Leaf gas exchange, chlorophyll fluorescence and pigment indexes of *Eugenia uniflora* L. in response to changes in light intensity and soil flooding

MARCELO S. MIELKE[1,2] and BRUCE SCHAFFER[3]

[1]   *Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Ilhéus/Itabuna km 16, Ilhéus, BA, 45650-000, Brazil*

[2]   *Corresponding author (msmielke@uesc.br)*

[3]   *University of Florida, IFAS, Tropical Research & Education Center, 18905 SW 280th Street, Homestead, FL 33031, USA*

Summary   The interactive effects of changing light intensity and soil flooding on the photosynthetic performance of *Eugenia uniflora* L. (Myrtaceae) seedlings in containers were examined. Two hypotheses were tested: (i) the photosynthetic apparatus of shade-adapted leaves can be rapidly acclimated to high light after transfer from shade to full sun, and (ii) photosynthetic acclimation to changing light intensity may be influenced by soil flooding. Seedlings cultivated in a shade house (40% of full sun, $\approx$ 12 mol m^{-2} day^{-1}) for 6 months were transferred to full sun (20–40 mol m^{-2} day^{-1}) or shade (30% of full sun, $\approx$ 8 mol m^{-2} day^{-1}) and subjected to soil flooding for 23 days or not flooded. Chlorophyll content index (CCI), chlorophyll fluorescence, leaf weight per area (LWA), photosynthetic light–response curves and leaf reflectance indexes were measured during soil flooding and after plants were unflooded. The CCI values increased throughout the experiment in leaves of shaded plants and decreased in leaves of plants transferred to full sun. There were no significant interactions between light intensity and flooding treatments for most of the variables analyzed, with the exception of Fv/Fm 22 days after plants were flooded and 5 days after flooded plants were unflooded. The light environment significantly affected LWA, and light environment and soil flooding significantly affected the light-saturated gross CO_2 assimilation rate expressed on area and dry weight bases ($A_{max-area}$ and A_{max-wt}, respectively), stomatal conductance of water vapor (g_{ssat}) and intrinsic water use efficiency (A/g_s). Five days after flooded plants were unflooded, the normalized difference vegetation index (NDVI) and the scaled photochemical reflectance index (sPRI) were significantly higher in shade than in sun leaves. Thirty days after transferring plants from the shade house to the light treatment, LWA was 30% higher in sun than in shade leaves, and $A_{max-area}$ and g_{ssat} were 59% and 99% higher, respectively, in shade than in sun leaves. Changes in CCI, NDVI and sPRI in leaves of *E. uniflora* seedlings transferred from shade to full sun appear to be associated with changes in pigment composition and protective mechanisms against excess light.

Introduction

Eugenia uniflora L. (Myrtaceae) is a shrub or small tree native to South America, ranging from Surinam to southern Brazil and Uruguay (Margis et al. 2002). This species is cultivated throughout the subtropics and tropics as a fruit and ornamental plant (Donadio et al. 2002, Oliveira et al. 2005). It is also often used for medicinal purposes (Schmeda-Hirschmann et al. 1987, Schapoval et al. 1994, Ogunwande et al. 2005, Oliveira et al. 2005) and by the cosmetic industry (Melo et al. 2007). *E. uniflora* is ecologically important as a colonizing species on disturbed areas as well as a food source for local fauna (Margis et al. 2002). Some studies with young plants have shown that *E. uniflora* is a light-demanding species (Scalon et al. 2001). However, other reports have described it as shade tolerant (Bianchini et al. 2003). There are many references demonstrating the presence of *E. uniflora* in gallery forests (Rodrigues and Nave 2000, Botrel et al. 2002, Bianchini et al. 2003, Pinto et al. 2005), indicating its potential use in gallery forest restoration projects. Despite its cultural, ecological and economic importance, little is known about the physiological responses of *E. uniflora* to environmental stresses.

Gallery forests are highly unstable ecosystems susceptible to intermittent changes in volume and flow of water through rivers that can result in soil flooding. Flooding-induced oxygen deficiency and low soil redox potential adversely affect several aspects of plant physiology such as changes in carbon assimilation and absorption of macronutrients as well as suppression of root metabolism (Kozlowski 1997, Pezeshki 2001, Kreuzwieser et al. 2004). Soil flooding also affects leaf formation and expansion and induces premature leaf senescence and abscission. Prolonged flooding often results in tree

death (Kozlowski 1997). Although stomatal closure is one of the first measurable responses of plants to soil flooding, flooding-induced changes in carbon assimilation can also be attributed to non-stomatal limitations to photosynthesis (Herrera et al. 2008) derived from changes in leaf pigments and alterations in carboxylating enzymes (Kozlowski 1997, Pezeshki 2001). Tolerance to anaerobic stress may be influenced by the developmental stage of the plant and its interaction with other environmental factors such as soil temperature (Ojeda et al. 2004) or light intensity (Wagner and Dreyer 1997, Gardiner and Krauss 2001, Lavinsky et al. 2007). As a consequence of soil flooding in gallery forests, soil instability and the fall of overstory trees resulting in gap formation may cause sudden increases in light intensity in the understory.

In all forests, light is a dynamic resource that may change within hours, days or weeks (Canham et al. 1990). Acclimation to the light environment is a key ecophysiological feature for establishment, growth and survival of tree species (Lee et al. 1996, Walters 2005, Valladares and Niinemets 2008). Leaves generally exhibit a great deal of plasticity in relation to acclimation to the light environment (Valladares and Niinemets 2008). In general, sun leaves have higher leaf weight per area than shade leaves (Valladares and Niinemets 2008), which is related to thicker leaves and/or reduced leaf area of sun leaves compared to shade leaves. The increased thickness of sun leaves is usually associated with a higher proportion of palisade parenchyma and photosynthetic cells per unit area and an increase in the amount of photosynthetic enzymes (Evans and Poorter 2001). Leaves acclimated to shade have different physiology, anatomy and morphology than sun-adapted leaves. Shade leaves have characteristics that increase light capture, such as a higher chlorophyll content, a lower chlorophyll a/b ratio and more nitrogen allocated to the light-harvesting complexes than sun leaves (Evans and Poorter 2001, Valladares and Niinemets 2008). Such characteristics are very important in low light environments but can lead to an excessive absorption of light energy when leaves are suddenly exposed to high light.

Photosynthetic acclimation may involve physiological adjustments within hours, days or weeks or structural changes within weeks or months (Bongers and Popma 1990, Walters 2005). While long-term acclimation to high light intensity involves the production of new foliage that is structurally and functionally different from that of the previously shaded leaves, short-term acclimation involves adjustments in the composition of the photosynthetic apparatus within individual cells or chloroplasts (Bongers and Popma 1990). The capacity of certain species to deal with changes from low to high light intensity also depends largely on the ability to minimize the magnitude and duration of photoinhibition of photosynthesis (Naidu and DeLucia 1998, Krause et al. 2001, Houter and Pons 2005, Guo et al. 2006). Photoinhibition is not solely dependent on high light intensity but is also influenced by other environmental stresses, such as water deficits (Araus and Hogan 1994) and soil flooding (Lavinsky

et al. 2007). Thus, soil flooding may influence the photosynthetic acclimation of *E. uniflora* leaves after transference from low to high light intensity.

Changes in pigment content and composition are within the main responses of leaves suddenly exposed to high light (Bailey et al. 2001, Walters 2005, Guo et al. 2006, Naramoto et al. 2006). Pigment content and composition can be accessed using handheld chlorophyll meters (Torres Netto et al. 2002, Pinkard et al. 2006, Hawkins et al. 2009) and leaf reflectance indexes (Sims and Gamon 2002). Chlorophyll meters measure light transmittance at two wavelengths corresponding to red and far-red, transforming this data to chlorophyll content index (Markwell et al. 1995). In contrast, reflectance spectroscopy supplies a wealth of information of the electromagnetic spectrum between approximately 350 and 1100 nm, providing the basis for different leaf reflectance indexes that have been developed for chlorophylls, xanthophylls and anthocyanins (Gamon and Surfus 1999, Nichol et al. 2006, Letts et al. 2008).

