Use of a SPAD meter to estimate chlorophyll content in *Eugenia uniflora* L. leaves as affected by contrasting light environments and soil flooding

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Abstract

In three separate experiments, the effectiveness of a *SPAD-502* portable chlorophyll (Chl) meter was evaluated for estimating Chl content in leaves of *Eugenia uniflora* seedlings in different light environments and subjected to soil flooding. In the first experiment, plants were grown in partial or full sunlight. In the second experiment plants were grown in full sunlight for six months and then transferred to partial sunlight or kept in full sunlight. In the third experiment plants were grown in a shade house (40% of full sunlight) for six months and then transferred to partial shade (25–30% of full sunlight) or full sunlight. In each experiment, plants in each light environment were either flooded or not flooded. Non-linear regression models were used to relate SPAD values to leaf Chl content using a combination of the data obtained from all three experiments. There were no significant effects of flooding treatments or interactions between light and flooding treatments on any variable analyzed. Light environment significantly affected SPAD values, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll [Chl (*a*+*b*)] contents in Experiment I ($p \leq 0.01$) and Experiment III ($p \leq 0.05$). The relationships between SPAD values and Chl contents were very similar among the three experiments and did not appear to be influenced by light or flooding treatments. There were high positive exponential relationships between SPAD values and Chl (*a*+*b*), Chl *a*, and Chl *b* contents.

Additional key words: forest restoration; gallery forests; light acclimation; Myrtaceae.

Introduction

Analysis of pigment composition of leaves is important in plant ecophysiological studies, providing key information about physiological responses to environmental factors such as light (Valladares and Niinemets 2008), drought (Yordanov *et al.* 2000), and flooding (Kozlowski 2002). Destructive methods for pigment determinations are accurate but expensive and time consuming (Arnon 1949, Hiscox and Israelstam 1979, Porra *et al.* 1989). In some cases, when stress factors induce very rapid changes in pigment content, the need for more intensive analysis is hampered by the time required for sample collection, extraction and quantification of pigment contents. This may become a problem when the distance and time required for bringing the material from the field to the laboratory affects the accuracy of results. Nondestructive methods for determining leaf Chl content present advantages by allowing rapid and repeated measurements of the same leaves over time (Cate and Perkins 2003, Nickum *et al*. 2010).

Hand-held Chl meters have been shown to be accurate for estimating foliar Chl and nitrogen contents in several plant species (Torres-Netto *et al.* 2002, Chang and Robison 2003, Marenco *et al.* 2009). Hand-held devices such as the *SPAD-502* Chl meter measure light transmittance at two wavelengths corresponding to red and far-red, and transform this data to SPAD values (Markwell *et al.* 1995). However the SPAD meter provides only a simple index and analysis of leaf pigment content and composition requires calibrating SPAD values with actual measurements of pigments using

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Abbreviations: Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; PPFD – photosynthetic photon flux density.
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destructive methods. The mathematical relationship between SPAD values and leaf pigment content varies among different types of leaf pigments (Torres-Netto *et al.* 2002, 2005) as well as different plant species (Pinkard *et al.* 2006, Marenco *et al*. 2009), and sometimes environmental growth conditions (Campbell *et al.* 1990).

Light and soil flooding are important environmental factors affecting leaf pigment composition and content (Mielke and Schaffer 2010a). In general, leaves developed in shade contain more Chl and have a lower Chl *a*/*b* ratio than leaves developed in full sun (Valladares and Niinemets 2008). Moreover, losses of Chl and leaf chlorosis are typical symptoms of stress observed in tropical or temperate tree species subjected to soil flooding (Gravatt and Kirby 1998, Gardiner and Krauss 2001). Studies with tree species in tropical and temperate ecosystems have shown that both light and soil flooding affect leaf senescence and the content and composition of leaf pigments (Gardiner and Krauss 2001, Lavinsky *et al.* 2007).

