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INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL)

A INTERAÇÃO ECOFISIOLÓGICA PLANTA-AMBIENTE: O PAPEL DA ACLIMATAÇÃO FOTOSSINTÉTICA NA RESPOSTA A FATORES AMBIENTAIS EM ESPÉCIES ARBÓREAS

MARIA TERESA PORTES

Tese apresentada ao Instituto de Biociências da Universidade Estadual Paulista "Julio de Mesquita Filho", Campus de Rio Claro, para a obtenção do título de Doutor em Ciências Biológicas (Área de concentração: Biologia Vegetal)

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RESUMO GERAL

As restrições impostas pelo ambiente obrigam as plantas a transitarem entre estados fisiologicamente distintos, podendo tal transição ser representada pelo processo de aclimatação. O aparato fotossintético apresenta alta sensibilidade ao ambiente, mas possui alta capacidade de aclimatação, a qual é necessária dada a sua função essencial no metabolismo vegetal e o seu elevado nível de integração com outras vias metabólicas. A investigação do papel da aclimatação fotossintética na resposta a diversas condições ambientais, em diferentes espécies arbóreas, foi o principal tema de estudo da presente tese. Foram realizados experimentos com espécies arbóreas nativas e cultivadas sob diferentes níveis de controle ambiental, ou seja, no campo, em casa de vegetação e em câmaras de crescimento. Os experimentos com espécies arbóreas nativas envolveram a avaliação da capacidade de aclimatação das espécies de diferentes grupos sucessionais, as quais supostamente apresentam demandas luminosas distintas e diferem em sua habilidade de ajuste e acoplamento ao ambiente. O experimento com a espécie cultivada foi realizado com *Eucalyptus globulus*, e avaliou o efeito da deficiência hídrica em plantas sob diferentes regimes térmicos. A conjunção dos resultados obtidos nos quatro experimentos realizados permitiu verificar que a aclimatação do aparato fotossintético foi influenciada pelo ambiente de crescimento das plantas e pela estratégia ecológica das espécies, mais conservativa ou mais flexível. Além disso, foi verificado que a estratégia ecológica das espécies não está, necessariamente, relacionada ao seu grupo sucessional, conforme freqüentemente descrito na literatura. Os diversos ajustes no aparato fotossintético, descritos no presente trabalho em diferentes espécies e condições ambientais, demonstraram a importância e a contribuição da aclimatação fotossintética no ajustamento fisiológico da planta a uma nova condição do ambiente. Assim sendo, a aclimatação fotossintética é um processo metabólico crucial para o restabelecimento da homeostase da planta, após uma determinada variação do ambiente.

Palavras-chave: ecofisiologia, aclimatação, aparato fotossintético, espécies tropicais arbóreas, *Eucalyptus globulus*, heterogeneidade ambiental

GENERAL ABSTRACT

The constraints imposed by the environment compel plants to transit between distinct physiological states, represented by the acclimation process. The photosynthetic apparatus is highly sensitive to the environment, however it presents a high acclimation capacity which is necessary given its essential role in plant metabolism and high level of integration with other pathways. The investigation of the role of photosynthetic acclimation in response of different tree species to diverse environmental conditions was the main subject of the present study. Experiments with tropical tree species and cultivated species were performed under different levels of control of environmental conditions, *i.e.* in the field, in the greenhouse, and in growth chambers. The experiments with tropical tree species involved the evaluation of the acclimation capacity of species belonging to different ecological groups, supposedly presenting distinct light demands and ability to adjust and couple to the environment. The experiment with cultivated species was carried out with *Eucalyptus globulus* and photosynthetic acclimation was evaluated under water deficit, in plants under different thermal regimes. The conjunction of the results obtained in the four experiments performed suggests that the acclimation of the photosynthetic apparatus was influenced by the growth environment jointly with the ecological strategy of the species, more conservative or more flexible. Moreover, it was verified that the ecological strategy of the species is not necessarily related with its ecological group as often stated in the literature. The diverse changes in the photosynthetic apparatus described in the present study in different species and environmental conditions, demonstrated the importance and the contribution of the photosynthetic acclimation in the physiological adjustment of a plant to its current environmental condition. Therefore, photosynthetic acclimation is a crucial process to the reestablishment of the plant's homeostasis after a given environmental variation.

Key words: ecophysiology, acclimation, photosynthetic apparatus, tropical tree species, *Eucalyptus globulus*, environmental heterogeneity

APRESENTAÇÃO

Invariavelmente, as plantas são expostas a condições ambientais adversas, tais como alterações na temperatura, disponibilidade hídrica, intensidade luminosa, disponibilidade de nutrientes, pragas e patógenos, entre outros. No entanto, a interação das plantas com seu ambiente biótico e abiótico levam ao desenvolvimento de mecanismos que possibilitam suportar e sobreviver a tais condições adversas. Logo, a capacidade de modificar características morfo-anatômicas, fisiológicas, bioquímicas e moleculares é um dos fatores que faz com que as plantas lidem com as mudanças ambientais, sendo especialmente importante para esses organismos sésseis.

Tal capacidade modulativa do fenótipo frente a condições ambientais distintas, promovida por ajustes na expressão do genótipo, é conhecida como plasticidade fenotípica, e a habilidade de expressá-la é característica da espécie. Assim, a aclimatação é uma forma de expressar a plasticidade fenotípica de uma espécie. A capacidade de aclimatação de uma espécie pode ser estimada através de estudos ecofisiológicos, que permitem acessar o controle do metabolismo e indicam os mecanismos fisiológicos pelos quais as plantas resistem e respondem a diferentes condições ambientais. Dentre os processos mais sensíveis e responsivos a estresses podemos destacar a fotossíntese, que é um componente fundamental do metabolismo, através do qual as espécies vegetais assimilam CO_2 e o fixam em moléculas orgânicas fundamentais para o crescimento e desenvolvimento desses organismos. Como a fotossíntese é um processo essencial para as plantas e apresenta um papel central no metabolismo vegetal, é esperado que o aparato fotossintético apresente elevada capacidade de resposta, ajuste e acoplamento ao ambiente. De fato, é observado que o aparato fotossintético apresenta alto nível de robustez, proporcionado por uma capacidade substancial de expressar plasticidade fenotípica.