With the aim of providing information to assist with using *E. uniflora* in gallery forest restoration programs, the interactive effects of changing light intensity and soil flooding on the photosynthetic performance of seedlings of *E. uniflora* in containers were examined. Two hypotheses were tested: (i) the photosynthetic apparatus of shade-adapted leaves can be rapidly acclimated to high light intensity after transfer from shade to full sun, and (ii) photosynthetic acclimation to changing light intensity may be influenced by soil flooding.

Materials and methods

Study site and plant material

The study was conducted at the Tropical Research and Education Center, University of Florida (TREC/UF) in Homestead, FL, USA (25.5° N 80.5° W). In spring 2008, 1.5-year-old seedlings of *E. uniflora* L. were acquired from a commercial nursery in Homestead. Plants were cultivated in 10-l plastic containers containing a standard nursery substrate of 65% pine bark, 25% Florida peat and 10% coarse sand by volume. According to a standard method used to propagate *E. uniflora* in commercial nurseries in south Florida, all containers had about 10–12 seedlings. After plants were transported to TREC/UF, careful selection was done to obtain uniform plants for the study. At the beginning of the experiment, the average height of the plants per container was 0.8–1.0 m, and the average stem diameter 0.10 m above the soil surface was 5–15 mm.

Experimental procedures

The experiment was conducted in a completely randomized design in a 2×2 factorial arrangement with two light levels (shade and full sun) and two flooding treatments (flooded and non-flooded). There were five replications for each treatment combination and two containers per replication. The experi-

ment was installed on a flat, open area at TREC/UF. Ten 3 × 2 m blocks (each block with sufficient area for ten containers) were selected perpendicular to the daily track of the sun permitting plants in full sunlight to receive almost all solar radiation during the day. Five blocks contained 3 × 2 × 1.7 m shade cages (shade replications) that were constructed with PVC tubes and covered with one layer of a neutral shade netting (25–30% of full sun). The other five blocks (sun replications) were left in the open. Replications were distributed randomly and far enough apart so that cages (shade treatment replications) did not shade plants in the sun treatment. During the experiment, all plants were irrigated three times per day with an automated, timer-controlled irrigation system. Prior to the experiment, containers were placed in a shade house (about 40% of full sun) in March 2008. The plants remained in the shade house until October 2008 when half of the containers were transferred to full sun and half to the shade cages. Immediately after transference, half of the containers in each light environment were flooded (flooded treatment), and half of them were maintained near field capacity by the automatic irrigation system (non-flooded treatment). Plants in the flooded treatment were flooded by submerging the containers with plants in 19-l plastic buckets filled with tap water to 50 mm above the soil surface, to ensure complete inundation of roots. The duration of the flood treatment was 23 days. Pigment indexes, chlorophyll fluorescence and leaf gas exchange were measured during the flooding period and 10 days after flooded plants were unflooded.

Microclimate and soil temperature

Photosynthetic photon flux (PPF) was measured in each light treatment with quantum sensors model LI-190SA connected to LI-1000 data loggers (Li-Cor Inc., Lincoln, NE). Air temperature (Ta) and relative humidity (RH) were monitored and recorded with a Hobo Pro V2 datalogger (Onset Computer, Bourne, MA), and the air vapor pressure deficit (VPD) was calculated according to Landsberg (1986). Microclimate was measured 11 days after transferring plants from the shade house to full sun or shade and throughout the experiment (in full sun and shade treatments). During the flooding period, soil temperature was recorded in three containers in each treatment with TIDBITV2 waterproof temperature dataloggers (Onset Computer, Bourne, MA). Simultaneous measurements in the shade house and the full sun treatments 11 days before transferring plants from the shade house to the sun and shade treatments showed that mean values of total daily PPF inside the shade house were about 12 mol m^{-2} day^{-1} or 40% of full sun.

Soil redox potential (Eh)

Soil redox potential was measured for flooded plants 6, 14 and 21 days after plants were initially flooded. The measurements were made at a soil depth of 20 cm using a platinum Ag+/AgCl combination electrode (Accumet, Fisher Scien-

tific, Pittsburgh, PA) attached to a portable pH/voltage meter (Accumet model AP62, Fisher Scientific, Pittsburgh, PA).

Chlorophyll content index and chlorophyll fluorescence

The leaf chlorophyll content index (CCI) and chlorophyll fluorescence were measured 1, 5, 14 and 22 days after the start of the flooding treatment on one recently matured, fully expanded leaf per plant/container. Each replication was the average value of two leaves (two containers per replication). The CCI was measured with a SPAD meter (Model 502, Minolta Inc., Osaka, Japan), and chlorophyll fluorescence was measured with a portable fluorescence system (model OS-30, Opti-Sciences Inc., Hudson, NH). The CCI and chlorophyll fluorescence measurements were done between 8:00 and 9:00 AM. Leaves were acclimated in the dark for 30 min prior to each chlorophyll fluorescence measurement. The initial (Fo), maximum (Fm) and variable (Fv) fluorescence and the maximum quantum efficiency of the photosystem II (Fv/Fm) were measured and calculated as described by Maxwell and Johnson (2000). Chlorophyll content in leaf tissues was expressed as CCI because previous experiments showed a good relationship ($r^2 = 0.90$) between leaf chlorophyll concentration and CCI values for *E. uniflora* (M.S. Mielke, B. Schaffer and C. Li, unpublished data).

Photosynthetic characteristics

Photosynthetic light responses to PPF were measured with a portable photosynthesis system (CIRAS-2, PP Systems, Amesbury, MA) 18 days after seedlings were initially flooded and 7 days after plants were unflooded (recovery). One recently matured, fully expanded leaf per container (one container per replication) was selected and acclimated to the environmental conditions inside the leaf cuvette for about 20 min. Leaf gas exchange measurements were done between 08:00 and 12:00 AM. The air flow rate, Ta, VPD, PPF inside the leaf chamber and reference CO_2 concentration during measurements were 200 ml min^{-2}, 25 °C, 1 kPa, 1000 μmol photons m^{-2} s^{-2} and 375 μmol mol^{-2}, respectively. Light–response curves were made decreasing PPF from 1000 to 0 μmol photons m^{-2} s^{-2} through nine steps. Net CO_2 assimilation (A_n) and stomatal conductance of water vapor (g_s) were determined at each PPF. Immediately after measurements, leaves were collected, and total leaf area and leaf dry weight were determined for each plant. Leaf area was determined with a leaf area meter (model LI-3000, Li-Cor Inc., Lincoln, NE), and leaf dry weight was obtained after oven-drying leaves at 75 °C until a constant weight was reached. Leaf weight per area (LWA) was calculated by dividing the leaf dry weight by the leaf area. The light–response curves were fitted by a NLIN routine of the SAS statistical software package (SAS Institute, Cary, NC) using the exponential model (Iqbal et al. 1997, Gomes et al. 2006):

$$A_n = A_{max}[1 - \exp(-\alpha PPF/A_{max})] - Rd \qquad (1)$$

where A_{max} is the light-saturated gross CO_2 assimilation rate, α is the apparent quantum yield of photosynthesis and Rd is the dark respiration rate. The A_{max}, expressed on a dry weight basis, was obtained by dividing A_{max} by LWA. The g_{ssat} was the value of g_s at a PPF of 1000 μmol photons m^{-2} s^{-2}, and intrinsic water use efficiency (A/g_s) was the ratio between A_{nsat} (measured at 1000 μmol photons m^{-2} s^{-2}) and g_{ssat}.