Gallery forests are ecosystems bordering waterways, which are very important for the maintenance of water resources, providing a variety of ecological services, such as control of floods and erosion, removal of nutrients from agricultural runoff, and alleviation of the effects of environmental pollution, and as habitats for local fauna (Kozlowski 2002). In many places, particularly in South America, the expansion of agricultural and urban areas have caused the partial or total removal of gallery forests, and restoration programs are required and have been implemented by governmental and nongovernmental organizations (Cullen Jr. *et al.* 2005, Tabarelli *et al.* 2005). In gallery forests, soil flooding and light availability are among the main environmental factors affecting tree seedling survival and growth. Changes in these factors occur because this type of ecosystem is very unstable and prone to intermittent changes in volume and flow of water through rivers, resulting in soil flooding and changes in light availability. Considering that changes in pigment content are within the main responses of leaves exposed to high light and soil flooding (Lavinsky *et al.* 2007, Mielke and Schaffer 2010b) the use of hand-held Chl meters could be very useful to provide information about the physiological performance of seedlings of tree species after planting in gallery forest restoration projects. To our knowledge, no studies have been published relating SPAD values and Chl contents and composition in leaves of plants exposed to soil flooding in different light environments.

E. uniflora L. (Myrtaceae) is a shrub or small tree, between 4 to 8 m in height, native to South America (Margis *et al.* 2002). *E. uniflora* is ecologically important as a colonizing species in disturbed areas and as a food source for local fauna (Margis *et al.* 2002). Studies have shown that *E. uniflora* is a high-light-demanding species and moderately sensitive to soil flooding (Mielke and Schaffer 2010a,b). There are countless references demonstrating the presence of *E. uniflora* in gallery forests (Rodrigues and Nave 2000), indicating its potential use in gallery forest restoration projects. Despite its ecological and social importance little is known about ecophysiological responses of *E. uniflora* to changing environments.

Considering the importance of this species for gallery forest restoration programs, there is a need to be able to rapidly evaluate physiological stress caused by flooding in changing light environments typically found in gallery forests. The use of a hand-held Chl meter may be ideal for rapid, nondestructive assessment of physiological stress of trees, such as *E. uniflora* in these forests. There are a scarcity of references on the use of hand-held Chl meters for estimating leaf pigments in trees subjected to soil flooding and different light availabilities. Therefore, the purpose of this study was to analyze the effectiveness of a portable Chl meter (*SPAD-502*) to estimate Chl content in leaves of *E. uniflora* seedlings subjected to soil flooding in different light environments.

Materials and methods

In spring 2008, *E. uniflora* L. seedlings were obtained from a commercial nursery located in Homestead, Florida, USA. Plants were cultivated in 10-l plastic containers (10–12 plants per container) with a standard nursery substrate of 65% pine bark, 25% Florida peat, and 10% coarse sand by volume. Plants were carefully selected to obtain a uniform sample. Three independent experiments were conducted at the Tropical Research and Education Center, University of Florida (TREC/UF), Homestead, Florida, USA (25.5°N, 80.5°W). The seedlings were about one year old at the time of transference to the TREC/UF. At this stage, the average height of the plants per container was 0.5–0.8 m and the average stem diameter 0.10 m above the soil surface was 3–10 mm. In an open field at TREC/UF, 3 × 2 m (each area sufficient for ten containers) blocks were selected perpendicular to the daily track of the sun permitting plants in full sunlight to receive almost all solar radiation during the day. Shade cages (3 × 2 × 1.7 m) were constructed with PVC tubes covered with one layer of a neutral shade netting (25–30% of full sunlight) and placed on 5 blocks. The remaining five blocks were left open (no cages). Cages were spaced far enough apart so that they did not shade the open blocks. All experiments were arranged in a completely randomized design with a 2 × 2 factorial arrangement consisting of two light treatments (partial and full sunlight), two flood treatments (flooded and nonflooded) and 5 replications (blocks) per treatment. During each experiment, plants were irrigated three times per day with an automatic irrigation system.

