Chlorophyll fluorescence parameters in populations of two legume trees: *Stryphnodendron adstringens* (Mart.) Coville (Mimosoideae) and *Cassia ferruginea* (Schrad.) Schrad. ex DC. (Caesalpinoideae)

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(ABSTRACT) – (Chlorophyll fluorescence parameters in populations of two legume trees: *Stryphnodendron adstringens* (Mart.) Coville (Mimosoideae) and *Cassia ferruginea* (Schrad.) Schrad. ex DC. (Caesalpinoideae)). The aim of this study was to investigate the photosynthetic performance in populations of two legume tree species, *Stryphnodendron adstringens* (Mimosoideae), typical from Cerrado, and *Cassia ferruginea* (Caesalpinoideae) from the Atlantic Rain Forest. The photosynthetic traits were assessed by measures of chlorophyll fluorescence in progenies of naturally pollinated plants from three populations of *S. adstringens* and a population of *C. ferruginea*. Plants of *S. adstringens* growing under similar conditions of *C. ferruginea* plants demanded higher light values for photosynthesis saturation, 600 µmol.m⁻².s⁻¹ and 350 µmol.m⁻².s⁻¹ respectively, and showed higher intrinsic photosynthetic efficiency of photosystem II, Fv/Fm of 0.814 versus 0.783 in *C. ferruginea*. The highest values of Fv/Fm observed in *S. adstringens* can explain the highest electron transport rates (ETR) obtained for this species. No significant differences were found among progenies from different *C. ferruginea* trees nor among populations of *S. adstringens*, and only in few cases, variation among progenies within populations were found for *S. adstringens* plants. The fact that fluorescence parameters distinguished species but not populations or most of progenies may be related to low intraspecific genetic variation of these chlorophyll fluorescence traits or due to lack of expression on genetic differences in plants under no stressful conditions.

Key words - chlorophyll fluorescence, legume trees, population approach, quantum yield

RESUMO – (Parâmetros da fluorescência da clorofila em populações de duas leguminosas arbóreas: *Stryphnodendron adstringens* (Mart.) Coville (Mimosoideae) e *Cassia ferruginea* (Schrad.) Schrad. ex DC. (Caesalpinoideae)). O objetivo do estudo foi avaliar a performance fotossintética em populações de duas leguminosas arbóreas, *Stryphnodendron adstringens* (Mimosoideae), uma espécie típica do Cerrado e *Cassia ferruginea* (Caesalpinoideae), que ocorre na Mata Atlântica. Os parâmetros de fotossíntese foram obtidos por medições de fluorescência da clorofila em progê尼斯es de plantas naturalmente polinizadas provenientes de três populações de *S. adstringens* e de uma população de *C. ferruginea*. Plantas de *S. adstringens* crescendo sob as mesmas condições que plantas de *C. ferruginea* demandaram, comparativamente, valores mais elevados de luz para a saturação da fotossíntese, respectivamente 600 µmol.m⁻².s⁻¹ e 350 µmol.m⁻².s⁻¹, e apresentaram uma mais elevada eficiência intrínseca do fotossistema II, Fv/Fm de 0.814 versus 0.783 em *C. ferruginea*. Os maiores valores de Fv/Fm observados em *S. adstringens* podem explicar as maiores taxas relativas de transporte de elétrons (ETR). Não foram encontradas diferenças significativas entre progê尼斯es de *C. ferruginea*, nem entre populações de *S. adstringens*, e apenas algumas poucas diferenças entre progê尼斯es dentro de populações de *S. adstringens*. O fato de parâmetros da fluorescência da clorofila distinguir espécies, mas não populações ou progê尼斯es pode estar relacionado à baixa variação genética destes caracteres de fluorescência da clorofila ou devido a ausência da expressão das diferenças genéticas em plantas sob condições não estressantes.

Palavras-chave - abordagem populacional, fluorescência da clorofila, leguminosas arbóreas, rendimento quântico

**Introduction**

Several studies on photosynthesis of neotropical species can be found in the literature (e.g. Hogan et al. 1995, Valadares et al. 1997, Allen & Pearcy 2000, Lemos Filho 2000) and most of them emphasize differences among species and/or responses to different environmental conditions. Specifically in Brazil, studies on photosynthesis of woody species have been performed mainly for cerrado plants, and were reviewed by Mattos (1998), Moraes & Prado (1998), Paulilo & Felipe (1998) and Franco (2000). In general, these ecophysiological studies use gas exchange methodology and point out to a significant decrease of net photosynthesis due stomatal conductance limitations, mainly during dry season. Moraes & Prado (1998) and Paulilo & Felipe (1998) presented photosynthetic data for many cerrado species and compared them with plants...
from different biomes, while Franco (2000) presented data comparing evergreen, briefly deciduous and deciduous cerrado species.