O processo fotossintético é caracterizado por apresentar alto nível de integração metabólica podendo, assim, influenciar vários outros processos, uma vez que seu produto é utilizado em praticamente todas as vias que compõem o metabolismo vegetal. Assim, o estudo do funcionamento da maquinaria

fotossintética é um campo atraente e fundamental da Ecofisiologia Vegetal, que possibilita analisar a dinâmica da interação planta-ambiente. Nas últimas décadas, foi obtido um importante e acentuado avanço na instrumentação e nas técnicas utilizadas em Ecofisiologia, especialmente para acessar as respostas fotossintéticas, avaliando tanto a etapa fotoquímica quanto a bioquímica. Os analisadores de gases por infravermelho (IRGAs) e os fluorômetros merecem destaque, uma vez que possibilitam estimar prontamente as oscilações do aparato fotossintético sob condições experimentais controladas ou no campo.

Os mecanismos fisiológicos envolvidos nos padrões de respostas a variações ambientais podem ajudar-nos a entender a significância funcional dessas variações, além de elucidar os limites de tolerância e aclimatação das espécies. Portanto, a avaliação da capacidade de aclimatação do aparato fotossintético, devido a sua importância no crescimento, desenvolvimento e produtividade vegetal, é fundamental quando consideramos a iminência de mudanças climáticas globais e a necessidade imediata de conservação dos ambientes florestais. Deste modo, estudos ecofisiológicos com espécies nativas e cultivadas assumem importância significativa no panorama atual da Biologia Vegetal.

Estudos com espécies arbóreas tropicais revelaram que espécies pioneiras e espécies mais tardias da sucessão florestal secundária apresentam diferentes capacidades de aclimatação, e tal constatação foi atribuída à heterogeneidade do ambiente onde tais grupos ecológicos são encontrados. A maioria dos autores considera que espécies pioneiras, comumente encontradas em clareiras, apresentam maior capacidade de aclimatação devido à elevada variabilidade do seu ambiente físico, enquanto espécies secundárias, encontradas no sub-bosque, apresentam menor habilidade em aclimatar-se a condições que contrastem do seu ambiente original, considerado menos variável. Contudo, um dos critérios utilizados na classificação de espécies arbóreas tropicais em grupos ecológicos é o grau de tolerância ao sombreamento, o qual é inferido pelo habitat onde a espécie ocorre mais frequentemente. Tal observação permite, então, atribuir um grupo ecológico à dada espécie, *grosso modo,* pioneira, secundária ou clímax. No entanto, esse critério é puramente visual podendo acarretar em distorções a cerca da classificação e, consequentemente, do potencial de aclimatação de espécies arbóreas. Estudos ecofisiológicos podem auxiliar no aprimoramento desses critérios de classificação e

podem ajudar a revelar os mecanismos que conferem a capacidade de aclimatação das espécies.

Espécies florestais cultivadas, como as do gênero *Eucalyptus*, apresentam aparato fotossintético altamente eficiente, capaz de converter elevadas quantidades de CO_2 por área foliar, tendo sido especialmente selecionadas para utilização na produção de madeira, papel e outros derivados de celulose. Dentre as espécies de interesse econômico do gênero *Eucalyptus* encontra-se *E. globulus*, que apresenta crescimento rápido e grande porte, sendo amplamente cultivada, principalmente, em regiões temperadas. Contudo, o custo da alta produtividade de *E. globulus* é o seu elevado consumo de água. Em um cenário de mudanças climáticas globais, onde a precipitação tende a diminuir e a temperatura tende a aumentar, o desempenho fotossintético de *E. globulus* ainda é incerto sob tais condições. Assim, a avaliação do efeito combinado de deficiência hídrica e temperaturas moderadamente elevadas se faz necessária a fim de investigar as respostas fisiológicas de *E. globulus* frente a condições adversas ao seu crescimento.

Tendo em vista contribuir para o entendimento dos mecanismos subjacentes à capacidade de aclimatação de espécies vegetais, a presente tese foi realizada com espécies arbóreas, nativas e cultivadas, cujo âmbito geral foi avaliar as respostas fisiológicas a condições ambientais adversas e, conseqüentemente, estimar a capacidade de aclimatação do aparato fotossintético. A tese é composta por quatro artigos, dos quais, até a presente data, dois estão publicados e um está submetido para publicação em periódicos científicos.

O primeiro capítulo, intitulado “Photosynthetic induction responses in tropical forest tree species: unexpected acclimation capacities to distinct light conditions”, submetido ao periódico *Journal of Vegetation Science*, descreve as respostas de indução fotossintética de quatro espécies arbóreas tropicais, classicamente pertencentes a diferentes grupos ecológicos, crescidas em três ambientes luminosos (pleno sol, 50% e 10% de irradiância). Esse capítulo mostra que a utilização e capacidade de aclimatação à luz das espécies estudadas não está necessariamente relacionada ao seu grupo ecológico, indicando que a tolerância ao sombreamento e o requerimento luminoso das espécies não são critérios apropriados para distinguir grupos ecológicos.

O segundo capítulo intitulado “Water deficit affects photosynthetic induction in *Bauhinia forficata* Link (Fabaceae) and *Esenbeckia leiocarpa* Engl. (Rutaceae) growing in understorey and gap conditions”, publicado no periódico *Brazilian Journal of Plant Physiology*, apresenta a influência da deficiência hídrica *in situ* na utilização de luz em duas espécies arbóreas tropicais, crescidas no sub-bosque e na clareira de um fragmento florestal de Floresta Estacional Semi-decidual em Narandiba/SP, em meses com disponibilidade hídrica contrastantes. Esse estudo demonstra que a deficiência hídrica limita a utilização de luz, sendo que tal limitação foi menos pronunciada no sub-bosque, onde as plantas foram capazes de reduzir a respiração e manter a taxa fotossintética próxima do ponto de compensação, apesar desse ambiente ter apresentado menor disponibilidade hídrica. Assim, a disponibilidade de água pode influenciar significativamente o estabelecimento de plântulas e a sobrevivência de plantas jovens durante períodos de seca, freqüentes em ambientes florestais que apresentam sazonalidade definida. Nesse estudo foi verificado que o ambiente de crescimento, mais do que o *status* sucessional das espécies, promoveu diferenças na utilização fotossintética da luz.

