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Photosynthetic characteristics of ornamental passion flowers grown under different light intensities

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Abstract

Responses of leaf gas exchange, fluorescence emission, chlorophyll concentration, and morpho-anatomical features to changes in photosynthetic photon flux density (PPFD) were studied in three wild ornamental species of *Passiflora* L. to select sun and shade species for landscaping projects. Artificial shade was obtained with different shading nylon nets, under field conditions, which allowed the reduction of 25, 50, and 75% of global radiation, along with a control treatment under full sunlight. For *Passiflora morifolia* the highest mean values of light-saturated net photosynthetic rate (P_{Nmax}) and light compensation point (LCP) were observed at 50 and 25% shade, respectively, while the highest values of dark respiration rate (R_D) and apparent quantum yield (α) were observed at 75% shade. For *Passiflora suberosa litoralis* the highest value of $P_{\max}$ was observed at full sunlight. The highest mean values for $P_{\max}$, R_D, and LCP for *Passiflora palmeri* var. *sublanceolata* were obtained at 25% shade. The highest values of net photosynthetic rate (P_N) for *P. morifolia*, *P. palmeri* var. *sublanceolata*, and *P. suberosa litoralis* were 21.09, 16.15, and 12.36 μmol(CO_2) m^{-2} s^{-1}, observed at 50 and 75% shade and full sunlight, respectively. The values of the minimal chlorophyll fluorescence (F_0) were significantly different in *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata*, increasing with the increase of the irradiance. In contrast, the values of maximum photochemical efficiency of PSII (F_v/F_m) were significantly different only in *P. suberosa litoralis*, being higher at 75%, progressively reducing with the increase of PPFD levels. The total concentration of chlorophyll (Chl) was higher in shaded plants than in the ones cultivated in full sunlight. On the other hand, the values of Chl *a/b* ratio were reduced in shaded plants. A significant effect of shade levels on leaf area (LA) and specific leaf area (SLA) was found for the three species, whose highest mean values were observed at 75% shade. The thickness of foliar tissues was significantly higher for the three species at full sunlight and 25% shade. These results suggested that *P. morifolia* and *P. palmeri* var. *sublanceolata* appeared to be adapted to moderate shade conditions. *P. suberosa litoralis* presented higher plasticity to greater variation of the irradiance levels, while the photoinhibition was one of the limiting factors for this species at full sunlight.

Additional key words: gas exchange; ornamental; Passifloraceae; photoinhibition; shade.

Introduction

Light is one of the main factors of physical environment which control growth and development of plants through photosynthesis, especially concerning CO_2 assimilation. In any habitat, PPFD varies in time and place, thus inducing plants to develop acclimation to the variation of light intensities (Zhang *et al.* 2003). Low light levels may lead to stress in plants, caused by the reduction of CO_2 assimilation, by decreases in carbohydrate production, and reduction of growth and development; whereas high light levels can damage the photosynthetic apparatus

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Abbreviations: Chl – chlorophyll; C_i/C_a – intercellular to atmospheric CO_2 concentration ratio; E – leaf transpiration rate; F_0 – minimal fluorescence of dark-adapted state; F_m and F_v – maximal and variable fluorescences of dark-adapted state, respectively; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance to water vapour; LA – leaf area; LCP – light compensation point; LDM – leaf dry mass; $P_{\max}$ – light-saturated rate of gross photosynthesis; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; R_D – dark respiration rate; SLA – specific leaf area; α – apparent quantum yield.

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in ways which are sometimes irreversible (Barth *et al.* 2001).

Many morpho-physiological characteristics are related to acclimatization to high irradiances, such as: (*1*) changes in respiration and photosynthetic rates (Zhang *et al.* 2003); (*2*) increase in the concentration and activity of Rubisco (Ramalho *et al.* 1999); (*3*) fast recovery from photoinhibition (DaMatta and Maestri 1997); (*4*) reduction in LA and SLA in order to protect itself from high irradiance (Matos *et al.* 2009); (*5*) increase in leaf blade thickness, due to the quantity of layers or growth of palisade tissue (Givnish *et al.* 2004); (*6*) deep development of spongy layer (Morais *et al.* 2004); and (*7*) presence of thicker cuticle, with higher quantity of epicuticular wax (Akunda *et al.* 1979).

The cultivation of ornamental *Passiflora* L. species in Brazil is almost nonexistent, but nowadays it has stood out in European and North American countries, in the market of hybrid seeds or seedlings (Abreu *et al.* 2009). In these countries, the passion flower is cultivated in gardens, on walls, fences, pergolas, greenhouses, and conservatories (Vanderplank 2000). The herbaceous or woody habit of passion flowers fascinates people mainly because of the great beauty of the flowers and the wide variety of leaf shapes, whereas some species are of ornamental value because of their foliage (Vanderplank 2000, Ulmer and MacDougal 2004). The use of protected environment in the production of ornamental plants has intensified in Brazil (Guiselini *et al.* 2004). However, studies about the morpho-physiological behavior of ornamental *Passiflora* species concerning the quantity of light absorbed are rare, which makes it more difficult to understand, more specifically, the way which the plants grow and develop. Therefore, photosynthetic studies in these species will contribute to the understanding of mechanisms of acclimation of ornamental plants to light and provide information about the optimum levels of irradiance for growing these plants.