Comparative studies using chlorophyll fluorescence measurements have also been published in Brazil, most of them about cerrado species. These works include comparison of mistletoes photosynthesis in relation to their hosts (Lütte 	extit{et al.} 1998), photoinhibition (Lemos Filho 2000) and more recently, for 	extit{Miconia albicans}, rapid changes in photosynthetic behaviour due to overnight rainfall at a dry spell amidst in the rainy season have been shown by Mattos 	extit{et al.} (2002). Despite their relevance, all ecophysiological and species comparative studies of photosynthesis, using both gas exchange or chlorophyll fluorescence, usually use samples with a few plants as representative of species behaviour.

As pointed by Arntz & Delph (2001), a population level perspective is necessary to assess one of the requirements for evolution by natural selection, which is the presence of variation for a given trait within a population. Thus, although studies of photosynthesis at a population level seem to be necessary for the understanding of adaptive evolution to different environments, this approach has not been taken for neotropical wood species.

In the present study we analyse the photosynthetic performance of progenies of naturally pollinated plants from different populations of two legume tree species, 	extit{Stryphnodendron adstringens} (Mimosoideae) and 	extit{Cassia ferruginea} (Caesalpinoideae), which are typical from cerrado and Atlantic forest respectively, evaluating photosynthesis through measurements of chlorophyll fluorescence.

**Material and methods**

The study was carried out with plants that were obtained from seeds collected from randomly chosen naturally pollinated plants in Southeastern region of Brazil. For 	extit{Stryphnodendron adstringens}, three populations were evaluated, one from Piraju, São Paulo State, and the others from Furnas and Gouveia, from Minas Gerais State. For 	extit{Cassia ferruginea} a single population from Parque Estadual do Rio Doce (PERD), Minas Gerais State, was analysed. In 	extit{S. adstringens}, the progenies of six trees from Piraju, four from Gouveia and five from Furnas were evaluated. In 	extit{C. ferruginea}, the progenies of 13 trees from PERD were evaluated. The experiment was performed in three entirely randomised block design. The seeds were scarified and germinated on the temperature of 28 ± 1 °C in the darkness. Six days later, seedlings with uniform development were selected and planted in 500 mL pots with a loamy dystrophic soil. After 10 weeks of sowing, each plant was supplied with 80 mL of half-strength Hoagland solution. In the glasshouse, the photosynthetic photon flux density (PPFD), determined with a LI-COR quantum sensor, was of about 200 to 400 µmol.m⁻².s⁻¹, measured between 9 to 11 am. After twenty weeks of growth, the plants were moved to a growing room and kept under temperature of 27 ± 2 °C, relative air humidity of about 60 ± 10%, 12 hours photoperiod with 60 µmol.m⁻².s⁻¹ of PPFD, provided by daylight F40D fluorescent lamps (OSRAM, USA). Those conditions are similar to the ones utilised by Queiroz 	extit{et al.} (2001).

After two days of adaptation to those environmental conditions, fluorescence parameters were determined in the central leaflet from the penultimate mature leaf, in order to avoid differences due to the ontogenetic stage, as recommended by Šesák & Šiffel (1997). Between one to four plants from each mother tree in each block were evaluated. At the end of the subjective night, the chlorophyll fluorescence measurements were performed with a pulse amplitude modulated photosynthesis yield analyser (Mini-PAM, Walz, Germany), obtaining the minimal fluorescence yield (Fo) and the maximum fluorescence during a pulse saturating irradiance (Fm), which allowed the determination of the potential quantum yield of photosystem II (PSII), Fv/Fm = (Fm - Fo)/Fm. Light saturation curves were also obtained using the light curve program of the instrument, evaluating one plant from each mother tree, in each block. Actinic light on leaves was increased up to ca. 2000 µmol.m⁻².s⁻¹ during 4 minutes in 8 steps of 30 seconds each. At each level of light supplied by the instrument, a pulse saturating irradiance was applied to obtain chlorophyll fluorescence parameters. The effective quantum yield is given as ΔF/F’m = (F’ m - F)/F’ m, where F is the minimal chlorophyll fluorescence of the light adapted leaf and F’ m the maximum light adapted fluorescence during a saturating light pulse. Apparent photosynthetic electron transport rates (ETR) are given as 0.5 × ΔF/F’m × PPFD, and the relative excessive PPFD was obtained as (Fv/Fm - F/F’m)/(Fv/Fm) (Bilger 	extit{et al.} 1995). In a study using a similar light curve program, Lüttge 	extit{et al.} (1998) pointed that due to the short time of light exposition in each step the photosynthesis of leaves was never in steady state and correct absolute values of ETR were not obtained. However, they considered that comparative assessments of photosynthetic performance of leaves are still valid.