Leaf reflectance

Five days after plants in the flooded treatment were un-flooded (27 days after transferring plants from the shade house to the sun or shade treatments), leaf reflectance on the adaxial leaf surface was measured on three plants per treatment. Each replication was the average value of three recently matured, fully expanded leaves per plant. In the early morning (08:00–09:00 AM), seedlings were transferred to a shed and kept under low light during all measurements. Leaf reflectance was measured with a portable spectrometer (Uni-spec, PP Systems, Amesbury, MA) using a bifurcated fiber optics cable and a leaf clip. Within the spectral range from 350 to 1100 nm, the normalized difference vegetation index (NDVI) which is an index of chlorophyll content, the red/green reflectance ratio (Red:Green) which is an index of anthocyanin content, and the photochemical reflectance index (PRI) which is an index of carotenoid content were calculated as described by Gamon and Surfus (1999):

$$NDVI = (R_{750} - R_{705})/(R_{750} + R_{705}) \qquad (2)$$
$$PRI = (R_{531} - R_{570})/(R_{531} + R_{570}) \qquad (3)$$
$$Red : Green = \sum R_{Red} / \sum R_{Green} \qquad (4)$$

where R is the reflectance and subscripts indicate the wavelengths in nm. To avoid negative values for PRI, a scaled value of PRI (sPRI) was calculated (Letts et al. 2008) as:

$$sPRI = (PRI + 1)/2 \qquad (5)$$

At the time of reflectance measurements, leaf chlorophyll fluorescence was measured with a portable fluorescence system (model OS-30, Opti-Sciences, Hudson, NH) as previously described.

Data analysis

Interactions between light and flooding treatments were analyzed by a two-way ANOVA. For soil redox potential comparisons between sun and shade treatments of only flooded plants, a T-test was used. All statistical analyses were done using the SAS statistical software package (SAS Institute, Cary, NC).

Results

The average, minimum and maximum Ta were similar for the shade and sun treatments (Table 1). The lowest Ta was observed during the flooding period. The average VPD was also similar for the sun and shade treatments but slightly higher in

Table 1. Summary of climatic variables during the experiment. The data are presented as the mean followed by minimum and maximum observed values.

		During flooding (n = 23 days)	After flooding (n = 11 days)
Ta (°C)	Sun	22.1 (6.1–29.3)	22.9 (9.9–31.6)
	Shade	22.1 (5.3–30.8)	23.0 (8.9–33.2)
VPD (kPa)	Sun	0.5 (0.0–1.6)	0.5 (0.0–1.8)
	Shade	0.5 (0.0–1.9)	0.5 (0.0–2.3)
PPF (mol m^{-2} day^{-1})	Sun	24.7 (10.7–33.5)	23.6 (18.3–35.1)
	Shade	7.9 (3.7–10.7)	7.2 (5.6–9.7)

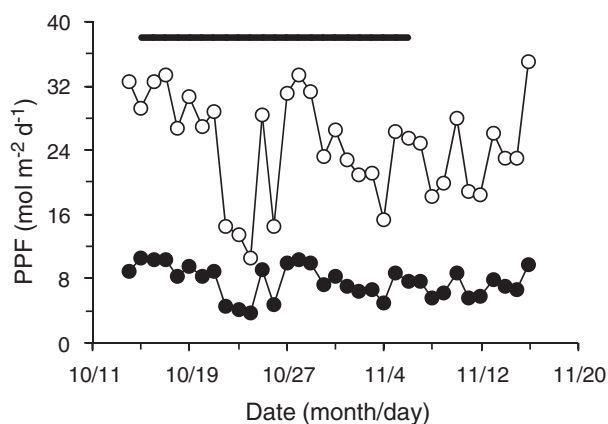Ta, air temperature; VPD, air vapor pressure deficit; PPF, daily total photosynthetic photon flux.

Figure 1. Total daily photosynthetic photon flux (PPF) during the entire treatment period (open circles, full sun and closed circles, shade). Black bar indicates the period in which the plants in sun and shade treatments were flooded.

shade than in full sun. Daily PPF tended to decrease during the experiment. The maximum values were observed just before transferring seedlings from the shade house to the experimental plots, reaching a maximum value of 40.2 mol photons m^{-2} day^{-2} in full sun (Table 1 and Figure 1). Based on the mean daily PPF in the sun and shade treatments, plants in the shade treatment received 30% of full sun. Figure 2 shows daily patterns of Ta, VPD and PPF in the full sun and shade treatments. On a clear day, patterns of Ta, VPD and PPF were very similar for the shade and full sun treatments, although as expected, the amount of light that reached the top of the plants was higher in the sun treatment. During the flooding period, no significant treatment effects were observed for the average or minimum soil temperature (Table 2). Soil Eh of flooded plants decreased to below −80 mV by the end of the first week of flooding in both sun and shade environments (Table 3), and no significant differences ($P > 0.05$, T-test) in Eh were observed between sun or shade treatments throughout the experiment.

The CCI values increased throughout the experiment in plants transferred from the shade house to the shade treat-
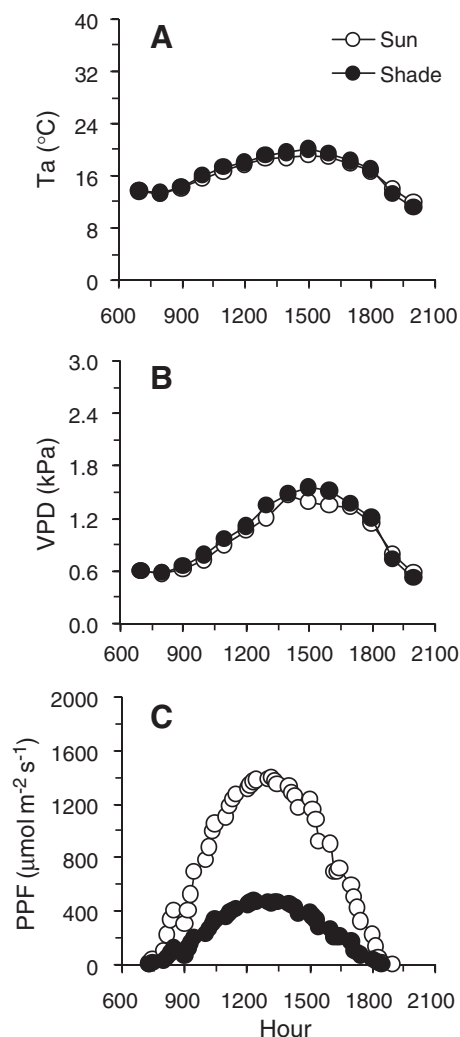
Figure 2. Daily time course of (A) air temperature (Ta), (B) vapor pressure deficit (VPD), and (C) photosynthetic photon flux (PPF) on 28 October 2008.

16% higher than that of sun leaves 22 and 27 days, respectively, after placing plants in the different light treatments. Similarly, Fv/Fm gradually decreased in plants transferred to full sun, reaching a minimum of about 0.6, 27 days after placing plants in the light treatments. The Fv/Fm was 9%, 14% and 21% higher in shade than in sun leaves 5, 22 and 27 days, respectively, after placing plants in the sun or shade treatments. Contrary to Fv/Fm, the Fo gradually increased throughout the experiment in plants transferred from the shade house to full sun. Twenty-seven days after transferring plants from the shade house to the sun and shade treatments (5 days after flooded plants were unflooded), the average Fo was 48% higher in sun than in shade leaves.