P. morifolia Mast occurs widely in Guatemala, Mexico, Venezuela, Bolivia, Colombia, Brazil, Ecuador, Peru, Paraguay, and Argentina. This species has beautiful white flowers with a purplish corona, which gives it, along with its intermediate plant size, favourable characteristics for growing in pots for indoor decoration (Vanderplank 2000, Milward-de-Azevedo and Baumgratz 2004). *P. suberosa litoralis* (Kunth) K. Porter-Utley occurs in almost all of Central and South America, Hawaii, New Guinea, Fiji and Samoa (Vanderplank 2000). Lobed leaves of *P. suberosa litoralis* are examples of the wide variety of shapes, sizes, and shades of green leaves in the genus. *P. palmeri* var. *sublanceolata* Killip is an ornamental tropical species; it is an herbaceous climber of medium size with beautiful flowers with pink and white petals and a purple corona. It is already being used as a parent in interspecific crosses to obtain hybrids for ornamental plants in the USA and Europe, as in the hybrids *P.* 'Aurora' and *P.* 'Pink Jewel' (Ulmer and Macdougal 2004).

Therefore, we hypothesized that: (*1*) the physiological features of *P. morifolia*, *P. suberosa litoralis*, and *P. palmeri* var. *sublanceolata*, three wild species of *Passiflora* with ornamental potential, are sensitive to changes in light environment; and (*2*) the structural changes in leaves are related to photosynthetic adaptations to different levels of PPFD. This study aimed to evaluate the effects of four levels of irradiance on gas exchange, fluorescence emission, chlorophyll concentration, and morpho-anatomical features in plant leaves of *P. morifolia*, *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata* to select sun- and shade species for landscaping projects, and provide support for introducing the cultivation of *Passiflora* in the tropical ornamental plants market in Brazil.

Materials and methods

Plant material and experimental conditions: The experiment was conducted at the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil, (14°45'S, 39°13'W). The three species under study have been cultivated at UESC's Active Germplasm Bank. The seeds were germinated in polystyrene trays of 128 cells, with washed sand and matured manure as substrate (1:1). Three weeks after the emergence, the plants were transplanted to plastic pots with capacity of 10 L, containing soil, organic matter, and washed sand as substrate (3:1:1), having wire frame supports, and were exposed to artificial shade levels. Irrigation was provided daily by a microsprinkler system. Artificial shade was obtained through the use of different shading nylon nets, fixed in wooden frames with dimensions of 5 × 5 × 2 m, under field conditions, which allowed the reduction of 25% (1,000–1,400 μmol m^{-2} s^{-1}, min – max), 50% (600–900 μmol m^{-2} s^{-1}), and 75% (200–400 μmol m^{-2} s^{-1}) of global radiation, along with a control treatment under full sunlight (1,500–1,900 μmol m^{-2} s^{-1}). The PPFD, temperature, and relative air humidity values (Fig. 1), measured at the upper extremity of plants between 08:00 and 18:00 h, were obtained through the use of one *LI-250* light meter with a linear quantum sensor *LI-191SA* (*LI-COR Biosciences Inc.*, Lincoln, NE, USA) per treatment, simultaneously. These evaluations were performed on five different days in an interval of seven months corresponding to the experimental time.

Gas exchange and PPFD-response curves: The leaf gas exchange was evaluated seven months after emergence, from 08:00 to 12:00 h, on five plants per species (a fully expanded and not self-shaded leaf per plant), using a portable photosynthesis system *LI-6400* (*LI-COR*

Biosciences Inc., Lincoln, NE, USA) equipped with an artificial irradiance source *6400-02B RedBlue*. Light-response curves were created with ten levels of PPFD [0, 10, 25, 50, 100, 200, 400, 600, 800, and 1,000 µmol(photon) m^{-2} s^{-1}] in the decreasing order, with 1–2 min intervals between each reading (CV < 0.8%). The CO_2 flux was adjusted to maintain a concentration of 380 µmol mol^{-1} inside the chamber and the leaf chamber temperature was maintained at 26°C.

The net photosynthetic rate (P_N), leaf transpiration rate (E), stomatal conductance to water vapour (g_s), and intercellular to atmospheric CO_2 concentration ratio (C_i/C_a) were calculated using the values of CO_2 and humidity variation inside the chamber (~50%), both measured by the infrared gas analyzer of the portable photosynthesis system when PPFD ≥ 600 µmol(photon) m^{-2} s^{-1} (saturating, but not inhibitory irradiance observed for the three species studied, in the four levels of shade). Nonlinear regression for exponential equations was used to estimate the photosynthetic parameters. The following equation was used for construction of P_N *vs.* PPFD curves (Iqbal *et al.* 1997): $P_N = P_{max}\ [1 - \exp(-\alpha\ \mathrm{PPFD}/\ P_{max})] - R_D$, where P_{max} is the light-saturated rate of gross photosynthesis, α is the apparent quantum yield, and R_D is the dark respiration rate, corresponding to the value of P_N when PPFD = 0 µmol(photon) m^{-2} s^{-1}. Based on the adjusted values, the light-saturated net photosynthetic rate (P_{Nmax}; $P_{max} = P_{Nmax} + R_D$), and the light compensation point were calculated (LCP).