O terceiro capítulo intitulado “Time-course of photosynthetic induction in four tropical woody species grown in contrasting irradiance habitats”, publicado no periódico *Photosynthetica*, evidencia o efeito do tempo de exposição ao escuro e do ambiente de crescimento na resposta de indução fotossintética em quatro espécies tropicais, pertencentes a diferentes grupos ecológicos, crescidas sob condições de clareira e sub-bosque em um fragmento florestal de Floresta Estacional Semi-decidual em Narandiba/SP. Tal estudo mostra que, *in situ,* espécies do mesmo grupo ecológico podem apresentar capacidades distintas de utilização de luz, indicando que a capacidade de expressar plasticidade fenotípica está mais relacionada a características intrínsecas das espécies do que ao grupo ecológico ao qual pertencem.

O quarto capítulo é intitulado “Exposure time to moderately high temperature affects photosynthetic response to water deficit in *Eucalyptus globulus* Labill.” e avaliou o efeito da deficiência hídrica em plantas sob diferentes regimes térmicos. Observou-se que a exposição prolongada à 35^{o} C impõe muitas restrições à atividade fotossintética, principalmente em plantas sob deficiência hídrica, enquanto uma curta exposição a 35^{o} C aumenta a eficiência fotossintética sob condições de

baixa disponibilidade de água, o que pode indicar o aumento da tolerância à deficiência hídrica, e até mesmo um mecanismo de tolerância cruzada em *E. globulus*. Tais resultados sugerem que a termo-aclimatação influencia na resposta à deficiência hídrica em *E. globulus*, indicando que as mudanças na temperatura e na disponibilidade de água, estimadas para o próximo século, podem influenciar substancialmente a produtividade de *E. globulus*.

No presente trabalho foram realizados estudos tanto com espécies arbóreas nativas quanto com espécies cultivadas de elevada importância econômica, sob condições controladas, em câmaras de crescimento e casa de vegetação, e também no campo. Tal experiência permitiu avaliar as respostas das plantas sob diferentes níveis de controle dos fatores ambientais. Foi possível verificar que a aclimatação é uma resposta intrínseca das espécies à variabilidade do ambiente, refletindo características ecológicas de seu nicho. O processo fotossintético tem um papel central no metabolismo vegetal e, indubitavelmente, apresenta elevada capacidade de responder e se aclimatar a alterações do ambiente. Assim, a extraordinária capacidade de modulação e ajuste da maquinaria fotossintética exerce um importante papel na performance e na função ecológica das espécies vegetais nos ecossistemas.

INTRODUÇÃO GERAL

Luz

O ambiente das plantas varia continuamente e, como organismos sésseis, é necessário que elas se ajustem constantemente às condições ambientais. Dado que as plantas são sistemas de desenvolvimento integrado, respostas à variação ambiental envolvem mudanças em uma *suite* de caracteres que são influenciadas pelo estado de desenvolvimento ontogenético do indivíduo, apresentando também influência sobre este (SULTAN, 2003; WEST-EBERHARD, 2003).

O ambiente de uma planta é constituído por fatores bióticos, que resultam da interação com outros organismos, e abióticos, que são os agentes físicos tais como luz, água, temperatura, umidade, gases e nutrientes (SCHULZE et al., 2005). Dentre os fatores ambientais que influenciam o crescimento e sobrevivência das plantas em florestas tropicais, a disponibilidade de luz é o mais limitante (CHAZDON, 1988; FETCHER et al., 1994). Dada a substancial importância da luz para os mecanismos que regulam a taxa de crescimento, desenvolvimento, estrutura, função e comportamento, a adaptação e aclimatação à luz é um fator crítico para a sobrevivência das plantas em qualquer ecossistema (NILSEN & ORCUTT, 1996).

A luz é um recurso de grande interesse para ecofisiologistas vegetais, não somente pela sua indiscutível importância para as plantas, mas também pela sua considerável complexidade e heterogeneidade nos ecossistemas (TANG, 1997). Diferente de outros fatores ambientais, a luz varia em qualidade, intensidade, direção e duração. Essa grande variação da luz demanda uma alta capacidade de resposta das plantas, uma vez que a luz influencia seu metabolismo de duas formas: fornecendo energia para o processo fotossintético e atuando como mediador na transferência de informação do ambiente para o organismo. Como mediador de informação, a luz está envolvida em vários processos regulatórios do crescimento e desenvolvimento vegetal, tais como fotomorfogênese, fototropismo e fotoperiodismo. Além disso, a luz atua em processos fisiológicos como regulação da abertura e fechamento estomático e atividade de enzimas relacionadas à fixação de carbono (TANG, 1997).

Na complexa matriz de microambientes que compõem as florestas tropicais, a disponibilidade de luz varia mais dramaticamente do que qualquer outro recurso (CHAZDON et al., 1996). A formação e fechamento de clareiras de diferentes dimensões criam um ambiente cuja luminosidade é altamente heterogênea (CHAZDON, 1988). Além disso, a radiação que chega ao sub-bosque representa somente 1-2% do total que incide no dossel e é, temporal e espacialmente, variável, sendo que até 90% do recurso luminoso está disponível na forma de *sunflecks* (CHAZDON, 1988). A intensidade dos *sunflecks* varia com a altura, abertura e densidade do dossel, e com a presença de nuvens e vento, cuja agitação permite que mais luz incida no sub-bosque, mas também gera *sunflecks* mais curtos e numerosos. Os *sunflecks* geralmente ocorrem em menos de 10% do dia, porém contribuem com mais de 60% da densidade do fluxo de fótons (DFF) diária em muitos sub-bosques (CHAZDON, 1988). Logo, o ganho de carbono em plantas do sub-bosque está condicionado à utilização eficiente de *sunflecks* (PEARCY, 1990). Em geral, é esperado que as espécies adaptadas a sombra sejam mais eficazes em utilizar *sunflecks* para fotossíntese (PEARCY et al., 1994; VALLADARES et al., 1997). Contudo, alguns aspectos fisiológicos, como a velocidade da indução fotossintética, parecem não estar tão estreitamente relacionados com a tolerância ao sombreamento de uma determinada espécie como se havia pensando anteriormente (NAUMBURG & ELLSWORTH, 2000).