Due to time limitations, one experimental block was analysed per day. Light saturation curves of fluorescence parameters were fitted using the software Graph Pad Prism. For all chlorophyll fluorescence parameters evaluated, mean and standard deviation were calculated for each population of 	extit{S. adstringens} and for 	extit{C. ferruginea} and compared by Analysis of Variance (ANOVA). Principal Component Analysis (PCA), using the software Pcord v.4, was carried out to investigate possible associations among the evaluated traits and differences among populations and among species.
In both species Fv/Fm data was transformed in arcsin√ to reach homogeneity of variance.

**Results and Discussion**

The curves representing the effective quantum yield (ΔF/F’m), photosynthetic electron transport rates (ETR) and the relative excessive PPFD determined for *C. ferruginea* and *S. adstringens* are shown respectively in figures 1 and 2. Comparing the two species, the photosynthetic performance was superior in *S. adstringens* plants, which presented higher values of ΔF/F’m and ETR even in lower levels of irradiance. The values of ETR showed that photosynthesis saturated in lower levels of PPFD for *C. ferruginea* (ca. 350 µmol.m⁻².s⁻¹) when compared to *S. adstringens* (ca. 600 µmol.m⁻².s⁻¹). However, the values obtained were smaller than the ones described by Lemos Filho (2000), who found values for photosynthesis saturation around 1500 µmol.m⁻².s⁻¹ in three species typical from cerrado growing under field conditions. The lower values of light saturation for the two studied species are probably

![Figure 1](image1.png)  
**Figure 1.** Effective quantum yield (ΔF/F’m) (A), apparent rate of photosynthetic electron transport from photosystem II (ETR) (B), and the relative excessive PPFD (C) in leaves of plants from a population of *C. ferruginea.*

![Figure 2](image2.png)  
**Figure 2.** Effective quantum yield (ΔF/F’m) (A), apparent rate of photosynthetic electron transport from photosystem II (ETR) (B), and the relative excessive PPFD (C) in leaves of plants from three populations of *S. adstringens.*

Y=0.6784*e^(-0.0052X)+0.0711  
r = 0.9677

Y=0.6479*e^(-0.0037*X)+0.1058  
r = 0.8669

Y=62.21*X/(168.2+X)-48.54*X/(905.0+X)  
r = 0.7210

Y=131.2*X/(276.3+X)-81.85*X/(575.4+X)  
r = 0.8606

Y=1.040*X/(139.3+X)  
r = 0.9729

Y=1.023*X/(204.2+X)  
r = 0.9322
related to the low PPFD in the environment where plants were cultivated.

It has been demonstrated that in sun adapted species, light saturation of photosynthesis occurs at higher light intensities than in shade adapted species (Bazzaz 1979). It is shown that compared to *C. ferruginea*, a higher value of radiation necessary for photosynthesis saturation was observed for *S. adstringens*. This fact is possibly related to the environment of natural occurrence of the studied species. In cerrado the canopy is typically discontinuous, and a lower leaf area index (LAI), around 1 in wet season (Miranda *et al.* 1997), allows a higher availability of light understory. On another hand, in the environment of natural occurrence of *C. ferruginea*, the Atlantic forest, the canopy is denser and higher LAI values, i.e. 4.9 during the rainy season in a semideciduous forest in Belo Horizonte (Paula & Lemos Filho 2001), results in lower light availability bellow the forest canopy.

According to the photosynthetic performance, a relative excessive PPFD above of 80% occurred in lower levels of photosynthetic active radiation for most of *C. ferruginea* plants (~ 500 μmol.m⁻².s⁻¹), while for *S. adstringens* plants, similar excess occurred at higher levels of radiation (~ 800 μmol.m⁻².s⁻¹). Considering that an increase in the excess of photosynthetic active radiation is directly related to an increase of non photochemical quenching (NPQ) (Björkman & Demmig-Adams 1995), it can be concluded that, comparatively with *S. adstringens*, cultivated plants of *C. ferruginea* under shade conditions, present a higher NPQ even when exposed to relatively low levels of irradiance.