Chl fluorescence emission was measured simultaneously on the same leaves (n = 5) used for the gas-exchange measurements, with a leaf chamber fluorometer *LI 6400-40*, a LED-based fluorescence accessory for the portable photosynthesis system *LI-6400* (*LI-COR Bioscience Inc.*, Lincoln, NE, USA). To assess the emission of Chl fluorescence in dark-adapted leaves, the leaf tissue was placed in standard *Hansatech* leaf clips for 30 min for solar radiation reflection, decrease leaf temperature and oxidation of the whole photosynthetic electron transport system. Following dark-adaptation, the leaf tissue was illuminated with a weak-modulated measuring beam (0.25 kHz, < 0.1 µmol m^{-2} s^{-1}, 630 nm, 1 s) to obtain the minimal fluorescence (F_0). A saturating white-light pulse (20 kHz; 6,000 µmol m^{-2} s^{-1}, 630 nm, 1 s) was applied to ensure maximum fluorescence emission (F_m). The maximum photochemical efficiency of PSII (F_v/F_m) was calculated by the equipment as [$F_v/F_m = (F_m - F_0)/F_m$)] (Roháček 2002, Baker 2008).

Chl concentration was determined in acetone extracts of leaf discs (collected from the same leaves used for the gas-exchange measurements) following Arnon (1949), with some modifications. The extraction of chloroplast pigments was carried out after the incubation of five leaf discs (0.5 cm^2) with 10 mL of 80% acetone at 4°C in the dark overnight, followed by maceration until it was completely extracted. The absorbance of extracts was read in a microplate spectrophotometer (*VersaMax, Molecular Devices Inc.*, Sunnyvale, CA, USA) at 645 and 663 nm and the chlorophyll contents were calculated using the equations proposed by Arnon (1949).

Morpho-anatomical features: Five plants per treatment were harvested to determine the LA and SLA after seven months of exposure to shade treatments. Leaf dry mass (LDM) was obtained after drying at 70°C until a constant mass was reached. LA was estimated using an automatic *Leaf Area Meter LI-3100* (*LI-COR Bioscience Inc.*, Lincoln, NE, USA). SLA was estimated as the quotient

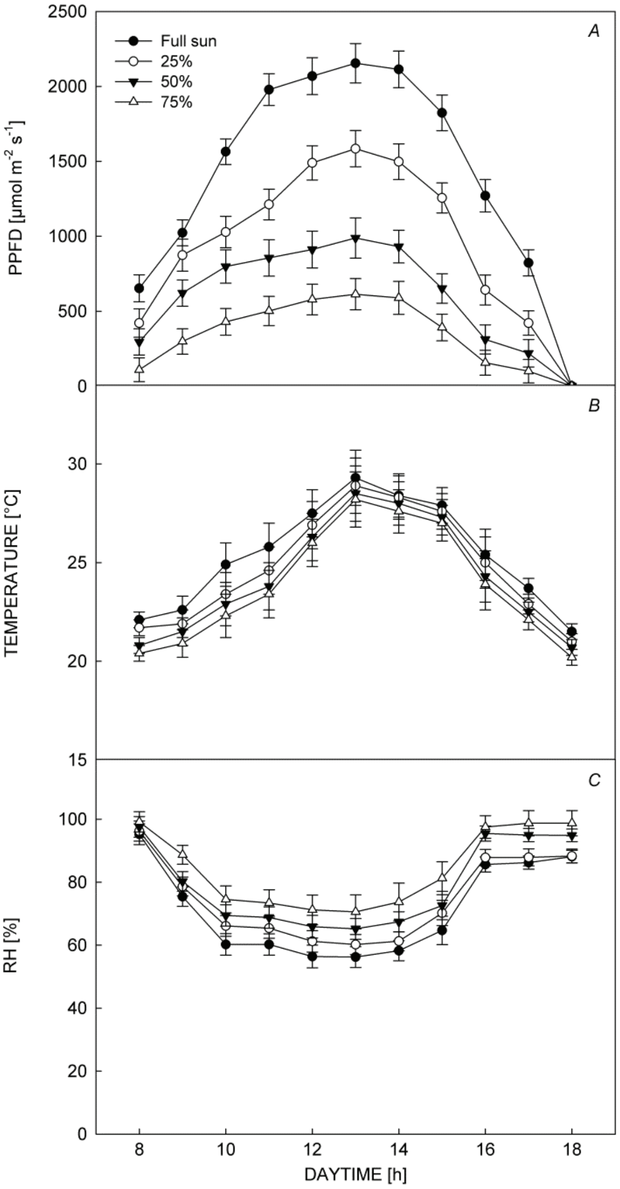

Fig. 1. Mean values of the daily changes in the (*A*) photosynthetic photon flux density (PPFD), temperature (*B*), and relative air humidity (*C*) in the full sunlight and shaded environments. *Vertical bars* denote the SE (n = 5).

between individual mean values of LA and LDM.

For light microscopy analysis, five mature leaves of five plants of each species of *Passiflora* at each light level were collected after seven months of exposure. Small pieces (2 mm^2) of leaves were fixed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). Leaf pieces were dehydrated in graded ethanol series and embedded in historesin (*Leica*, Heidelberg, Germany). Sections for light microscopy were stained with 1% toluidine blue and photographed with an *Olympus BX50* light microscope (Center Valley, PA, USA). For each species and light level, the thicknesses of leaf blade, upper and lower epidermis, cuticle, palisade and spongy parenchyma, and total mesophyll were measured from the light micrographs using the *Sigma Scan* software (*SPSS Inc*., San Jose, CA, USA).

Statistical analysis: The experiment was conducted in a completely randomized design, using a 4 × 3 factorial arrangement which corresponded to four levels of light availability (full sunlight and 25, 50, and 75% shade) and three *Passiflora* species, with five replicates, and one plant per experimental unit. The results were submitted to a variance analysis (*ANOVA*), followed by *Tukey*'s mean comparison test (p<0.05). All statistical procedures were performed using the *Statistica 6.0* software (*Statsoft Inc*., College Station, TX, USA).