Indução Fotossintética

Quando a irradiância aumenta, após um período de baixa incidência luminosa, o aumento correspondente na assimilação de CO_2 não é instantâneo e apresenta certa defasagem (OSTERHOUT & HASS, 1919; RABINOWITCH, 1956). Tal atraso está relacionado com o processo de indução fotossintética, que envolve a ativação de fatores bioquímicos e estomáticos (PEARCY, 1990).

Assim, para que as plantas utilizem a luz eficientemente, sob condições heterogêneas de irradiância, é necessária uma rápida indução fotossintética após a iluminação da folha (PEARCY, 1990). A indução fotossintética depende, sobretudo, de fatores bioquímicos e estomáticos, sendo distinguidas três fases desse processo:

(*1*) durante os primeiros 1–2 min de exposição à luz ocorre o aumento da atividade das enzimas envolvidas na regeneração de ribulose–1,5–bisfosfato (RuBP), que é o aceptor primário de CO_2 no ciclo de Calvin (KIRSCHBAUM & PEARCY, 1988; PEARCY et al., 1994). Acredita-se que a limitação na regeneração de RuBP, sob condições de baixa irradiância, é causada pela rápida inibição da frutose–1,6–bisfosfatase (FBPase) e, possivelmente, de outras enzimas envolvidas na regeneração de RuBP (SASSENRATH-COLE & PEARCY, 1992; MARTIN et al., 2000); (*2*) cerca de 10 min após o aumento na irradiância, a ribulose–1,5–bisfosfato carboxilase–oxigenase (Rubisco) é ativada progressivamente (WOODROW & MOTT, 1989). A ativação incompleta da Rubisco, na reação primária de carboxilação, é considerada a limitação bioquímica chave nessa fase da indução fotossintética (WOODROW & MOTT, 1989; MOTT & WOODROW, 2000); (*3*) a abertura estomática é a fase mais lenta desse processo, uma vez que sua completa indução pode levar mais de uma hora (TINOCO-OJANGUREN & PEARCY, 1993; KIRSCHBAUM et al., 1998; ALLEN & PEARCY, 2000a). A abertura estomática pode impor uma limitação secundária na indução fotossintética por retardar a taxa de atividade da Rubisco, devido à baixa concentração intercelular de CO_2 (VALLADARES et al., 1997; ALLEN & PEARCY, 2000b). Deste modo, o estado de indução fotossintética é dependente de vários mecanismos regulatórios, que operam em diferentes escalas de tempo (PEARCY, 1990).

A cinética da indução fotossintética também é influenciada por: (*1*) a aclimatação da planta ao seu ambiente de crescimento, que resulta em alterações bioquímicas e anatômicas na folha (CAO & BOOTH, 2001; LICHTENTHALER & BABANI, 2004); (*2*) o histórico de exposição luminosa da folha que determina o seu estado de indução (HAN et al., 1999; CAI et al., 2005), uma vez que afeta a taxa de ativação das enzimas fotossintéticas e a abertura estomática; e (*3*) fatores ecológicos, tais como temperatura (KÜPPERS & SCHNEIDER, 1993) e potencial hídrico (TINOCO-OJANGUREN & PEARCY, 1993; ALLEN & PEARCY, 2000a).

Os diferentes ambientes luminosos existentes em condições naturais impõem restrições distintas na eficiência de utilização da luz por espécies vegetais. O ambiente de clareira nas florestas tropicais apresenta alta incidência luminosa e demanda evaporativa, exigindo estratégias que evitam a perda de água excessiva e a fotoinibição. Sob condições de sub-bosque, onde a luminosidade é altamente

variável, a manutenção de um elevado estado de indução fotossintética é determinante na capacidade de explorar pulsos de luz eficientemente (PEARCY, 1990).

Sucessão Ecológica em Florestas Tropicais

Florestas tropicais apresentam grande diversidade de condições ambientais, tendo sido descritas como um mosaico sucessional com diferentes fases estruturais e florísticas induzidas, principalmente, pela abertura de clareiras no dossel. A sucessão florestal é, geralmente, explicada com base nas diferenças de demanda luminosa das espécies arbóreas (PACALA et al., 1996), que variam de tolerantes a intolerantes ao sombreamento. Estas espécies são, em geral, classificadas em dois grupos de acordo com a distribuição espacial e temporal no mosaico florestal: (*1*) o das espécies iniciais da sucessão ecológica, as quais demandam alta luminosidade e são intolerantes ao sombreamento (espécies pioneiras e secundárias iniciais), e (*2*) o das espécies mais tardias da sucessão, que são tolerantes ao sombreamento (espécies secundárias tardias) (PICKETT et al., 1987). A posição das espécies ao longo do *continuum* de ambientes luminosos em uma floresta tropical é determinada, em grande parte, pelo compromisso entre crescimento e sobrevivência (WRIGHT et al., 2003; POORTER & BONGERS, 2006), sendo que diferenças nas características fotossintéticas das espécies permitem a utilização diferencial da radiação e a ocupação de diferentes nichos (PRESS et al., 1996).