When leaves are exposed to more light than can be utilised through the process of photosynthesis, PSII function can be affected in a stress condition known as photoinhibition (Barber & Anderson 1992). In fact, when considering 0.8 as a threshold value for photoinhibition, as suggested by Lüttge *et al.* (1998), the measurements show that in average, *C. ferruginea* plants are photoinhibited (table 1). However, due to the low light level in experimental condition, this would not be possible. In a similar way, Böihar-Nordenkampf & Öquist (1993) reported values of Fv/Fm of 0.725 in non photoinhibited plants of french bean. Although not photoinhibited, compared to *S. adstringens*, plants of *C. ferruginea* show inferior values of Fv/Fm (p < 0.05) and higher values of relative excessive PPFD at a lower light levels (figures 1, 2), suggesting that shade adapted plants of this species may suffer greater photoinhibition damages if exposed to upper levels of light.

For all studied fluorescence parameters, significant differences (p < 0.05) were found when compared the two species (table 1). Progenies from different trees of *C. ferruginea* did not differ significantly in fluorescence traits (p > 0.05). *S. adstringens* populations did not differ for evaluated traits (p > 0.05), although some differences among progenies within populations could be detected (p < 0.05). Analysis of variance showed differences among Furnas progenies when Fo values were compared and among Piraju progenies when Fm values were compared. Higher values of intrinsic photosynthetic efficient of photosystem II, given by the ratio Fv/Fm, were observed in *S. adstringens* when compared to *C. ferruginea* plants. This higher potential quantum yield of photosystem II could explain the highest values of the maximum apparent rate of photosynthetic electron transport (ETRmax), obtained from the light saturation curve, observed for *S. adstringens* plants.

<table>
<thead>
<tr>
<th>Species</th>
<th>Populations</th>
<th>Fo</th>
<th>Fm</th>
<th>Fv/Fm</th>
<th>ETRmax</th>
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<td><em>S. adstringens</em></td>
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<td>398±101</td>
<td>2123±537</td>
<td>0.811±0.019</td>
<td>54.4±17.6</td>
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<tr>
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<td>Gouvea</td>
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<td>2154±541</td>
<td>0.815±0.024</td>
<td>50.8±12.3</td>
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<tr>
<td></td>
<td>Furnas</td>
<td>381±115</td>
<td>2094±540</td>
<td>0.816±0.036</td>
<td>50.8±16.6</td>
</tr>
<tr>
<td><em>C. ferruginea</em></td>
<td>PERD</td>
<td>665±90</td>
<td>3079±245</td>
<td>0.783±0.023</td>
<td>32.0±10.2</td>
</tr>
</tbody>
</table>

Table 1. Chlorophyll fluorescence parameters averages and standard deviations (Fo, minimal fluorescence yield; Fm, maximal fluorescence yield under a light saturating pulse; Fv/Fm, potential quantum yield of photosystem II; ETRmax, maximum apparent rate of photosynthetic electron transport), analyzed in three populations of *S. adstringens* and a population of *C. ferruginea*. 

Together the two factors explained 87.2% of the total variance. The figure clearly shows differences among the studied species, but within a species, patterns differing populations or progenies could not be detected, as plants within populations showed a wide range of positions in the figure, usually overlapping...
each other. This pattern agrees with the results found in analysis of variance.

Our results revealed that fluorescence measurements might be considered an efficient technique to distinguish species, but probably not populations or progenies of native tree species, when growing under a non-stressful environment. Similar observations have been reported by Portes & Magalhães (1993) that could not find differences among genotypes of *Phaseolus vulgaris* and by Campostrini & Maestri (1998) that could not distinguish *Coffea canephora* genotypes in relation to parameters of fluorescence emission. All these results are in agreement with Nicotra et al. (1997) reports, that physiological traits, as photosynthetic performance, are under strong stabilising pressures and so may show little or no genetic variation. Though, it should be noticed that we conducted our experiment in favorable growth environmental conditions and Singsaas et al. (2001) concluded that the intrinsic efficiency of photosynthesis do not vary substantially among plant species or growth conditions, unless plants experience stressful environments. In fact it has been pointed out by many other authors (Havaux & Lannoye 1985, Selmani & Wasson 1993, Fragella et al. 1995), that under a stressful situation, genetic differences on chlorophyll fluorescence parameters between populations could be expressed.

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References