Experiment I was conducted from spring to summer (April, 2008 to September, 2008). Five containers were placed in either partial (inside a cage) or full sunlight for a period of 55 days. After that period, half the containers in each light environment were subjected to soil flooding and the remaining plants were not flooded. Plants in the flooded treatment were flooded by placing each container in a 19-l plastic bucket filled with tap water to 50–100 mm above the soil surface to ensure complete inundation of the root system. After 36 days of flooding, flooded plants were removed from the buckets and the substrate was drained. Experiments II and III were conducted from late summer to fall (September, 2008 to November, 2008). In Experiment II, 40 containers were kept in full sunlight from March 2008 to October 2008. In October 2008, half of the containers were transferred to the same shade cages used in Experiment I, and half remained in full sunlight. In Experiment III, 40 containers were placed in a shade house (about 40% of full sunlight) in March 2008. The plants remained in the shade house until October 2008, when half the containers were transferred to full sunlight and half to the same shade cages described for Experiment I. In Experiments II and III half of the plants in each light treatment were flooded for 23 days and the remaining plants were maintained with soil near field capacity as described for Experiment I.

Throughout each experiment, the photosynthetic photon flux density (PPFD) was measured in each light treatment with model *LI-190SA* quantum sensors connected to *LI-1000* data loggers (*Li-Cor*, Lincoln, Nebraska, USA). Daily average, minimum, and maximum air temperatures and relative humidity were obtained from a weather station of the Florida Automated Weather Network (http://fawn.ifas.ufl.edu/) located 50 m from the experimental plots. Air temperature, relative humidity and total daily PPFD during the three experiments are shown in Table 1. In each experiment, the light intensity in the partial sunlight treatment was about 26% of that of the full sun treatment. The PPFD values in Experiments II and III were half as high as those obtained in Experiment I, in both partial (6.9 *vs*. 10.8 mol m^{-2} d^{-1}) and full sunlight (26.0 *vs.* 41.0 mol m^{-2} d^{-1}).

In Experiment I, leaves were collected in four of the five blocks (three leaves per treatment/container, totalizing 12 leaves per treatment or 48 leaves in total) and 10 leaves in advanced stage of senescence (yellow leaves) were also harvested only from plants growing in full sunlight. Leaves were harvested about one week after substrate drainage, while the plants still had symptoms of stress, such as premature senescence of old leaves, as well as low net photosynthesis and stomatal conductance in the new and mature leaves (data not shown). Due to high and intermittent rainfall at the end of flooding period in Experiment I, leaves were placed in a cooler filled with ice and immediately taken to the laboratory where estimates of leaf Chl content were determined with a *SPAD-502* Chl meter (*Minolta Camera*, Osaka, Japan). The time between the collection of leaves and SPAD measurements was approximately 5 min. Soon after SPAD measurements were made, the leaf pigments were extracted and quantified. For Experiments II and III leaves were collected in all five blocks. Five leaves per treatment (one leaf per repetition for each treatment) were collected and quantified following the same procedures used in Experiment I. Extractions were done 18 and 20 d after plants were flooded, for Experiments II and III, respectively. In Experiment I, leaves that had developed in partial or full sunlight were used. In Experiment II, we used leaves developed in full sunlight and leaves acclimatized for 18 d to partial sunlight. In Experiment III, we used only leaves that had developed in the shade house but were collected after an acclimation period of 20 d to the partial or full sunlight. All measurements were made in the morning.

In all experiments one SPAD measurement per leaf was made, always on the middle of one side of the leaf blade. After SPAD measurements, one 1.1-cm^2 (about 222 mg of fresh mass) leaf disc was collected from each leaf with a leaf punch (from the same areas where SPAD values were measured). We used cardboard with a hole of about 1.1 cm^2 to be sure that leaf discs were taken from the exactly same place where SPAD measurements were made. The leaves used in all experiments had areas varying between 6 and 10 cm^2. For all SPAD measurements, the factory default compensation value (0.0) was used. Chls were extracted by grinding the leaf discs in 1 ml of 100% acetone with a pinch of calcium carbonate in a mortar. The extract was poured into a test tube and the mortar was rinsed with 100% acetone. The rinsate was then poured into the test tube to bring the final

Table 1. Air temperature, relative humidity and total daily photosynthetic photon flux density (PPFD) during the experiments. The data indicate the mean followed by minimum and maximum observed values.