A tolerância a sombra é extensamente usada como um critério para classificar espécies em grupos ecológicos, ainda que sua definição seja tema de debate (KITAJIMA & BOLKER, 2003; NIINEMETS, 2006). Apesar desse critério de classificação ter sido utilizado por muitas décadas, estudos comparativos em regiões tropicais com alta diversidade reportaram que existem poucas espécies realmente tolerantes a sombra intensa ou que necessitem de muita luz, indicando que a maioria das espécies é intermediária em sua demanda luminosa (WRIGHT et al., 2003). Além disso, a classificação das espécies vegetais segundo sua tolerância a sombra é, em grande parte, apoiada em observações visuais carentes de dados quantitativos, ainda que as observações de distintos autores tendam a coincidir

(VALLADARES et al., 2004). COOMES & GRUBB (2000) reportaram que as escalas mais robustas de classificação de tolerância a sombra, e classificação relativa das espécies, são a de ELLENBERG (1991) para Europa e a de BAKER (1949) para América do Norte. Portanto, não existe um critério definido para classificar espécies tropicais em grupos ecológicos, a qual tem sido feita apenas considerando o habitat de ocorrência mais freqüente da espécie. Sendo assim, as espécies têm sido classificadas subjetivamente em diferentes grupos funcionais, sem uma quantificação apropriada da sua demanda luminosa.

Plasticidade Fenotípica

Plasticidade fenotípica é a propriedade de um dado genótipo produzir diferentes fenótipos em resposta a condições ambientais distintas (BRADSHAW, 1965; SCHLICHTING, 1986; SCHLICHTING & PIGLIUCCI, 1998; SULTAN, 2000; PIGLIUCCI, 2001). Tal propriedade é considerada como o principal meio de adaptação do indivíduo à heterogeneidade do ambiente (SCHLICHTING, 1986), supostamente porque a plasticidade pode conferir homeostase, o que possibilitaria a manutenção da funcionalidade do organismo. Uma alta plasticidade fenotípica aumenta as possibilidades de sobrevivência às novas condições ambientais geradas após uma perturbação como, por exemplo, a abertura de uma clareira (BAZZAZ, 1979; JOHNSON et al., 1997). Por outro lado, a baixa plasticidade pode ser fruto de uma especialização a condições ambientais adversas (LORTIE & AARSSEN, 1996), constituindo uma estratégia mais conservativa da espécie.

São numerosos os estudos que têm abordado a relação entre o caráter sucessional de uma espécie e sua capacidade de expressar plasticidade fenotípica frente à variabilidade luminosa (BAZZAZ & CARLSON, 1982; WALTERS & REICH, 1996; VALLADARES et al., 2000b, 2002b). Segundo a hipótese de recursos múltiplos proposta por BAZZAZ (1979), o ambiente das espécies iniciais da sucessão apresenta maior variabilidade dos fatores físicos, o que demanda um grau elevado de flexibilidade fisiológica. De fato, muitos estudos mostraram que espécies pioneiras apresentam alta plasticidade, devido provavelmente à elevada disponibilidade de recursos no ambiente dessas espécies que permite o rápido

investimento em aclimatação (GRIME et al., 1986). Além disso, espécies pioneiras têm folhas com ciclo de vida curto (REICH et al., 1992) e, conseqüentemente, são capazes de se ajustarem ao ambiente pela rápida substituição de suas folhas (ACKERLY, 1997; VALLADARES et al., 2000).

Muitas características das plantas são altamente plásticas devido ao seu papel fundamental na bioquímica, fisiologia ou desenvolvimento de um organismo (SULTAN, 1995). Por isso, a plasticidade do aparato fotossintético é fundamental uma vez que seu funcionamento é essencial para sobrevivência das plantas, principalmente em ambientes variáveis e heterogêneos (BRADSHAW, 1965; PINTADO et al., 1997). Desse modo, é esperado que ambos os grupos de espécies, iniciais e tardias da sucessão expressem elevada plasticidade, e tal predição vem sendo suportada por evidências de que os ajustes no aparato fotossintético não estão necessariamente relacionados ao *status* sucessional ou à demanda luminosa da espécie (TURNBULL, 1991; POPMA et al., 1992; VALLADARES et al., 2000; NAUMBURG & ELLSWORTH, 2000). Além disso, tem sido sugerido que a plasticidade nas características foliares esteja relacionada não somente com o nicho de regeneração de uma espécie, mas dependa também do histórico de exposição à irradiância durante seu ciclo de vida (POPMA et al., 1992; SULTAN, 2000).

Nos últimos anos o número de trabalhos que discutem plasticidade fenotípica aumentou consideravelmente, possivelmente devido à importância de estimar as respostas das plantas sob um cenário de mudanças globais (POTVIN & TOUSIGNANT, 1996; REHFELDT et al., 2001) e de idéias emergentes sobre a importância da plasticidade para o entendimento das interações entre espécies (CALLAWAY et al., 2003). Os campos da ecologia e desenvolvimento estão adquirindo rapidamente novos *insights* em evolução vegetal, sendo a plasticidade um mecanismo chave para o entendimento do desenvolvimento vegetal em um contexto ecológico (FARNSWORTH, 2004; SULTAN, 2005).

Aclimatação Fotossintética

As plantas desenvolveram mecanismos de resposta às variações do ambiente em diversas escalas, variando de semanas a meses e de poucos segundos a horas

(WALTERS, 2005). Por exemplo, alterações na razão raiz:parte aérea e anatomia foliar varia de semanas a meses (BALLARÉ, 1999; WESTON et al., 2000), enquanto alterações na atividade de proteínas do aparato fotossintético ocorrem de segundos a horas (DEMMIG-ADAMS & ADAMS, 1992). Entre esses extremos há um nível de resposta ao ambiente que envolve ajustes na composição do aparato fotossintético, que constitui o processo de aclimatação fotossintética (WALTERS, 2005). STRAUSS-DEBENEDETTI & BAZZAZ (1991) definiram aclimatação como mudanças que aparecem em fenótipos já expressos, o que é considerado um caso especial de plasticidade.