At the end of the flooding period (22 days after plants were flooded), soil flooding significantly affected CCI ($P \leq 0.05$), Fv/Fm, Fo and Fm ($P \leq 0.01$; Table 4). The average CCI, Fv/Fm and Fm were 2%, 18% and 9% higher, respectively, in non-flooded than in flooded plants. At the same time, the average Fo was 25% higher in flooded than in non-flooded plants. The significant effects of soil flooding persisted 5 days after flooded plants were unflooded for Fv/Fm and Fo.

Significant interactions between light and flooding treatments ($P \leq 0.05$) on Fv/Fm were observed at the end of the flooding period and 5 days after flooded plants were unflooded. The average value of Fv/Fm was significantly higher ($P < 0.05$) in non-flooded than in flooded plants at the end of flooding period in full sun treatment (Figure 3A). Five days after soil drainage, the average Fv/Fm was significantly higher in non-flooded than in flooded plants in shade treatment (Figure 3B). The lowest Fv/Fm values were always observed in flooded plants transferred from the shade house to full sun.

Twenty-seven days after transferring plants from the shade house to the sun or shade treatments (5 days after flooded plants were unflooded), light treatment significantly affected NDVI and sPRI ($P \leq 0.01$) (Table 5). The average values of NDVI and sPRI were 7% and 21% higher in shade than in sun treatments, respectively. At the same time, no significant differences were observed among light or flooding treatments for Red:Green ratio.

Eighteen days after transferring plants from the shade house to the sun or shade treatments and seedlings were initially flooded, there were no significant interactions ($P > 0.05$) between light and flooding treatments for any photosynthetic variable measured (Table 6). There were significant differ-

ment (Table 4), which was a response to increased shading after placing the plants in the shade cages (Table 1). No significant treatment differences ($P > 0.05$) were observed for CCI on the first day after placing plants in the sun or shade treatments, but significant differences in CCI ($P \leq 0.05$) between sun and shade treatments were observed 5, 14, 21 and 27 days after placing plants in the sun or shade treatments (Table 4). The average CCI of shade leaves was 11% and

Table 2. Means ($\pm$ SD) and ANOVA probability values comparing the effects of light environment and flooding on average, minimum and maximum soil temperatures.

Soil temperature (°C)	Light (L)		Flooding (F)		ANOVA		
	Shade	Sun	Flooded	Non-flooded	L	F	L × F
Average	22.7 ± 2.5	23.6 ± 2.5	22.9 ± 2.8	23.3 ± 2.2	ns	ns	ns
Minimum	20.6 ± 3.2	20.7 ± 3.2	20.4 ± 3.3	20.9 ± 3.0	ns	ns	ns
Maximum	24.8 ± 2.1	26.8 ± 2.2	25.8 ± 2.7	25.7 ± 2.0	*	ns	ns

ns, $P > 0.05$; *$P \leq 0.01$.

Table 3. Soil redox potential [Eh (mV)] of flooded plants in shade and full sun treatments.

Days of flooding	Shade	Sun	Significance[2]
6	-82 ± 25[1]	-81 ± 48	ns
14	-66 ± 20	-60 ± 25	ns
21	-47 ± 30	-47 ± 8	ns

[1]Values are means ± SD.

[2]ns indicates no significant difference between treatments according to a standard T-test ($P > 0.05$, $n = 5$).

ences between sun and shade treatments for LWA, $A_{\text{max-area}}$, Rd, g_{ssat} ($P \leq 0.05$), $A_{\text{max-wt}}$ and A/g_s ($P \leq 0.01$) and significant effects of flooding on $A_{\text{max-area}}$, $A_{\text{max-wt}}$, g_{ssat} and A/g_s ($P \leq 0.01$). Average $A_{\text{max-area}}$, $A_{\text{max-wt}}$, α, g_{ssat} and A/g_s were higher in the shade than in the sun treatment, whereas LWA and Rd were higher in sun plants than shade plants. The average LWA was 14% higher in sun than in shade leaves. The average $A_{\text{max-area}}$ was 85% higher in shade than in sun leaves (5.46 versus 2.96 µmol m^{-2} s^{-2}) and 188% higher in non-flooded than in flooded plants (6.25 versus 2.17 µmol m^{-2} s^{-2}). At the same time, the average g_{ssat} was 73% higher in shade than in sun leaves (32.6 versus 18.8 mmol m^{-2} s^{-2}) and 195% higher in non-flooded than in flooded plants (38.4 versus 13.0 mmol m^{-2} s^{-2}).

Thirty days after transferring plants from the shade house to the sun or shade treatments (7 days after flooded plants

were unflooded), there were significant differences between sun and shade treatments for LWA, $A_{\text{max-area}}$, α ($P \leq 0.05$), $A_{\text{max-wt}}$, and g_{ssat} ($P \leq 0.01$), while there were significant differences between flooding treatments for $A_{\text{max-area}}$, $A_{\text{max-wt}}$, g_{ssat} ($P \leq 0.01$) and α ($P \leq 0.05$) (Table 6). There were no significant interactions ($P > 0.05$) between light and flooding treatments for any photosynthetic variable measured. Average LWA was 30% higher in sun than in shade leaves. The average $A_{\text{max-area}}$ was 59% higher in shade than in sun leaves (7.42 versus 4.66 µmol m^{-2} s^{-2}) and 70% higher in non-flooded than in flooded plants (7.60 versus 4.48 µmol m^{-2} s^{-2}). At the same time, the average g_{ssat} was 99% higher in shade than in sun leaves (56.3 versus 28.3 mmol m^{-2} s^{-2}) and 92% higher in non-flooded than in flooded plants (53.8 versus 28.1 mmol m^{-2} s^{-2}).

Discussion

When soil is flooded, oxygen deprivation changes soil physicochemical properties, reduces the soil redox potential (Eh) and changes overall plant metabolism (Kozlowski 1997, Pezeshki 2001). Soil flooding affects stomatal conductance, carbon assimilation, root metabolism and absorption of macronutrients (Pezeshki 2001, Kozlowski 1997, Kreuzwieser et al. 2004), by interrupting the flow of energy in the photosynthetic process and affecting the photosyn-

Table 4. Averages (± SD) and significance levels of a two-way ANOVA comparing the effects of light environment (L) and flooding (F) treatments and interactions between light and flooding (L × F) treatments on chlorophyll content index (CCI) and chlorophyll fluorescence of *E. uniflora* seedlings.

Variable	DAT	Light (L)		Flooding (F)		ANOVA		
		Shade	Sun	Flooded	Non-flooded	L	F	L × F
CCI	1	55.9 ± 2.2	54.2 ± 2.0	55.5 ± 2.1	54.6 ± 2.4	ns	ns	ns
	5	58.4 ± 2.0	55.6 ± 2.3	56.3 ± 2.8	57.8 ± 2.2	*	ns	ns
	14	61.2 ± 3.6	55.4 ± 3.1	56.6 ± 3.5	60.2 ± 4.6	**	**	ns
	22	61.7 ± 3.5	55.4 ± 3.1	57.4 ± 5.3	60.2 ± 4.6	**	*	ns
	27(5)	63.8 ± 4.3	55.2 ± 3.0	59.8 ± 6.5	59.1 ± 5.4	**	ns	ns
Fv/Fm	1	0.76 ± 0.04	0.78 ± 0.10	0.77 ± 0.06	0.77 ± 0.09	ns	ns	ns
	5	0.74 ± 0.01	0.68 ± 0.05	0.71 ± 0.05	0.71 ± 0.04	**	ns	ns
	14	0.69 ± 0.05	0.66 ± 0.06	0.67 ± 0.06	0.68 ± 0.05	ns	ns	ns
	22	0.72 ± 0.04	0.63 ± 0.10	0.62 ± 0.10	0.73 ± 0.03	**	**	*
	27(5)	0.75 ± 0.08	0.62 ± 0.02	0.65 ± 0.05	0.71 ± 0.11	**	*	*
Fo	1	197 ± 28	172 ± 58	186 ± 38	183 ± 55	ns	ns	ns
	5	208 ± 9	254 ± 44	240 ± 48	222 ± 26	**	ns	ns
	14	220 ± 32	220 ± 72	236 ± 63	204 ± 40	ns	ns	ns
	22	229 ± 34	247 ± 48	264 ± 39	211 ± 24	ns	**	ns
	27(5)	207 ± 69	307 ± 23	284 ± 38	229 ± 90	**	*	ns
Fm	1	831 ± 39	804 ± 115	830 ± 109	805 ± 53	ns	ns	ns
	5	797 ± 32	806 ± 100	827 ± 83	776 ± 52	ns	ns	ns
	14	712 ± 44	641 ± 116	706 ± 100	647 ± 78	ns	ns	ns
	22	807 ± 40	685 ± 73	713 ± 94	779 ± 62	**	**	ns
	27(5)	815 ± 18	806 ± 16	815 ± 16	807 ± 19	ns	ns	ns