Results

PPFD-response curves and leaf gas exchange: No significant differences were observed for the parameters derived from the response curves to PPFD among the different light levels for *P. morifolia*. For *P. suberosa litoralis*, a significant difference (p<0.05) was verified among the P_{Nmax} values in different light levels, whose highest value was observed at full sunlight. For *P. palmeri* var. *sublanceolata*, the artificial shade influenced significantly (p<0.05) the parameters of response curves to PPFD, except for α. For this species the highest mean values of P_{Nmax}, R_{D}, and LCP were obtained at 25% shade (Table 1).

The highest values of P_{N} for *P. morifolia*, *P. suberosa litoralis*, and *P. palmeri* var. *sublanceolata*, were 21.09, 12.36, and 16.15 μmol(CO$_2$) m^{-2} s^{-1}, observed at 50% shade, full sunlight and 75% shade, respectively. There was increase of g_{s}, together with the increase of P_{N}, reaching the highest values at full sunlight for *P. suberosa litoralis* and at 50% shade for *P. morifolia* and *P. palmeri* var. *sublanceolata*. The P_{N} increase phase also agreed with the increase of $C_{\mathrm{i}}/C_{\mathrm{a}}$ and E (Table 2).

Chl fluorescence and concentration: The values of F$_0$ were significantly different (p<0.05) for *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata*, and increased with PPFD (Fig. 2*A*). The F$_{\mathrm{v}}$/F$_{\mathrm{m}}$ values were significantly different between the shade levels only in *P. suberosa litoralis*, being higher at 75% shade, reducing progressively with the increase of PPFD levels (Fig. 2*B*). The total Chl concentration was higher in more shaded plants than in those cultivated at full sunlight, with statistically different means (p<0.05) for the three studied species

Table 1. Light-saturated net photosynthetic rate (P_{Nmax}) [μmol(CO$_2$) m^{-2} s^{-1}], dark respiration rate (R_{D}) [μmol m^{-2} s^{-1}], light compensation point (LCP) [μmol m^{-2} s^{-1}], and apparent quantum yield (α) [mol mol^{-1}] estimated for *P. morifolia*, *P. suberosa litoralis*, and *P. palmeri* var. *sublanceolata* under different shade conditions, to seven months after emergence. These are means ± SE values from 5 replicates. Means comparison were done using *Tukey*'s test (p<0.05). For each variable *lowercase letters* indicate comparison among treatments and *uppercase* ones comparison among species. CV – variation coefficient.

Species	Shading	P_{Nmax}	R_{D}	LCP	α
P. morifolia	Full sun	22.40 ± 3.7^{Aa}	1.78 ± 0.4^{Aa}	35.9 ± 6.4^{Aa}	0.05 ± 0.005^{Aa}
	25%	22.79 ± 1.9^{Aa}	1.92 ± 0.2^{Aa}	38.5 ± 2.5^{Aa}	0.05 ± 0.003^{Aa}
	50%	27.06 ± 1.5^{Aa}	2.12 ± 0.3^{Aa}	37.5 ± 7.9^{Aa}	0.06 ± 0.007^{Aa}
	75%	20.25 ± 1.4^{Aa}	2.31 ± 0.2^{Aa}	36.2 ± 5.3^{Aa}	0.07 ± 0.010^{Aa}
CV [%]		18.64	21.87	23.52	24.67
P. suberosa litoralis	Full sun	15.13 ± 1.6^{Ba}	2.06 ± 0.2^{Aa}	31.8 ± 3.8^{Aa}	0.07 ± 0.010^{Aa}
	25%	10.85 ± 1.1^{Ba}	1.92 ± 0.3^{Aa}	32.9 ± 1.9^{Ba}	0.06 ± 0.005^{Aa}
	50%	12.93 ± 0.4^{Ca}	2.19 ± 0.2^{Aa}	31.3 ± 2.1^{Aa}	0.07 ± 0.003^{Aa}
	75%	8.15 ± 1.2^{Bb}	1.42 ± 0.3^{Bb}	26.9 ± 6.9^{Ba}	0.05 ± 0.003^{Aa}
CV [%]		27.17	26.42	21.50	21.27
P. palmeri var. *sublanceolata*	Full sun	10.49 ± 0.1^{Cc}	0.87 ± 0.07^{Bc}	20.6 ± 0.5^{Bb}	0.04 ± 0.003^{Aa}
	25%	23.28 ± 1.4^{Aa}	2.17 ± 0.1^{Aa}	47.7 ± 6.9^{Aa}	0.05 ± 0.005^{Aa}
	50%	19.03 ± 0.6^{Bb}	1.71 ± 0.2^{Aab}	22.9 ± 2.5^{Bb}	0.08 ± 0.010^{Aa}
	75%	18.75 ± 0.8^{Ab}	1.06 ± 0.1^{Cbc}	15.6 ± 0.3^{Cb}	0.07 ± 0.010^{Aa}
CV [%]		27.96	40.60	52.69	31.72

Table 2. Net photosynthetic rate (P_N), stomatal conductance to water vapour (g_s), leaf transpiration rate (E), and intercellular to atmospheric CO_2 concentration ratio (C_i/C_a), measured under saturating irradiance (PPFD ≥ 600 μmol m^{-2} s^{-1}) and ambient CO_2 concentration (C_a = 380 μmol mol^{-1}) from *P. morifolia*, *P. suberosa litoralis*, and *P. palmeri* var. *sublanceolata* under different shade conditions, seven months after emergence. These are means ± SE values from five replicates. Means comparison were done using *Tukey*'s test ($p<0.05$). For each variable *lowercase letters* indicate comparison among treatments and *uppercase* ones comparison among species. CV – variation coefficient.