		Experiment I	Experiments II and III
Temperature [°C]	Average	26.5 (22.3–28.9)	21.5 (15.1–26.7)
	Min	22.2 (14.3–27.8)	17.1 (6.5–24.0)
	Max	31.3 (26.0–33.9)	26.3 (19.1–30.9)
Relative humidity [%]		80 (64–92)	80 (56–91)
PPFD [mol m^{-2} d^{-1}]	Partial sunlight	10.8 (1.3–15.8)	6.9 (0.9–11.4)
	Full sunlight	41.0 (5.0–59.9)	26.0 (3.2–43.5)

extract to 5 ml. The extract was filtered through a 0.45 μm syringe filter to remove debris. The absorbance of the filtered extract was then determined with a spectrophotometer (*DU-640, Beckman Coulter,* Fullerton, California, USA). Chl *a* and Chl *b* contents were measured at absorbance wavelengths of 663 (A_{663}) and 645 nm (A_{645}) and concentrations were calculated using the equations given by Hendry and Price (1993).

Data on the effects of light environment and flooding treatments on SPAD values and Chl contents were analyzed by two-way *ANOVA*, with four replications for Experiment I and five replications for Experiments II and III. Nonlinear [$y = a\exp(bx)$] regression models were used to relate SPAD values to leaf Chl content. For nonlinear regression, all measurements were used. All statistical analyses were done using the *SAS* statistical software package (*SAS Institute*, Cary, North Carolina, USA).

Results

Light environment significantly affected the SPAD values, Chl *a*, Chl *b* and Chl (*a*+*b*) contents in Experiment I ($p \leq 0.01$) and Experiment III ($p \leq 0.05$) (Table 2). There were no significant effects of flooding or interactions between light- and flooding treatments on any variable analyzed. In Experiment I, the average values of SPAD, Chl *a*, Chl *b*, and Chl (*a*+*b*) were respectively 41%, 87%, 99%, and 90% higher for leaves of plants in partial than in full sunlight. In Experiment III the average values of SPAD, Chl *a*, Chl *b*, and Chl (*a*+*b*) were respectively 22%, 38%, 32%, and 37% higher for leaves in the partial than in the full sunlight treatment.

The lowest Chl (*a*+*b*) content (223.6 μmol m^{-2}) and SPAD value (36.1) were observed for plants in full sun that were not flooded in Experiment I, whereas the highest values were observed in plants in partial sunlight that were not flooded in Experiment II [674.2 μmol m^{-2} and 57.8 for Chl (*a*+*b*) and SPAD value, respectively] (Fig. 1). Senescent leaves (collected only in full sun, in Experiment I) had the lowest SPAD value and Chl *a*, Chl *b* and Chl (*a*+*b*), among all leaves examined. Based on all data (n = 98) the SPAD values and Chl *a*, Chl *b* and Chl (*a*+*b*) contents varied from 11.6 to 67.5, 42.1 to 629.5 μmol m^{-2}, 9.8 to 255.2 μmol m^{-2}, 51.9 to 884.6 μmol m^{-2}, and 23.3 to 205.1 μmol m^{-2}, respectively.

In all three experiments, SPAD values were positively correlated with Chl *a*, Chl *b*, and Chl (*a*+*b*) content (Fig. 1). The coefficients of determination (r^2) for the regression models relating SPAD to Chl *a* and Chl *b* were between 0.92 for Chl *a* (Fig. 1*A*) and 0.74 for Chl *b* (Fig. 1*B*). A high r^2 value (0.89) was also observed for the relationship between SPAD values and Chl (*a*+*b*) (Fig. 1*C*).