DAT, days after transferring plants from the shade house to the sun and shade treatments. Values between parentheses mean the number of days after unflooding plants in the flooded treatment. For the measurements made 5 days after unflooding plants in the flooded treatment, $n = 3$. ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$.
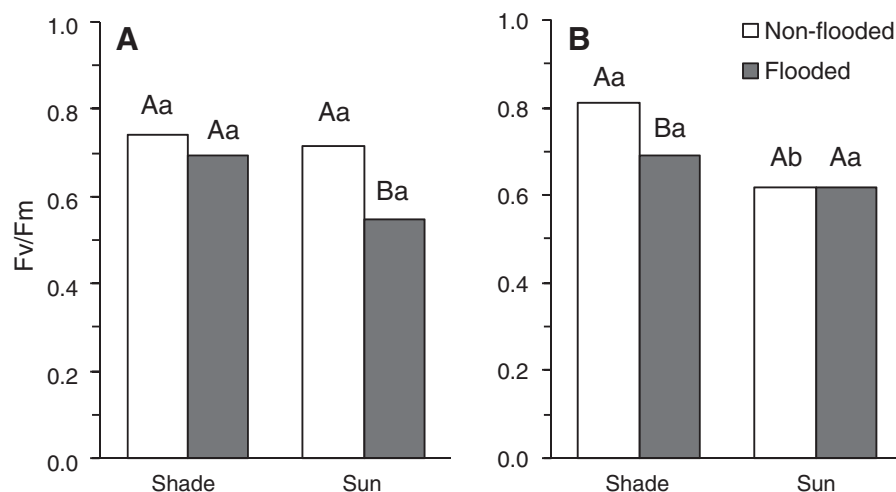
Figure 3. Maximum quantum efficiency of photosystem II (Fv/Fm) measured in the early morning (8:00–9:00 AM) 21 (A, $n = 5$) and 27 days (B, $n = 3$) after transferring plants from the shade house to the sun and shade treatments (5 days after flooded plants were unflooded). Different capital letters indicate differences between flooding treatments within light environments and different lower case letters represent differences between light intensity treatments within flooding treatments. Means followed by the same letter are not significantly different according to a standard *T*-test ($P < 0.05$).

Table 5. Average (± SD) and significance levels of a two-way ANOVA comparing the effects of light environment (L) and flooding (F) treatments, and interactions between light and flooding (L × F) treatments on reflectance derived indexes of *E. uniflora* seedlings. Measurements were done 27 days after transferring plants from the shade house to the sun and shade treatments, or 5 days after flooded plants were unflooded.

Variable	Light (L)		Flooding (F)		ANOVA		
	Shade	Sun	Flooded	Non-flooded	L	F	L × F
NDVI	0.70 ± 0.04	0.58 ± 0.03	0.65 ± 0.08	0.63 ± 0.07	**	ns	ns
sPRI	0.508 ± 0.005	0.474 ± 0.006	0.492 ± 0.020	0.490 ± 0.019	**	ns	ns
Red:Green	0.48 ± 0.04	0.46 ± 0.03	0.48 ± 0.05	0.46 ± 0.01	ns	ns	ns

NDVI, normalized difference vegetation index; sPRI, scaled photochemical reflectance index; Red:Green, red/green reflectance ratio. ns, $P > 0.05$; **$P \leq 0.01$.

thetic acclimation to changing light. At soil redox values below +350 mV, oxygen begins to disappear from the soil, inducing several changes in the overall plant metabolism (Pezeshki and DeLaune 1998). In the present experiment, 6 days after the soil was initially flooded, soil Eh decreased to values below −80 mV and remained around this value in both shade and sun treatments during the remainder of the flooding period, indicating that the roots of flooded plants were under anaerobic conditions (Pezeshki 2001).

Different authors have reported a decrease in chlorophyll content (Guo et al. 2006, Naramoto et al. 2006) or a decrease followed by recovery (Guo et al. 2006) during photosynthetic acclimation from low to high light. One month after transferring plants from the shade house to the sun and shade treatments, the CCI in the leaves of plants transferred to full sun that were not flooded was still higher than previously observed for *E. uniflora* leaves adapted to full sun (M.S. Mielke and B. Schaffer, unpublished data). While the average CCI of plants transferred from the shade house to full sun showed little change throughout the experiment, CCI increased ap-

proximately 12% from the first day after placing plants in shade to the last measurement date. Moreover, the gradual increase of CCI in the shade treatment and the small decrease in the sun treatment indicates that there was no chlorophyll degradation in leaves of *E. uniflora* after exposure to high light intensity (full sun).

The Fv/Fm is widely used as an indicator of photoinhibition of photosynthesis (Maxwell and Johnson 2000, Baker 2008). A rapid decrease in Fv/Fm followed by a subsequent recovery has been reported for trees transferred from low to high light (Krause et al. 2001, Cai et al. 2005, Houter and Pons 2005, Guo et al. 2006, Naramoto et al. 2006). In the current experiment, rapid decreases or recovery of Fv/Fm were not observed, and Fv/Fm gradually decreased in plants transferred to full sun, reaching minimum values of about 0.6, 27 days after transferring plants from the shade house to the sun treatment. The Fv/Fm values obtained 1 week after transferring plants from the shade house to the sun or shade treatments, both in non-flooded and flooded plants, were relatively high compared to other reports. For example, in a

Table 6. Average ($\pm$ SD) and significance levels of a two-way ANOVA comparing the effects of light environment (L) and flooding (F) treatments, and interactions between light and flooding (L $\times$ F) treatments on parameters derived from light–response curves and light-saturated stomatal conductance of water vapor (g_{ssat}) of *E. uniflora* seedlings.