A aclimatação não ocorre somente devido à variação na luz, mas é também observada, em maior ou menor extensão, em reposta a muitos outros aspectos do ambiente, incluindo temperatura, seca, concentração de CO_2 atmosférico, infecções por patógenos, entre outros. É intrigante que, mesmo fatores que apresentam efeitos indiretos na fotossíntese são capazes de induzir ajustes na composição dos cloroplastos (WALTERS, 2005). Essa habilidade do aparato fotossintético responder a uma ampla gama de estímulos levou à hipótese de que os sinais que induzem a aclimatação são derivados da própria fotossíntese, e não de sua detecção por vias independentes. Tal estratégia regulatória permite que a planta detecte alterações no aparato fotossintético, independentemente da causa, e responda para compensá-las, permitindo a integração de diversos sinais e promovendo a eficiência do processo fotossintético (ANDERSON et al., 1995; WALTERS, 2005).

Existe uma variação substancial na extensão e natureza das respostas de aclimatação entre espécies. A maioria das espécies apresenta, no mínimo, alguma variação na capacidade fotossintética, mas a escala de mudanças observadas na composição do cloroplasto varia dramaticamente. Inicialmente, acreditava-se que esta variação estava relacionada com o crescimento das espécies no sol ou na sombra (BOARDMAN, 1977; ANDERSON & OSMOND, 1987), mas recentemente tornou-se claro que a capacidade de aclimatação é pronunciada em espécies crescidas em ambas as condições (MURCHIE & HORTON, 1997). Portanto, a aclimatação parece fornecer uma indubitável vantagem competitiva, principalmente em ambientes heterogêneos ou sujeitos a variações ambientais marcantes, uma vez que plantas com maior potencial de aclimatação são, provavelmente, mais bem sucedidas em ambientes variáveis (WALTERS, 2005).

Eucalyptus globulus

O gênero *Eucalyptus* é um dos mais cultivados em plantações florestais para produção de madeira e polpa celulósica em todo mundo (JAMES & DEL LUNGO, 2005). Dentre tais espécies de crescimento rápido, *E. globulus* se destaca como a principal espécie cultivada em plantações industriais de regiões temperadas (DOUGHTY, 2000; POTTS et al., 2004). Em Portugal, por exemplo, cerca de 700.000 ha são destinados ao plantio de *E. globulus* (POTTS et al., 2004). O cultivo de *E. globulus* se destina principalmente a produção de polpa celulósica, uma vez que as propriedades químicas e físicas de sua madeira, como baixo conteúdo de lignina e alto conteúdo de celulose, são muito apropriadas para tal uso. Entretanto, existe um aumento crescente do emprego de *E. globulus* na produção de folhas e de outros produtos sólidos de madeira (RAYMOND, 2000; GREAVES et al., 2004).

Produtos derivados de madeira têm um potencial de mitigar as emissões de gases do efeito estufa, agindo como reservatório e dreno de carbono (WHITTOCK et al., 2007), substituindo materiais de construção mais dependentes de energia (por exemplo, concreto e aço) e também como biocombustíveis (NÚÑEZ-REGUEIRA et al., 2002). Assim, a reconhecida importância das florestas em atenuar as mudanças climáticas tem levado muitos países a estimarem a quantia de carbono florestal, tanto para aumentar o conhecimento científico quanto para fornecer informações que sirvam de base para o estabelecimento de políticas climáticas nacionais e internacionais e para o sequestro de carbono. Muitos desses estudos contabilizam não somente o carbono em ecossistemas florestais, mas também o carbono contido em produtos florestais (PUSSINEN et al., 1997; SKOG & NICHOLSON, 2000).

Contudo, é importante salientar que a alta produtividade de espécies do gênero *Eucalyptus* está vinculada a elevadas taxas de consumo de água (WHITEHEAD & BEADLE, 2006). Caso a previsão de aumento médio na temperatura de 2-4^{o} C no próximo século se concretize, provavelmente ocorrerá seca em muitas áreas do mundo devido a mudanças na precipitação (IPCC, 2007), de modo que a produtividade de *E. globulus* pode ser severamente afetada.

OBJETIVOS

Objetivo Geral:

Considerando a importância da aclimatação na resposta a diversas condições ambientais, e o papel fisiológico e ecológico do aparato fotossintético para o desenvolvimento vegetal, a presente tese teve como objetivo geral avaliar a capacidade de aclimatação, principalmente do aparato fotossintético, de espécies arbóreas nativas e cultivadas sujeitas a diferentes condições luminosas, disponibilidade hídrica e temperatura.

Objetivos Específicos:

- Testar a hipótese clássica de que espécies iniciais da sucessão florestal apresentam maior capacidade de aclimatação do que espécies mais tardias;

- Avaliar a eficiência na utilização de luz em espécies iniciais e tardias da sucessão florestal aclimatadas a diferentes condições luminosas;

- Analisar a deficiência hídrica na indução fotossintética em espécies arbóreas nativas de diferentes grupos ecológicos, crescidas em condições de sub-bosque e clareira, em meses com disponibilidade hídrica distintas;

- Avaliar a capacidade de explorar a heterogeneidade luminosa através do perfil de indução fotossintética de espécies nativas de diferentes grupos ecológicos crescidas na clareira e no sub-bosque, expostas a diferentes períodos de escuro;

- Investigar os efeitos da deficiência hídrica e temperatura, e especialmente sua interação, no aparato fotossintético de plantas jovens de *E. globulus*;

- Avaliar os efeitos da exposição breve e prolongada a uma temperatura moderadamente alta em plantas jovens de *E. globulus*.