Discussion

In full sunlight the average Chl contents tended to be higher in Experiments II and III than in Experiment I, which can be explained by the higher total daily PPFD in summer than in fall (Table 1). In Experiment I the response to different light environments was typical for sun or shade leaves (Valladares and Niinemets 2008). This indicates that *E. uniflora* has a great plasticity and high capacity to acclimate to low- or high-light environments (Valladares *et al.* 2000). However, based on the results of Experiment II, it is possible to conclude that when the leaves of *E. uniflora* were developed in full sunlight they have a limited capacity to acclimate to a new environment when transferred from full to partial sunlight. In contrast, as observed from the results obtained in Experiment III, *E. uniflora* leaves developed in partial sunlight had characteristics that allowed partial acclimation when the plants were transferred from partial to full sunlight.

Leaf chlorosis and Chl degradation are symptoms of stress observed in tropical or temperate tree species subjected to soil flooding (Gravatt and Kirby 1998, Gardiner and Krauss 2001, Lavinsky *et al.* 2007). As shown in Table 2, despite the small decreases in Chl *a* and Chl (*a*+*b*) observed in flooded plants, the greatest differences were found between light treatments and no significant interactions were observed between light- and flooding treatments. Recent results have shown that *E. uniflora* is moderately sensitive to soil flooding (Mielke and Schaffer 2010a,b). Although flooded plants had typical symptoms of flooding stress such as senescence of old leaves, reductions in photosynthesis and stomatal conductance and decreases in biomass (Mielke and Schaffer 2010b), other stress symptoms such as leaf chlorosis and Chl degradation did not appear to be associated with susceptibility to soil flooding in this species.

The leaf Chl contents observed in Experiments I, II, and III are in agreement with previous reports for temperate (Gitelson *et al.* 2003, Naramoto *et al.* 2006), subtropical (Jifon *et al.* 2005), and tropical trees (Torres-Netto *et al.* 2005). For example, Gitelson *et al.* (2003) reported values of total Chl ranging from 97 to 832 μmol m^{-2} for *Fagus sylvatica*, and from 10 to 470 μmol m^{-2} for *Aesculus hippocastanum*, and Torres-Netto *et al.* (2005) reported maximum values of Chl *a*, Chl *b*, and Chl (*a*+*b*) contents of 600, 180, and 800 μmol m^{-2} in *Coffea canephora* trees.

High r^2 values were reported for the regression models relating SPAD values to Chl (*a*+*b*) content for other tree species (Richardson *et al.* 2002, Torres-Netto *et al.* 2002, Cate and Perkin 2003, van den Berg and Perkin 2004, Torres-Netto *et al.* 2005, Pinkard *et al.* 2006, Amarante *et al.* 2008, Marenco *et al.* 2009). Nonlinear

Table 2. Significance levels from the two-way *ANOVA* to compare the effects of light environments (L) and flooding (F) treatments, and interactions between light and flooding (L × F) treatments on SPAD values and chlorophyll (Chl) contents [μmol m^{-2}] for leaves of *E. uniflora* seedlings in three independent experiments (Exp). Data are the means ± standard deviations of all replications within each factor (light environment or soil flooding). * – $p \leq 0.05$; ** – $p \leq 0.01$; ns – $p > 0.05$.

Source	Variable	Light (L)		Flooding (F)		ANOVA		
		Partial sunlight	Full sunlight	Flooded	Nonflooded	L	F	L × F
Exp I	SPAD	51.0 ± 5.8	36.3 ± 4.1	41.6 ± 6.4	45.7 ± 11.0	**	ns	ns
	Chl *a*	320.7 ± 45.3	171.1 ± 36.9	234.5 ± 69.9	257.3 ± 104.9	**	ns	ns
	Chl *b*	104.6 ± 30.6	52.5 ± 17.3	87.3 ± 40.4	69.9 ± 31.3	**	ns	ns
	Chl (*a*+*b*)	425.3 ± 59.7	223.7 ± 53.1	321.8 ± 109.7	327.2 ± 132.5	**	ns	ns
Exp II	SPAD	57.7 ± 5.7	56.7 ± 5.9	56.6 ± 5.1	57.8 ± 6.4	ns	ns	ns
	Chl *a*	462.7 ± 93.7	392.0 ± 68.5	421.3 ± 78.2	433.4 ± 100.3	ns	ns	ns
	Chl *b*	188.7 ± 38.6	173.7 ± 28.4	182.0 ± 31.3	180.4 ± 38.0	ns	ns	ns
	Chl (*a*+*b*)	651.4 ± 132.0	565.7 ± 95.6	603.3 ± 107.8	613.8 ± 137.6	ns	ns	ns
Exp III	SPAD	44.9 ± 5.5	36.9 ± 7.9	41.3 ± 6.3	40.5 ± 9.4	*	ns	ns
	Chl *a*	281.0 ± 55.6	203.2 ± 64.1	231.4 ± 55.1	252.8 ± 85.3	*	ns	ns
	Chl *b*	121.4 ± 26.1	91.7 ± 27.8	107.2 ± 25.3	105.8 ± 36.1	*	ns	ns
	Chl (*a*+*b*)	402.4 ± 79.9	294.8 ± 90.7	338.7 ± 79.3	358.6 ± 120.6	*	ns	ns