DAT	Variable	Light (L)		Flooding (F)		ANOVA		
		Shade	Sun	Flooded	Non-flooded	L	F	L $\times$ F
18	LWA (g m^{-2})	86.2 $\pm$ 6.3	98.4 $\pm$ 10.2	91.1 $\pm$ 10.3	93.6 $\pm$ 10.9	*	ns	ns
	$A_{max-area}$ (μmol m^{-2} s^{-2})	5.46 $\pm$ 3.13	2.96 $\pm$ 2.29	2.17 $\pm$ 1.71	6.25 $\pm$ 2.50	*	**	ns
	Rd (μmol m^{-2} s^{-2})	0.65 $\pm$ 0.19	1.20 $\pm$ 0.52	0.90 $\pm$ 0.53	0.94 $\pm$ 0.44	*	ns	ns
	α (μmol μmol^{-2})	0.038 $\pm$ 0.012	0.036 $\pm$ 0.023	0.041 $\pm$ 0.022	0.033 $\pm$ 0.013	ns	ns	ns
	A_{max-wt} (nmol g^{-2} s^{-2})	63.8 $\pm$ 36.5	29.2 $\pm$ 21.1	24.8 $\pm$ 20.5	68.2 $\pm$ 31.0	**	**	ns
	g_{ssat} (mmol m^{-2} s^{-2})	32.6 $\pm$ 18.5	18.8 $\pm$ 13.5	13.0 $\pm$ 7.4	38.4 $\pm$ 14.5	*	**	ns
	A/g_s (μmol mol^{-2})	136.7 $\pm$ 38.3	59.2 $\pm$ 76.7	61.3 $\pm$ 80.8	134.6 $\pm$ 34.3	**	**	ns
30 (7)	LWA (g m^{-2})	83.4 $\pm$ 8.1	108.6 $\pm$ 12.5	94.1 $\pm$ 11.2	97.9 $\pm$ 21.2	*	ns	ns
	$A_{max-area}$ (μmol m^{-2} s^{-2})	7.42 $\pm$ 2.56	4.66 $\pm$ 2.27	4.48 $\pm$ 2.31	7.60 $\pm$ 2.28	*	**	ns
	Rd (μmol m^{-2} s^{-2})	0.57 $\pm$ 0.42	0.99 $\pm$ 0.59	0.59 $\pm$ 0.41	0.97 $\pm$ 0.61	ns	ns	ns
	α (μmol μmol^{-2})	0.041 $\pm$ 0.011	0.033 $\pm$ 0.010	0.032 $\pm$ 0.013	0.042 $\pm$ 0.005	*	*	ns
	A_{max-wt} (nmol g^{-2} s^{-2})	89.9 $\pm$ 32.2	42.1 $\pm$ 17.5	49.8 $\pm$ 28.8	82.2 $\pm$ 34.9	**	**	ns
	g_{ssat} (mmol m^{-2} s^{-2})	53.6 $\pm$ 20.9	28.3 $\pm$ 18.9	28.1 $\pm$ 17.9	53.8 $\pm$ 21.6	**	**	ns
	A/g_s (μmol mol^{-2})	131.8 $\pm$ 27.9	139.2 $\pm$ 46.7	140.3 $\pm$ 36.6	130.7 $\pm$ 39.9	ns	ns	ns

DAT, days after transferring plants from the shade house to the sun and shade treatments; LWA, leaf weight per area; A_{max}, light-saturated gross CO_2 assimilation rate; α, apparent quantum yield; Rd, dark respiration rate; g_{ssat}, stomatal conductance to water vapor at light saturation; A/g_s, intrinsic water use efficiency. Value between parentheses indicates the number of days after unflooding plants in the flooded treatment. ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$.

study of photosynthetic acclimation of six late-successional woody species from a tropical monsoon forest in China, Cai et al. (2005) reported that decreases in Fv/Fm measured at midday occurred about 5–10 days after plants were transferred from low light (4.5% full sun) to high light (24.5% full sun) where two understory shrubs (*Lasianthus attenuatus* and *Lasianthus hookeri*) and a canopy tree (*Pometia tomentosa*) had values between 0.5 and 0.6. Naramoto et al. (2006) also reported that leaves of *Fagus crenata* seedlings that developed in shade (0.5 mol m^{-2} day^{-1}) and were transferred to high (21 mol m^{-2} day^{-1}) and medium (9.8 mol m^{-2} day^{-1}) light reached Fv/Fm values measured in the early morning of about 0.08 and 0.37, 2 days after transfer from shade to high and medium light, respectively.

Photoinhibition of photosynthesis is also related to the amount of light during leaf growth and development before transference from low to high light. In our experiment, *E. uniflora* seedlings had grown in the shade house with 40% of full sun (about 12 mol m^{-2} day^{-1}) before transfer to the shade or full sun treatments. In a study with three species of a tropical genus *Garcinia*, Guo et al. (2006) reported that plants transferred from 4.5% to 40% of full sun exhibited a more drastic decrease in Fv/Fm 5 days after transference than plants transferred from 12.5% to 40% of full sun. Thus, the absence of drastic decreases in Fv/Fm after transferring seedlings of *E. uniflora* from the shade house to the sun or shade treatments may have been related to the relatively high light availability during leaf growth.

Differences in Fv/Fm between non-flooded and flooded plants indicated that flooding affected the photochemistry of photosynthesis in flooded plants in the shade treatment and flooded and non-flooded plants in the full sun treatment.

These differences were related to increases in Fo associated with no changes in Fm and CCI. Increases in Fo indicate photoinactivation of photosystem II reaction centers, which may be related to non-photochemical quenching accompanied by the excitation energy dissipation as heat rather than photochemical or oxidative damage and loss of photosystem II reaction centers (Baker 2008). The differences in Fv/Fm and Fo between sun and shade plants at the end of flooding period indicates that flooding plants after transferring them from the shade house to full sunlight had affected the flow of energy through the photosynthetic processes, making these plants susceptible to photoinhibition (Long et al. 1994). Gradual and constant decreases in Fv/Fm and increases in Fo may also indicate that the photosynthetic apparatus of *E. uniflora* leaves has a low capacity to acclimate to changes in light.

Differences between the average LWA in sun and shade treatments 18 days after transferring plants from the shade house to the sun and shade treatments were an average of 14% for non-flooded and flooded plants, whereas after 33 days differences increased to 44% for non-flooded plants. Similar results were observed by Naidu and DeLucia (1997) with leaves of *Acer saccharum* and *Quercus rubra* grown in a forest understory and transferred to a canopy gap. These differences may have been related to morphological changes during the transition from shade to sun, leading to increase in leaf thickness or carbohydrate accumulation as a result of increases in photosynthesis (Naidu and DeLucia 1997). Contrary to expectations, the largest increases in LWA occurred in non-flooded plants. Although we did not measure leaf starch content or leaf thickness, the differences in LWA between shade and sun plants may have been associated with morphological and biochemical changes. Starch

accumulation in leaves associated with changes in the source/sink relationship has been regarded as one of the main factors responsible for decreases in photosynthesis in plants subjected to soil flooding (Pezeshki 2001). Thus, for *E. uniflora*, a greater accumulation of starch would be expected in leaves of flooded plants than those of non-flooded plants if all plants had presented similar values of carbon assimilation. The lowest values of carbon assimilation observed in flooded plants may explain, at least in part, the lack of significant difference in LWA between flooded and non-flooded plants.

Values of $A_{max-area}$, A_{max-wt} and g_{ssat} in leaves of non-flooded plants were higher in leaves of plants transferred from the shade house to the shade than in leaves of plants transferred from the shade house to full sun. Previous reports indicate that sun-acclimated leaves have higher $A_{max-area}$, g_{ssat} and Rd and lower A_{max-wt} and α than shade-acclimated leaves (Valladares and Niinemets 2008). Leaf gas exchange results of the present study indicate that leaves of *E. uniflora* seedlings transferred from shade to full sun were unable to fully acclimate to the new light conditions even 1 month after transferring them from a shade house to the sun treatment. Naramoto et al. (2006) also found that the photosynthetic capacity of plants transferred to medium light did not reach the expected average gas exchange values of leaves that developed under those light conditions, while the photosynthetic capacity of leaves of plants transferred from medium to high light was lower than that of plants kept in medium light. In that case, the decrease in photosynthetic capacity was interpreted as damage to the photochemical and biochemical phases of photosynthesis because there were concomitant and dramatic decreases in Fv/Fm.