Species	Shading	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	C_i/C_a
P. morifolia	Full sun	20.90 ± 0.9^{Aa}	0.214 ± 0.005^{Aa}	3.27 ± 0.02^{Ab}	0.53 ± 0.02^{Aa}
	25%	17.31 ± 0.9^{Ab}	0.182 ± 0.010^{Ab}	3.49 ± 0.20^{Bb}	0.56 ± 0.01^{Ba}
	50%	21.09 ± 0.6^{Aa}	0.231 ± 0.003^{Aa}	4.88 ± 0.30^{Aa}	0.58 ± 0.01^{Ba}
	75%	15.59 ± 0.6^{Ab}	0.137 ± 0.007^{Bc}	3.08 ± 0.20^{Bb}	0.45 ± 0.01^{Bb}
CV [%]		17.35	23.48	27.33	12.48
P. suberosa litoralis	Full sun	12.36 ± 0.8^{Ba}	0.094 ± 0.004^{Ba}	1.83 ± 0.07^{Ba}	0.40 ± 0.03^{Ba}
	25%	8.47 ± 0.5^{Bbc}	0.058 ± 0.002^{Bc}	1.01 ± 0.07^{Cb}	0.22 ± 0.02^{Cb}
	50%	10.60 ± 0.5^{Cab}	0.075 ± 0.003^{Bb}	1.56 ± 0.08^{Ba}	0.36 ± 0.03^{Ca}
	75%	6.47 ± 0.4^{Bc}	0.038 ± 0.003^{Cd}	0.76 ± 0.08^{Cb}	0.15 ± 0.05^{Cb}
CV [%]		29.81	40.03	37.36	50.79
P. palmeri var. *sublanceolata*	Full sun	8.92 ± 0.2^{Cb}	0.072 ± 0.007^{Bb}	1.59 ± 0.10^{Bb}	0.42 ± 0.05^{Bb}
	25%	15.64 ± 0.6^{Aa}	0.291 ± 0.010^{Aa}	5.09 ± 0.20^{Aa}	0.74 ± 0.01^{Aa}
	50%	16.12 ± 0.4^{Ba}	0.303 ± 0.020^{Aa}	4.91 ± 0.10^{Aa}	0.73 ± 0.01^{Aa}
	75%	16.15 ± 0.3^{Aa}	0.274 ± 0.020^{Aa}	4.63 ± 0.30^{Aa}	0.70 ± 0.02^{Aa}
CV [%]		20.35	38.67	33.10	20.46

(Fig. 2*C*). *P. morifolia* presented higher value of total Chl concentration at 50% shade, whereas *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata* obtained higher values at 75% shade. Chl *a/b* ratio correlated with the shade levels in a statistically significant way for all species, reaching values around 2.0 for plants under shade (Fig. 2*D*).

Morpho-anatomical features: A significant effect ($p<0.05$) of shade levels on LA and SLA was found for the three species, whose highest mean values were observed at 75% shade (Fig. 2*E,F*). Significant differences ($p<0.05$) were also observed for all anatomical characteristics. The thickness of upper and lower epidermis, spongy and palisade layers, cuticle, mesophyll, and total thickness of leaf blade were significantly higher, for the three species studied, at full sunlight and 25% shade (Table 3). The leaf mesophyll at the same shade levels, with dorsiventral organization, was thicker, made of palisade layer with narrow and long cells, with almost no intercellular space between them. The spongy layer was presented by around five layers of small cells, with irregular shape, distributed in large intercellular spaces. However, in the plant leaves kept at 50 and 75% shade, a deep reduction of length of palisade layer cells was observed, proportional to the reduction of the leaf thickness, as well as a higher compactation of spongy layer, with significant reduction of intercellular spaces, granting a compacted aspect to the leaf blade.

Discussion

The response pattern of *P. palmeri* var. *sublanceolata* to the increasing of P_{max} with the increase of PPFD levels, followed by a decrease of P_{Nmax} at full sunlight, has been reported for other shade-tolerant species (Aleric and Kirkman 2005). Plants adapted to shade environments are more photosynthetically efficient at low levels of PPFD, and lose such efficiency in high levels of PPFD (Leverenz 1995). Photosynthesis in shaded environments requires the maximization of absorbed light, together with low rates of carbon losses through respiration (Zhang *et al.* 2003), and these plants present extremely reduced values of LCP, mainly due to their low values of R_D (Chen and Klinka 1997).

The low values of P_{Nmax} and R_D observed for *P. palmeri* var. *sublanceolata* at full sunlight (Table 1) indicate that these plants were stressed. Such values are a consequence of the photoinhibition phenomenon, which is the slow and reversible reduction of photosynthetic efficiency in high levels of PPFD, which causes the partial decrease of the ability of converting the radiant energy into dry mass (Laing *et al.* 1995). Long exposure to excess of light may cause photodestruction of the photosynthetic pigments and the death of cells or organism. Generally, shade-tolerant species are more sensitive to photoinhibition than sun-adapted species (Barth *et al.* 2001). The fact that *P. palmeri* var. *sublanceolata* plants have survived at full sunlight, but with significantly low photosynthetic rates, indicates that such species may resist in such conditions, but the cultivation at full sunlight does not seem to be the most proper way for the

