *S. australis* is the only species that presents photoinhibition, with a maximal quantum yield of 0.68 (Adams & al. 1998, Adams & al. 1999).

In the Cerrado, Labouriau &al. 1964 studied several plants regarding nocturnal transpiration and showed that *S. cissoides* keep their stomata closed at night, with a negligible transpiration. Considering the recent taxonomic review of the genus (Andreata 1997), it is most likely that the studied species was in fact *S. goyazana*.

The foliar anatomical structure of *S. goyazana* is typical of a well hydrated leaf (Palhares & al. 2009a, Palhares & Zaidan 2010). The species of *Smilax* present large xylematic vessels, with more than 20 μm in diameter, and thus, susceptible to embolism. However, the roots of some species are able to produce a positive water pressure of 0.1 MPa in the apex of the aerial part (Cobb & al. 2007). Although positive pressure has not been observed in the present study, the leaf water potential did not significantly vary along the course of the day or during the annual seasons. Thus, the stability of the rates of carbon assimilation, transpiration and electron transport is related to the stability of the water potential, promoted, probably, by a very efficient radicular system, since water reservoir structures were not found (Palhares & Silveira 2005).

Regarding Male and Female Differences

Being a dioecious genus, studies comparing the photosynthetic responses of male and female plants of the genus *Smilax* were not found. Although the female plants of *S. goyazana* bears fruits throughout the rainy period, with gradual maturation and dispersion (Munhoz & Felfili 2005, 2007), the carbon assimilation rates does not differ from the male plants.

Although not reaching 5% of probability, the electron transport rate tended to be higher in female plants, but the chlorophyll and carotenoid measurements showed no differences related to the sex of the plant. This also happens to the dioecious species *Clusia hilariana*, typical of sandy coastal dunes (LIEBIG & al. 2001). Such physiological aspect remains to be better understood.

Regarding the Cerrado

NOGUEIRA & al. 2004 studied photosynthetic profiles related to the successional stage of reforestation in an area placed at the transition of the Cerrado domain and the Atlantic rain forest domain: in general, the secondary and climax species present lower net carbon assimilation rates and lower water-use efficiency. So, although *S. goyazana* is a dominant non-grass species of the herbaceous layer of the Cerrado, the photosynthetic responses indicate it is a secondary/climax plant. Notwithstanding, their seeds germinate only under strict conditions that are probably present only after the establishment of a mature ecosystem (PALHARES & al. 2009b), thus corroborating the idea of a climax species.

The values of gas exchange at night was close to zero while in other Cerrado species there is a respiration rate of about –1.0 to –2.0 μmol $CO_2.m^{-2}.s^{-1}$ (SCHOLZ & al. 2006). However, the hypothesis of an eventual weak CAM metabolism to recycle the nocturnal carbon (GURALNICK & JACKSON 2001, SAGE 2002) was refused, as the titratable acidity showed no CAM cycle is present.

The chlorophyll and carotenoid contents were similar to other Cerrado plants, and the ratio chlorophyll a/b, around 3.0, is typical of leaves adapted to plentiful sunlight (CARVALHO & al. 2007). Also, even though the light saturation point was only 1/5 of the maximum solar irradiance available in the environment, *S. goyazana* tolerates the high irradiances without showing photoinhibition, as it presented a maximum quantum yield of the typical value for most plant species (KRAUSE & WEIS 1991, GENTY & al. 1990).

Regarding CO_2 Supression

The levels of atmospheric CO_2 has constantly enhanced during the last decades. 20 years ago, the concentration of atmospheric CO_2 was around 320 ppm (PRADO & al. 2004) and, 10 years ago, around 360 ppm (MIRANDA & al. 1997). Experimentally, the photosynthetic responses differ between leaves that are suddenly exposed to higher levels of CO_2 and leaves that are grown under the same higher levels (MATTA & al. 2001, AIDAR & al. 2002). In the present study, the simple reduction of the external carbon to the levels of some years ago (320 ppm) provoked in some leaves the loss of carbon instead of assimilation, and the biochemical mechanisms related to this remains to be investigated.

In this way, the experiment of reducing atmospheric carbon, where none of the leaves recovered the net CO_2 assimilation rate, contrary to what is expected in laboratory conditions (LONG & BERNACCHI 2003), leads to the inference that the main mechanism for draining the light excess in *S. goyazana* is the photorespiration, as commonly found in other Cerrado species (FRANCO & al. 2007).

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