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Capítulo I

Artigo submetido ao periódico *Journal of Vegetation Science*

Photosynthetic induction responses in tropical forest tree species: unexpected acclimation capacities to distinct light conditions

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Abstract - We tested the classical hypotheses that: *i)* early successional species (ES), rather than late successionals (LS), have higher acclimation capacity considering the high variability in its typical habitat, *ii)* nonetheless LS show higher light utilization efficiency under low light conditions. We compared photosynthetic responses of four species widely found in Brazilian forests and classified into distinct ecological groups. To assess acclimation capacity, gas-exchange measurements of photosynthetic induction time courses were compared among plants grown in three light environments: 10%, 50% and full sun light. Induction curves were fitted with a sigmoid model, yielding photosynthetic parameters such as induction state (IS), maximum CO_2 assimilation rate (A_{max}) and time to attain 50% (T_{50}) and 90% (T_{90}) of A_{max}, being compared by means of a two-way ANOVA with Tukey's post-hoc test. Chlorophyll content (Chl *a* and *b* content), electron transport rate (ETR) and specific leaf weigh (SLW) were also measured and compared. Principal component analysis (PCA) and shade adjustment coefficient (SAC) were used to estimate overall physiological responses. Three acclimation responses were detected: *i*) *Schinus terebinthifolius* is light-demanding but with high acclimation capacity to low light, *ii*) *Hymenaea courbaril* is shade-tolerant but with low acclimation capacity to high light, *iii*) *Cecropia pachystachya* and *Tabebuia impetiginosa* are light-demanding, but with low acclimation capacity to low light. The LS *T. impetiginosa* presented high acclimation to high light while the ES *C. pachystachya* showed low acclimation to low light, not supporting classical hypothesis *i)*. The ES *S. terebinthifolius* had high light utilization efficiency under low light, while the LS *T. impetiginosa* showed low photosynthetic acclimation to low light *ii)*.Thus, shade tolerance and light preference are not necessarily linked to successional status and are not appropriate criteria to distinguish ecological groups.

Key words: Brazilian tropical tree species, photosynthetic acclimation, light utilization, ecological groups

Resumo - (Respostas de indução fotossintética em espécies tropicais arbóreas: capacidades inesperadas de aclimatação a condições luminosas distintas.) Nós testamos a hipótese clássica de que: *i)* espécies iniciais da sucessão (ES) apresentam maior capacidade de aclimatação do que espécies mais tardias (LS), considerando a alta variabilidade em seu habitat típico, *ii)* entretanto LS apresenta maior eficiência na utilização de luz sob condições de baixa irradiância. Nós comparamos as respostas fotossintéticas de quatro espécies amplamente encontradas nas florestas Brasileiras e classificadas em diferentes grupos ecológicos. Para acessar a capacidade de aclimatação, foram comparadas as medidas de trocas gasosas nos cursos de indução fotossintética entre as espécies crescidas em três ambientes com luminosidades distintas: 10% e 50% de irradiância e pleno sol. As curvas de indução fotossintética foram ajustadas com um modelo sigmoidal, gerando parâmetros fotossintéticos tais como o estado de indução fotossintética (IS), taxa máxima de assimilação de CO_2 (A_{max}) e o tempo para atingir 50% (T_{50}) e 90% (T_{90}) do A_{max}, os quais foram comparados por uma ANOVA - 2 fatores com o teste de Tukey como teste *a posteriori*. O conteúdo de clorofila (Chl *a* e *b*), a taxa de transporte de elétrons (ETR) e o peso foliar específico (SLW) foram também medidos e comparados. A análise de componentes principais (PCA) e o coeficiente de ajustamento à sombra (SAC) foram usados para estimar as respostas fisiológicas globais. Três respostas de aclimatação foram detectadas: *i*) *S. terebinthifolius* apresentou um perfil de elevada demanda luminosa combinado com a elevada capacidade de aclimatação à sombra, *ii*) *H. courbaril* apresentou um perfil de tolerância à sombra aliado à baixa capacidade de aclimatação ao sol e *iii*) *C. pachystachya* e *T. impetiginosa* apresentaram um perfil de alta demanda luminosa combinado à baixa capacidade de aclimatação à sombra. Os resultados obtidos não suportaram as hipóteses clássicas testadas. A LS *T. impetiginosa* mostrou alta capacidade de aclimatação ao sol enquanto a ES *C. pachystachya* apresentou uma baixa capacidade de aclimatação à sombra, não suportando a hipótese *i.* A ES *S. terebinthifolius* apresentou elevada capacidade de utilização de luz quando crescida na sombra, enquanto a LS *T. impetiginosa* apresentou baixa capacidade de aclimatação à sombra, não suportando a hipótese *ii.* Assim, a tolerância ao sombreamento e a preferência luminosa da espécie não está necessariamente relacionada ao seu *status* sucessional e não constituem critérios apropriados para a distinção de grupos ecológicos.

Palavras-chave: espécies arbóreas tropicais Brasileiras, aclimatação fotossintética, utilização de luz, grupos ecológicos

INTRODUCTION

Tropical forest species of different ecological groups rely on distinct light utilization capacities to cope with the high spatio–temporal variability in light. The efficiency of light utilization depends essentially on the photosynthetic induction state, determined by the light–mediated activation of photosynthetic enzymes, mainly Rubisco, stomatal opening and their maintenance under low–light (CHAZDON, 1988; PEARCY et al., 1994). Previous studies have suggested that shade–tolerant species are more efficient in sunfleck utilization due to the ability to achieve and maintain a high photosynthetic induction state when compared to shade-intolerant species (SCHNEIDER et al., 1993; KÜPPERS et al., 1996). It is a crucial trait since sunflecks contribute with 10–80% of total diurnal photosynthetically active radiation (CHAZDON et al., 1996), being determinant to the carbon gain of plants in understory environments (SIMS & PEARCY, 1993; PEARCY & YANG, 1998). Although, it was also previously suggested that early successional species (ES), considered shade–intolerant, would have higher physiological flexibility than late successionals (LS), shade–tolerant, since it is generally assumed that variability in the physical environment is higher in early successional habitats, especially in air and soil temperature, soil humidity and light intensity (BAZZAZ, 1979). FREDEEN & FIELD (1996) and STRAUSS–DEBENEDETTI & BAZZAZ (1996) described that ES typically have greater ability to acclimate photosynthesis to low light than LS to high light, also exhibiting higher and more flexible metabolic rates (BAZZAZ, 1979; BAZZAZ & PICKETT, 1980; STRAUSS-DEBENEDETTI & BAZZAZ, 1996). However there is no general consensus and a number of exceptions to these predictions were reported (VALLADARES et al., 2000; NAUMBURG & ELLSWORTH, 2000; PORTES et al., 2008), indicating that differences in photosynthetic induction dynamics and acclimation capacity are not necessarily related to shade tolerance. Shade tolerance is widely used as a criterion to classify species into ecological groups, even though their precise definition remains a matter of debate (SACK & GRUBB, 2001; KITAJIMA & BOLKER, 2003; NIINEMETS, 2006).