Eighteen days after transferring plants from the shade house to the sun or shade treatments, flooding induced a more pronounced decrease in A_{nsat} than g_{ssat}, and a strong decrease in the A/g_s was observed in flooded plants. Effects of soil flooding persisted 7 days after flooded plants were unflooded. In contrast, an increase in A/g_s was observed following increases in net CO_2 assimilation with little change in g_{ssat}. Comparing data of flooded plants 18 days after transferring plants from the shade house to the sun or shade treatments and 7 days after flooded plants were unflooded, increments in light-saturated net CO_2 assimilation rate ($A_{max-area}$ − Rd) and g_{ssat} were in the order of 206% and 116%, respectively. Those results indicate that stomatal limitations to photosynthesis persisted 7 days after soil drainage. Reductions in net CO_2 assimilation, Rd and g_{ssat} are common responses of tree species to soil flooding (Schaffer et al. 1992, Kozlowski 1997, Gravatt and Kirby 1998, Pezeshki and DeLaune 1998, Nuñez-Elisea et al. 1999, Gardiner and Krauss 2001, Mielke et al 2003, Lavinsky et al. 2007). In the present study, such changes were observed in *E. uniflora* transferred to sun and shade during soil flooding and after unflooding plants in the flooded treatment. In many flood-tolerant plants, increases in A/g_s occur due to decreases in g_s associated with maintenance of high photosynthetic rates (Mielke et al. 2005, Lavinsky et al. 2007).

The results obtained from reflectance indexes (NDVI, sPRI and Red:Green ratio) indicate that the content and composition of pigments changed in response to transferring *E. uniflora* from the shade house to full sun. The NDVI is an excellent index for estimating chlorophyll content and can be used in studies on leaf, canopy and ecosystem levels (Sims and Gamon 2002). Significant differences in NDVI between the sun and shade treatments corroborate the results observed for CCI. Leaves of *E. uniflora* growing in full sun generally have a reddish color due to anthocyanin accumulation. Anthocyanins are non-chloroplastidic pigments that have been related to photoprotection by absorbing the excess of blue light in leaves exposed to full sun (Close and Beadle 2005). However, the absence of significant differences in the Red:Green ratio between sun and shade treatments is an indication that the acclimation of leaves of *E. uniflora* to light does not involve changes in the synthesis of anthocyanins. In contrast, changes in sPRI in leaves of *E. uniflora* seedlings transferred to full sun appeared to be associated with changes in pigment composition and protective mechanisms against excess light.

The PRI has been successfully used to detect changes in leaf photosynthetic light use efficiency (Penuelas et al. 1997, Sims and Gamon 2002, Nichol et al. 2006, Letts et al. 2008). Even though different formulas have been used by different authors for PRI (Penuelas et al. 1997, Sims and Gamon 2002, Nichol et al. 2006, Letts et al. 2008) and comparisons among different plants in different studies are complicated by various PRI definitions, some studies have shown that changes in PRI are related to changes in the xanthophyll cycle and the pool size of xanthophylls and chlorophylls (Sims and Gamon 2002, Nichol et al. 2006). Nichol et al. (2006) found significant correlations among PRI, the effective quantum yield ($\Delta F/Fm'$) and non-photochemical quenching (NPQ) in a mangrove canopy, indicating that changes in PRI may be indicative of thermal energy dissipation by the xanthophyll cycle. It has been suggested that the increase in the quantity of xanthophylls and the activity of the xanthophyll cycle is related to the dissipation of excess of light energy (Demmig-Adams and Adams 1992).

In summary, our results indicate that the photosynthetic apparatus of *E. uniflora* leaves developed in shade has a relatively limited capacity to acclimate to changes in light intensity. There were no significant interactions between light intensity and flooding treatments for most of the variables analyzed with the exception of Fv/Fm 22 days after flooding and 5 days after flooded plants were unflooded. Changes in A/g_s indicated that both stomatal and non-stomatal limitations to photosynthesis were related to the low capacity of photosynthetic acclimation of flooded *E. uniflora* after transference from a shade house to full sun. Changes in CCI, NDVI and sPRI in leaves of *E. uniflora* seedlings transferred to full sun appear to be associated with changes in pigment composition and protective mechanisms against excess light. *E. uniflora* is a shrub or small tree commonly observed in gallery forests (Rodrigues and Nave 2000, Botrel et al. 2002, Bianchini

et al. 2003, Pinto et al. 2005), and, to our knowledge, there are no other studies addressing the interactions between soil flooding and photosynthetic acclimation in trees. The results of our study indicate that these factors, alone or in combination, may significantly affect the physiological performance of tree seedlings and understory shrubs in gallery forests during flooding conditions.

References

Araus, J.L. and K.P. Hogan. 1994. Leaf structure and patterns of photoinhibition in two neotropical palms in clearings and forest understory during the dry season. Am. J. Bot. 81:726–738.

Bailey, H., R.G. Walters, S. Jansson and P. Horton. 2001. Acclimation of *Arabidopsis thaliana* to light environment: the existence of separate low light and high light responses. Planta 213:794–801.

Baker, N.R. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59:89–113.

Bianchini, E., R.S. Popolo, M.C. Dias and J.A. Pimenta. 2003. Diversidade e estrutura de espécies arbóreas em área alagável do município de Londrina, sul do Brasil. Acta Bot. Bras. 17:405–419.

Bongers, F. and J. Popma. 1990. Leaf dynamics of seedlings of rain forest species in relation to canopy gaps. Oecologia 82:122–127.

Botrel, R.T., A.T. Oliveira-Filho, L.A. Rodrigues and N. Curi. 2002. Influência do solo e topografia sobre as variações da composição florística e estrutura da comunidade arbóreo-arbustiva de uma floresta estacional semidecidual em Ingaí. MG. Rev. Bras. Bot. 25:195–213.

Canham, C.D., J.S. Denslow, W.J. Platt, J.R. Runkle, T.A. Spies and P.S. White. 1990. Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. Can. J. For. Res. 20:620–631.

Close, D.C. and C.L. Beadle. 2005. Xanthophyll-cycle dynamics and rapid induction of anthocyanin synthesis in *Eucalyptus nitens* seedlings transferred to photoinhibitory conditions. J. Plant Physiol. 162:37–46.

Demmig-Adams, B. and W.W. Adams. 1992. Photoprotection and other responses of plants to light stress. Ann. Rev. Plant Physiol. Mol. Biol. 43:599–626.

Donadio, L.C., F.V. Moro and A.A. Servidone. 2002. Frutas Brasileiras. Jaboticabal, Novos Talentos. 288 p.

Evans, J.R. and H. Poorter. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen in maximizing carbon gain. Plant Cell Environ. 24:755–767.

Gamon, J.A. and J.S. Surfus. 1999. Assessing leaf pigment content and activity with a reflectometer. New Phytol. 143:105–117.

Gardiner, E.S. and K.W. Krauss. 2001. Photosynthetic light response of flooded cherrybark oak (*Quercus pagoda*) seedlings grown in two light regimes. Tree Physiol. 21:1103–1111.

Gomes, F.P., M.A. Oliva, M.S. Mielke, A.-A. F. de Almeida and H. G. Leite. 2006. Photosynthetic irradiance-response in leaves of dwarf coconut palm (*Cocos nucifera* L. 'nana', Arecaceae): comparison of three models. Sci. Hort. 109:101–105.

Guo, X. R., K. F. Cao and Z. F. Xu. 2006. Acclimation to irradiance in seedlings of three tropical rain forest Garcinia species after simulated gap formation. Photosynthetica 44:193–201.

Gravatt, D.A. and C.J. Kirby. 1998. Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. Tree Physiol. 18:411–417.

Hawkins, T.S., E.S. Gardiner and G.S. Comer. 2009. Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. J. Nat. Conserv. doi:10.1016/j.jnc.2008.12.007.