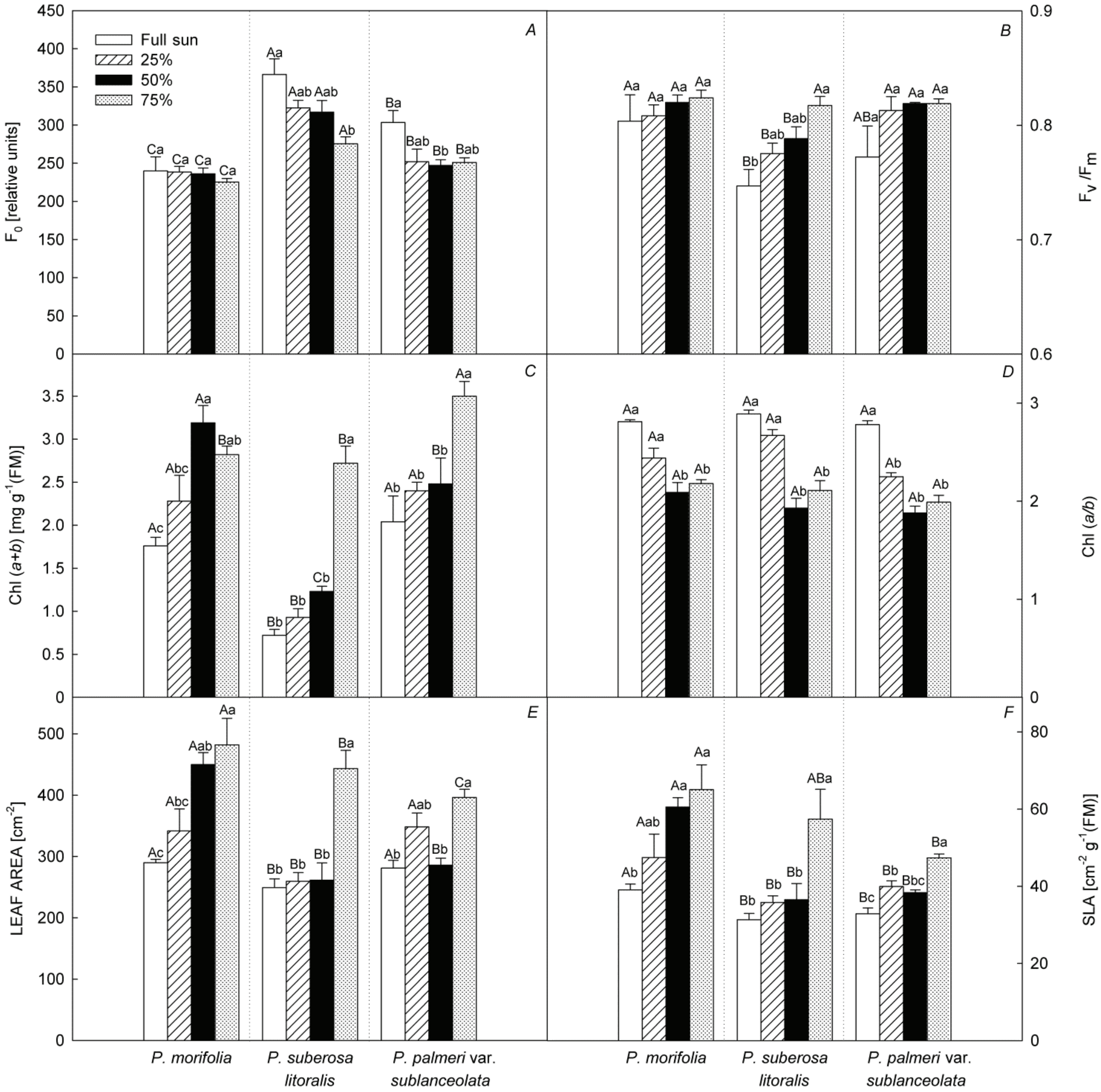

Fig. 2. Minimal fluorescence, F_0 (*A*), maximum photochemical efficiency of PSII, F_v/F_m (*B*), total chlorophyll (Chl) concentration (*C*), Chl *a/b* ratio (*D*), total leaf area (*E*), and specific leaf area, SLA (*F*) of *P. morifolia*, *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata* under different shade conditions, to seven months after emergence. Artificial shade was obtained with different shading nylon nets, under field conditions, which allowed the reduction of 25, 50, and 75% of global radiation, along with a control treatment under full sunlight. *Bars* represent means ($n = 5$) and means comparison was done using *Tukey*'s test ($p<0.05$). *Vertical bars* denote the SE. For each variable *lowercase letters* indicate comparison among treatments and *uppercase* ones comparison among species.

growth and development of these plants.

The highest value of P_{Nmax} of *P. suberosa litoralis* at full sunlight, with decrease in subsequent levels of shade, shows the acclimation of this species to high light environments. This indicates that high light intensity energy was necessary to reach P_{Nmax} and plants showed physiological plasticity which enables the adaptation to higher energy uptake (Aleric and Kirkman 2005). High values of R_D, LCP, and α at full sunlight have also been observed for this species (Table 1). The maintenance of high photosynthetic rates have a high energetic cost, being beneficial only under high irradiance conditions, in which the quantity of Rubisco and PSII reaction center increase with the expansion of the light-harvesting complex II (Hikosaka and Terashima 1995).