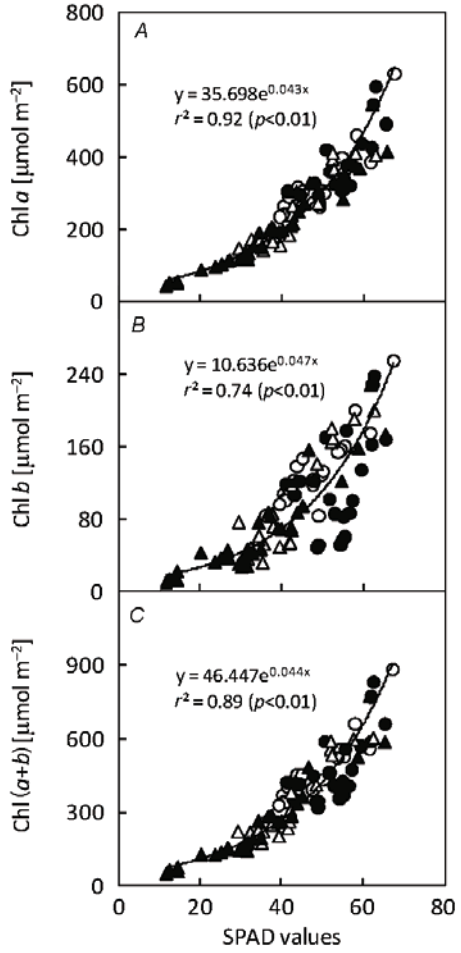

Fig. 1. Relationships between SPAD values and *A*: chlorophyll *a* (Chl *a*), *B*: chlorophyll *b* (Chl *b*) and *C*: total chlorophyll [Chl (*a*+*b*)] in *E. uniflora* leaves. (n = 98). Partial sunlight + nonflooded (●), full sunlight + nonflooded (▲), partial sunlight + flooded (○) and full sunlight + flooded (Δ).

relationships between SPAD values and Chl content were reported for *Betula papyrifera* (Richardson *et al.* 2002), *Carica papaya* (Torres-Netto *et al.* 2002), *Coffea canephora* (Torres-Netto *et al.* 2005), *Eucalyptus nitens* and *E. globulus* (Pinkard *et al.* 2006), *Vitis labrusca* hybrid (Steele *et al.* 2008) and *Lindera* sp. (Hawkins *et al.* 2009). These nonlinear responses have been attributed to decreases in the accuracy of the SPAD meter when Chl content is high (Steele *et al.* 2008). According to Udling *et al.* (2007), the nonlinear relationships between Chl content and SPAD values are due to a nonuniform distribution of Chl across the leaf and high light scattering causing deviations from linearity at high and low SPAD values. In our study, nonlinear regression was successfully used for estimating Chl contents from SPAD measurements.