Herrera, A., W. Tezara, O. Marín and E. Rengifo. 2008. Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. Physiol. Plant. 134:41–48.

Houter, N.C. and T.L. Pons. 2005. Gap size effects on photoinhibition in understorey saplings in tropical rainforest. Plant Ecol. 179:43–51.

Iqbal, R.M., A-R. Rao, E. Rasul and A. Wahid. 1997. Mathematical models and response functions in photosynthesis: an exponential model. Handbook of photosynthesis. M. Marcel Dekker, New York, pp 803–810.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monog. 1:1–29.

Krause, G.H., O.Y. Koroleva, J.W. Dalling and K. Winter. 2001. Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. Plant Cell Environ. 24:1345–1352.

Kreuzwieser, J., E. Papadopoulou and H. Rennenberg. 2004. Interaction of flooding with carbon metabolism of forest trees. Plant Biol. 6:299–306.

Landsberg, J.J. 1986. Physiological ecology of forest production. Academic, London.

Lavinsky, A.O., C.S. Sant'Ana, M.S. Mielke, A-A.F. de Almeida. F.P. Gomes, S. França and D.C. Silva. 2007. Effects of light availability and soil flooding on growth and photosynthetic characteristics of *Genipa 15americana* L. seedlings. New For. 34:41–50.

Lee, D.W., B. Krishnapilay, M. Mansor, H. Mohamad and S.K. Yap. 1996. Irradiance and spectral quality affect Asian tropical rain forest tree seedling development. Ecology 77:568–580.

Letts, M.G., C.A. Phelan, D.R.E. Johnson and S.B. Rood. 2008. Seasonal photosynthetic gas exchange and leaf reflectance characteristics of male and female cottonwoods in a riparian woodland. Tree Physiol. 28:1037–1048.

Long, S.P., S. Humphries and P.G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:633–662.

Lüttige, U. 2008. Physiological ecology of tropical plants. Springer, Berlin, pp 15–458.

Margis, R., D. Felix, J.F. Caldas, F. Salgueiro, D.S.D. De Araujo, P. Breyne, M. Van Montagu, D. De Oliveira and M. Margis-Pinheiro. 2002. Genetic differentiation among three neighboring Brazil-cherry (*Eugenia uniflora* L.) populations within the Brazilian Atlantic rain forest. Biodivers. Conserv. 11:149–163.

Markwell, J., J.C. Osterman and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth. Res. 46:467–472.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence: a practical guide. J. Exp. Bot. 51:659–668.

Melo, R.M., V.F.S. Corrêa, A.C.L. Amorim, A.L.P. Miranda and C.M. Rezende. 2007. Identification of impact aroma compounds in *Eugenia uniflora* L. (Brazilian Pitanga) leaf essential oil. J. Braz. Chem. Soc. 18:179–183.

Mielke, M.S, A.-A.F. Almeida, F.P. Gomes, A.G. Aguilar and P.A.O. Mangabeira. 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. Env. Exp. Bot. 50:221–231.

Mielke, M.S., A.-A.F. Almeida, F.P. Gomes, P.A.O. Mangabeira and D.C. Silva. 2005. Effects of soil flooding on leaf gas exchange and growth of two neotropical pioneer tree species. New For. 29:161–168.

Naidu, S.L. and E.H. DeLucia. 1998. Physiological and morphological acclimation of shade-grown tree seedlings to late-season canopy gap formation. Plant Ecol. 138:27–40.

Naramoto, M., S-i. Katahata, Y. Mukai and Y. Kakubari. 2006. Photosynthetic acclimation and photoinhibition on exposure to high light in shade-developed leaves of *Fagus crenata* seedlings. Flora 201:120–126.

Nichol, C.J., U. Rascher, S. Matsubara and B. Osmond. 2006. Assessing photosynthetic efficiency in an experimental mangrove canopy using remote sensing and chlorophyll fluorescence. Trees 20:9–15.

Nuñez-Elisea, R., B. Schaffer, J.B. Fisher, A.M. Colls and J.H. Crane. 1999. Influence of flooding on net CO_2 assimilation, growth and stem anatomy of *Annona* species. Ann. Bot. 84:771–780.

Ogunwande, I.A., N.O. Olawore, O. Ekundayo, T.M. Walker, J.M. Schmidt and W.N. Setzer. 2005. Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. Intern. J. Aromatherapy 15:147–152.

Ojeda, M.G., B. Schaffer and F.S. Davies. 2004. Flooding, root temperature, physiology, and growth of two *Annona* species. Tree Physiol. 24:1019–1025.

Oliveira, A.L., R.B. Lopes, F.A. Cabral and M.N. Eberlin. 2005. Volatile compounds from pitanga fruit (*Eugenia uniflora* L.). Food Chem 99:1–5.

Penuelas, J., J. Llusia, J. Pinol and I. Filella. 1997. Photochemical reflectance index and leaf radiation-use efficiency assessment in Mediterranean trees. Int. J. Remote. Sens. 13:2863–2868.

Pezeshki, S.R. 2001. Wetland plant responses to soil flooding. Env. Exp. Bot. 46:299–312.

Pezeshki, S.R. and R.D. DeLaune. 1998. Responses of seedlings of selected woody species to soil oxidation-reduction conditions. Env. Exp. Bot. 40:123–133.

Pinkard, E.E., V. Patel and C. Mohammed. 2006. Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using a non-destructive meter. For. Ecol. Manage. 223:211–217.

Pinto, J.R.R., A.T. Oliveira-Filho and J.D.V. Hay. 2005. Influence of soil and topography on the composition of a tree community in a central Brazilian valley Forest. Edinburgh J. Bot. 62:69–90.

Rodrigues, R.R. and A.G. Nave. 2000. Heterogeneidade florística das matas ciliares. *In* Matas Ciliares. Eds. R.R. Rodrigues and H.F. Leitão Filho. EDUSP, São Paulo, pp 45–71.

Scalon, S.P.Q., H. Scalon Filho, M.R. Rigoni and F. Veraldo. 2001. Germinação e crescimento de mudas de pitangueira (*Eugenia uniflora* L.) sob condições de sombreamento. Rev. Bras. Frutic. 23:652–655.

Schaffer, B., P.C. Andersen and R.C. Ploetz. 1992. Responses of fruit crops to flooding. Hort. Reviews 13:257–313.

Schapoval, E.S., S.M. Silveira, L. Miranda, C.B. Alice and A.T. Henriques. 1994. Evaluation of some pharmacological activities of *Eugenia uniflora* L. J. Ethnopharmacol. 44:137–142.

Schmeda-Hirschmann, G., C. Theoduloz, L. Franco, B.E. Ferro and A. Rojas de Arias. 1987. Preliminary pharmacological studies on *Eugenia uniflora* leaves: xanthine oxidase inhibitory activity. J. Ethnopharmacol. 21:183–186.

Sims, D.A. and J.A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81:337–354.

Torres Netto, A., E. Campostrini, J.G. Oliveira and O.K. Yamanishi. 2002. Portable chlorophyll meter for the quantification of photosynthetic pigments, nitrogen and the possible use for assessment of the photochemical process in *Carica papaya* L. Braz. J. Plant Physiol. 14:203–210.

Valladares, F. and U. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annu. Rev. Ecol. Evol. Syst. 39:237–257.

Wagner, P.A. and R. Dreyer. 1997. Interactive effects of waterlogging and irradiance on the photosynthetic performance of seedlings from three oak species displaying different sensitivities (*Quercus robur*, *Q. petraea* and *Q. rubra*). Ann. Sci. For. 54:409–429.

Walters, R.G. 2005. Towards an understanding of photosynthesis acclimation. J. Exp. Bot. 56:435–447.