The stomata usually open in response to the increase

Table 3. Summary of anatomical variables of *P. morifolia*, *P. suberosa litoralis*, and *P. palmeri* var. *sublanceolata* under different shade conditions, to seven months after emergence. UE – upper epidermis; LE – lower epidermis; CU – cuticle; PL – palisade layer; SL – spongy layer; TM – total mesophyll; LT – leaf thickness. These are means ± SE values from five replicates. Means comparison was done using *Tukey*'s test (p<0.05). For each variable *lowercase letters* indicate comparison among treatments and *uppercase* ones comparison among species. CV – variation coefficient.

Species	Shading	UE [μm]	LE [μm]	CU [μm]	PL [μm]	SL [μm]	TM [μm]	LT [μm]
P. morifolia	Full sun	23.2 ± 1.8^{Ba}	16.2 ± 1.3^{Ba}	2.6 ± 0.8^{Aa}	65.4 ± 2.1^{Bb}	77.8 ± 2.5^{Ba}	112.5 ± 3.7^{Cb}	122.5 ± 3.9^{Bb}
	25%	19.9 ± 0.9^{Ba}	15.8 ± 1.1^{Ba}	2.2 ± 0.5^{Bab}	78.6 ± 2.6^{Ba}	72.8 ± 2.7^{Ba}	129.4 ± 4.1^{Ba}	137.2 ± 5.3^{Ba}
	50%	12.9 ± 1.1^{Bb}	11.7 ± 0.9^{Bb}	1.9 ± 0.8^{Ab}	51.1 ± 2.2^{Bc}	58.6 ± 3.3^{Bc}	92.2 ± 5.5^{Bd}	95.4 ± 6.7^{Bd}
	75%	20.4 ± 1.2^{Aa}	13.8 ± 0.9^{Bab}	1.7 ± 0.8^{Ab}	50.1 ± 1.8^{Bc}	66.1 ± 2.2^{Bb}	102.1 ± 2.1^{Bc}	108.5 ± 1.6^{Bc}
CV [%]		19.9	18.5	12.8	37.5	19.5	25.8	21.7
P. suberosa litoralis	Full sun	31.3 ± 1.6^{Aa}	19.4 ± 1.1^{Aa}	3.1 ± 0.7^{Aa}	107.6 ± 3.8^{Aa}	134.9 ± 5.3^{Aa}	220.3 ± 4.1^{Aa}	255.2 ± 4.8^{Aa}
	25%	30.6 ± 1.8^{Aa}	20.8 ± 1.2^{Aa}	3.4 ± 0.8^{Aa}	92.7 ± 2.2^{Ab}	127.3 ± 9.1^{Aa}	205.8 ± 7.7^{Ab}	219.4 ± 9.9^{Ab}
	50%	31.3 ± 1.4^{Aa}	20.1 ± 1.1^{Aa}	2.4 ± 0.6^{Ab}	83.7 ± 2.2^{Ac}	104.3 ± 5.1^{Ab}	204.9 ± 7.7^{Ab}	212.4 ± 5.2^{Ab}
	75%	25.0 ± 1.5^{Ab}	17.9 ± 1.6^{Ab}	2.2 ± 0.8^{Ab}	71.7 ± 4.7^{Ad}	92.2 ± 5.4^{Ac}	187.2 ± 8.4^{Ac}	196.9 ± 5.5^{Ac}
CV [%]		20.4	22.2	14.5	35.7	24.7	22.1	21.2
P. palmeri var. *sublanceolata*	Full sun	15.6 ± 1.3^{Ca}	11.7 ± 0.7^{Ca}	2.1 ± 0.3^{Ba}	51.8 ± 1.8^{Ca}	78.2 ± 8.4^{Ba}	125.5 ± 9.7^{Ba}	134.3 ± 10.5^{Ba}
	25%	16.8 ± 0.9^{Ba}	12.3 ± 0.5^{Ca}	1.4 ± 0.4^{Bb}	48.6 ± 1.7^{Ca}	71.8 ± 4.3^{Ba}	106.1 ± 4.0^{Cb}	119.5 ± 5.0^{Cb}
	50%	11.9 ± 0.8^{Bb}	9.3 ± 0.4^{Bb}	1.6 ± 0.7^{Ab}	34.5 ± 1.6^{Cb}	62.3 ± 3.6^{Bb}	93.2 ± 3.7^{Bc}	96.1 ± 5.4^{Bc}
	75%	11.6 ± 0.7^{Bb}	11.5 ± 0.4^{Ca}	1.1 ± 0.9^{Bb}	34.9 ± 1.9^{Cb}	60.1 ± 2.2^{Bb}	90.3 ± 4.2^{Cc}	94.1 ± 4.5^{Cc}
CV [%]		30.1	30.1	12.9	31.1	45.1	39.4	35.7

of PPFD. Changes in g_s are related to both the control of water loss and CO_2 assimilation for maintenance of photosynthetic rate (Zhang *et al.* 2003). The reduction of g_s may restrict the rate of CO_2 fixation, with consequent decrease of its concentration in the substomatal cavities and in the intercellular spaces. The photoinhibition observed in *P. palmeri* var. *sublanceolata* may have caused the reduction of g_s at full sunlight (0.07 mol m^{-2} s^{-1}). That may be harmful to the plant, provided that the captured radiant energy is not used in the photosynthetic processes, causing, consequently, photodestruction of chlorophyll molecules (Costa *et al.* 1998).