Despite the high coefficients of determination (r^2 values) of the nonlinear regression models for estimating the relationship between SPAD values and Chl *a* or Chl (*a*+*b*), a relatively low r^2 value was observed for the relationship between SPAD values and Chl *b* (Fig. 2*B*). In this case, the high degree of variability among measurements may have been related to the light environment during leaf development in Experiment I (Table 1). Leaves developed in high light have a tendency to have higher Chl *a*/*b* ratios than leaves developed in low light (Niinemets and Valladares 2008). These differences may be associated with changes in the ratio between light-harvesting complexes and reaction centers because Chl *b* is located on the light-harvesting Chl *a*/*b* binding protein complexes of photosystem II (LHCII) (Pearcy 2000). In Experiment I, leaves had a higher Chl *a*/*b* ratio than in Experiments II or III. The average Chl *a*/*b* ratios were about 3.5 in Experiment I and 2.5 in Experiments II and III; and a total of 13 values for the Chl *a*/*b* ratio exceeded 4.0 in Experiment I. Those

differences seemed to be related to the light environment in Experiment I, which was almost twice that of Experiments II or III (Table 1). After removing Chl *a*/*b* ratios equal to or higher than 4.0 from the analysis, the r^2 value for the relationship between SPAD and Chl *b* increased to 0.83; but did not change substantially for SPAD *vs*. Chl *a* or SPAD *vs*. Chl (*a*+*b*) (data not shown).

Another possible explanation for the influence of data from Experiment I on the relatively low r^2 value for the regression model relating SPAD to Chl *b* is the fact that SPAD measurements were made on detached leaves in the laboratory. Some studies have demonstrated that the time of measurement, irradiance, and leaf water status may affect the accuracy of SPAD measurements (Hoel and Solhaug 1998, Martínez and Guimet 2004). In those studies, the authors concluded that SPAD values were more accurate if measurements were made in the morning; as we did in all the three experiments. Also, previous studies showed that SPAD values tended to increase when leaves were transferred from high to low light (Hoel and Solhaug 1998, Martínez and Guimet 2004), as well as when leaves were dehydrated (Martínez and Guiamet 2004). In Experiment I, the relatively poor relationship between SPAD values and Chl *b* occurred when SPAD values were lower than expected, and the equation changed from $y = 10.636e^{0.047}$ ($r^2 = 0.74$, $n = 98$) (Fig. 1*B*) to $y = 11.747e^{0.047}$ ($r^2 = 0.83$, $n = 85$) when Chl *a*/*b* values ≥ 4.0 were removed from the analyses. Therefore, we do not believe that the experimental procedures in Experiment I caused distortions in the model for estimating of Chl *b*.

Other factors, such as the method of pigments extraction (Tait and Hik 2003) may affect the relationship between SPAD values and Chl concentrations. Traditional methods for extraction of leaf pigments involve the use of acetone (Arnon 1949). However, in recent decades the extraction of pigments using acetone has been gradually replaced by other methods using solvents such as dimethylsulphoxide (DMSO) (Hiscox and Israelstam 1979, Tait and Hik 2003) and N,N-dimethylformamide (DMF) (Inskeep and Bloom 1985, García and Nicolás 1998). For example, Zotarelli *et al.* (2003) using a *SPAD-502* meter to estimate total Chl content in *Zea mays*, reported r^2 values of 0.66 and 0.88 for linear equations when Chls were extracted in 80% acetone or DMF, respectively. According to Hiscox and Israelstam (1979), extracting Chls with DMSO without maceration has the advantage of being faster than extraction with acetone and Chl extracts are more stable in DMSO than in acetone. We had good results extracting leaf pigments with acetone. Even the relatively low r^2 value for the relationship between SPAD and Chl *b* apparently did not have a direct relation to the method of extraction since, as discussed above, the values for the Chl *a*/*b* ratios remained fairly stable throughout Experiments II and III.

In summary, the *SPAD-502* Chl meter can be used to assess leaf Chl content and composition in *E. uniflora* subjected to flooding stress at different light intensities. In the three experiments the relationships between SPAD values and Chl contents were very similar and seemed not be influenced by the environmental growth conditions. The *SPAD-502* Chl meter can be used as a simple and accurate method to provide information on the physiological performance of *E. uniflora* seedlings after planting in gallery forests for restoration projects.

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