For *P. palmeri* var. *sublanceolata*, the conditions of maximum irradiation were not favorable concerning maximization of g_s, which also contributed to reduction of *E.* According to Farquhar (1978), *E* is reduced with increase of vapor pressure deficit, which suggests that the stomata are sensitive to environmental changes. Both light and air temperature influence the stomatal responses. The C_i/C_a ratio is a good indicator of stomatal limitation of photosynthesis (Farquhar and Sharkey 1982). The C_i/C_a ratio values change according to the habitat, with a tendency to be higher in mesomorphic than in xeromorphic species (Lloyd and Farquhar 1994), a fact which has been observed in this study, in which shade-tolerant species *P. morifolia* and *P. palmeri* var. *sublanceolata* present higher values of C_i/C_a than *P. suberosa litoralis* (Table 2).

F_0 and F_v/F_m are good indicators of the effect of environmental stress in photosynthesis. Generally, the values of F_v/F_m varied from 0.75 to 0.85, which shows efficient conversion of light energy at PSII level (Baker 2008). However, in plants of *P. suberosa litoralis* and *P. palmeri* var. *sublanceolata* cultivated at full sunlight, the reduction of F_v/F_m may indicate some kind of damage in the photosynthetic apparatus, and the photosynthetic rate of *P. palmeri* var. *sublanceolata* was influenced by the reduction of the ratio F_v/F_m, which proves the photoinhibition (Fig. 2*B*).

We have verified that *P. palmeri* var. *sublanceolata* presented higher values of total Chl than the other species (Fig. 2*C*). Such differences may be important under shades which are higher than 40%, once in such conditions the photosynthetic rate must be proportional to Chl concentration (Gabrielsen 1948). *P. palmeri* var. *sublanceolata* and *P. morifolia* would have conditions to present high growth rates, higher than *P. suberosa litoralis* ones even under shade conditions.

The highest Chl concentration in most shade levels may be considered a compensation effect at the lowest PPFD quantity available. Leaves cultivated under low light densities present higher Chl amounts per mass unit. Usually, leaves of shade plants have bigger chloroplasts and contain more Chl (Björkman 1981). The increase in the chloroplast size and Chl amount per chloroplast in shaded leaves compensates the decrease in a number of chloroplasts per leaf-area unit (Boardman 1977). Generally, Chl *a/b* ratio tends to be reduced with decrease of PPFD due to a higher relative proportion of Chl *b* in shaded environments. This is explained by the fact that Chl *b* is more slowly degraded in shade plants than Chl *a* (Engel and Poggiani 1991). This was observed in this work for the three studied species (Fig. 2*D*).

The increase in LA and SLA (Fig. 2*E,F*) is caused by changes in leaf dimensions and shape as a response to increasing shade levels. The leaf expansion under low irradiance is frequently reported and indicates the way that the plant compensates for the decrease in light, making better use of this resource by increasing the surface area (Campos and Uchida 2002). Increased SLA may improve light harvesting per unit of resources invested in construction of photosynthetic tissues (Lusk *et al.* 2008); it represents an adaptive mechanism, demonstrating the most efficient utilization of photoassimilates, as a larger photosynthetic area is produced per unit of accumulated dry matter (DaMatta 2004, Chaves *et al.* 2008). Therefore, the low LA and SLA values observed in passion flowers at full sunlight (Fig. 2*E,F*) may have benefited these plants, decreasing the exposure of plant tissues to the sun and reducing water loss and self-shade (Matos *et al.* 2009).

The results found in this study agree with the ones observed by several authors concerning the anatomical differences between sun and shade leaves (Campos and Uchida 2002, Givnish *et al.* 2004, Montanari *et al.* 2004, Morais *et al.* 2004). Due to the ability of light propagation for more inner regions of mesophyll, several layers of palisade parenchyma generally occur together with thicker leaves which are under high-light conditions (Smith *et al.* 1997). The highest values of mesophyll thickness and, therefore, of palisade layer at full sunlight (Table 3) are related to the increase in thickness of palisade layer, once the number of layers remained the same, for the three species under investigation.

A well developed system of intercellular spaces in the leaf mesophyll makes the gas exchange easier, benefiting the photosynthetic efficiency. The specialization of palisade parenchyma, which brings more efficient photosynthesis, is not related only to a higher number of chloroplasts in cells, but also to the dimensions of their free surface area (Costa *et al.* 1998). In plants cultivated at full sunlight, the palisade layer was more differentiated, with higher free surface area, despite the fact that the volume of intercellular spaces of spongy layer is much higher when compared to palisade layer. However, the increase of leaf blade thickness at full sunlight brings high energy cost, with consequent biomass investment (Oguchi *et al.* 2003).

Conclusions: Plants of *P. morifolia* and *P. palmeri* var. *sublanceolata* are adapted to moderate shade conditions. The leaf anatomical plasticity of *P. morifolia* benefited the maintenance of high photosynthetic rates under full sunlight conditions, whereas the photoinhibition was one of limiting factors for *P. palmeri* var. *sublanceolata* at full sunlight. The high rates of photosynthesis, transpiration, and stomatal conductance of *P. suberosa litoralis* at full sunlight, together with the variation of the anatomical characteristics observed, showed the acclimation ability of such species to environments with high PPFD levels.

The differential adaptation of the three studied species concerning CO_2 assimilation showed the ability of individual morpho-physiological plasticity, which became an essential tool for the plants to adapt to heterogeneous environments. This information is very useful to select sun- and shade species for landscaping projects, and provide support for introducing the cultivation of *Passiflora* in the tropical ornamental plants market in Brazil. This study suggests that *P. suberosa litoralis* can be used as an ornamental plant in outdoor gardens, green live fences or pergola green covers, and that *P. morifolia* and *P. palmeri* var. *sublanceolata* can be better used as indoor vase plants in shaded environments.

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