The ecological classification of plant species according to shade tolerance is based on personal observations, mostly devoid of quantitative data (VALLADARES, 2004). Furthermore, there are no defined criteria for classifying tropical tree species

into ecological groups, almost exclusively relying on the habitat of most frequent occurrence. Hence, more studies are necessary to find ecophysiological parameters that characterize ecological groups and the successional status of tropical tree species. While variations in the photosynthetic induction responses between species of different ecological groups have been previously studied (*e.g.* CHAZDON & PEARCY, 1986a; POORTER & OBERBAUER, 1993; KUPPERS et al., 1996; STRAUSS-DEBENEDETTI & BAZZAZ, 1996; VALLADARES et al., 1997; RIJKERS et al., 2000), little is known about photosynthetic induction characteristics in Brazilian tree species. Evaluating the acclimation capacity of such tropical forest tree species is crucial to reveal the physiological properties allowing the occupation of distinct environments.

Divergences from the classical predictions based on ecological groups may be related to previously unrecognized capacities to adjust the photosynthetic apparatus to light conditions experienced during growth, within limits of genetic constitution (KRAUSE et al., 2001), evidenced as phenotypic plasticity expressed according to the life history (SULTAN, 2000). Phenotypic plasticity is the property of a given genotype to produce different morphological and/or physiological phenotypes in response to different environmental conditions (BRADSHAW, 1965; SCHLICHTING, 1986; SCHLICHTING & PIGLIUCCI, 1998; SULTAN, 2000; PIGLIUCCI, 2001), considered as a major mean of individual adaptation to environmental heterogeneity (SCHLICHTING, 1986). STRAUSS-DEBENEDETTI & BAZZAZ (1991) define acclimation as changes arising in already expressed phenotypes, usually at the modular level (*i.e.* leaf), and regard it as a special case of plasticity. Species vary substantially, not only in the photosynthetic capacity under a given growth irradiance, but also in the ability to alter photosynthetic rates upon changes in irradiance, corresponding to photosynthetic acclimation (BOARDMAN, 1977; ANDERSON & OSMOND, 1987). Whereas some species increase their photosynthetic capacity after transfer from low to high irradiance, others do not (JURIK et al., 1979; SIMS & PEARCY, 1992; NEWELL et al., 1993) however, the relation to ecological group remains unclear.

The present study investigated the photosynthetic responses of four species widely found in Brazilian forests and commonly known to belong to distinct ecological groups in order to investigate differences in the acclimation capacity to contrasting

light environments among tropical forest tree species. We focused on comparisons of photosynthetic induction time courses, and parameters derived therefrom, among the four species grown in three contrasting light environments. Based on the classical expectations regarding early and late successional species, the aim was to test the hypotheses: *i)* ES show greater acclimation capacity than LS, *ii)* nonetheless LS show higher light utilization efficiency under low light conditions.

MATERIAL AND METHODS

Characterization of light environments and species: The study was carried out at Universidade do Oeste Paulista (UNOESTE) in Presidente Prudente, São Paulo, Brazil (22º07'32"S; 51º23'20"W, 475 m asl). The local climate is Aw type characterized as tropical with wet summer and dry winter, according to the Köppen classification. This region has a mean annual temperature of 23.1 ºC and mean annual rainfall of 1244 mm.

Three light environments were created, two of them with neutral shade cloth of different transmittances, which reduces the incident irradiance. The first environment was left uncovered, set under full sunlight and received a maximum of PPFD of 1900 μmol m^{-2} s^{-1}. The second had approximately 50% of full sunlight, with a maximum PPFD of 900 μmol m^{-2} s^{-1}, while the third had approximately 10% of full sunlight, with a maximum PPFD of 180 μmol m^{-2} s^{-1}. Temperature and relative humidity were recorded at five minutes intervals in a HOBO (Onset, Bourne) data logger, allowing to further characterize the different light environments. The mean temperature varied from 22° C in the morning to 32° C at midday in the 10% light environment, 22° C to 34° C in the 50% light environment and 21° C to 38° C in the full sun. Mean relative humidity varied from 35% in the morning to 29% at midday in the 10% light environment, 36% to 27% in the 50% light environment and from 36% to 26% in the full sun.

The species investigated in this study are broadly classified as early successionals (ES), *Schinus terebinthifolius* Raddi (Anacardiaceae) and *Cecropia pachystachya* Trec. (Cecropiaceae), and late successsionals (LS), *Tabebuia impetiginosa* (Mart.) Standl. (Bignoniaceae) and *Hymenaea courbaril* L.

(Leguminosae - Caesalpinoideae) (LORENZI, 1992). Saplings of each species were planted in 20 L pots containing potting soil and were randomly allocated to one of the three light levels created and there grown, being watered daily to full soil capacity. All measurements were taken in healthy and fully developed leaves in 6 individuals per species (1 leaf per individual), of approximately 1 year old, in all three light environments.

Leaf gas exchange measurements: Combined CO_2 and H_2O exchange measurements were carried out from 0900 to 1500 h in days with no or few clouds. Measurements of net CO_2 assimilation (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}) and dark respiration (R_d, μmol m^{-2} s^{-1}) were recorded using an open system portable infrared gas analyzer (CIRAS-2, PPSystems, Hertfordshire, UK). The system was set to record at 10 s intervals, flow rate of 200 mL min^{-1} and constant temperature of 28° C. The whole plant was kept in the dark during 1 hour prior to measurements, aiming to induce deactivation of the photosynthetic apparatus and to uniformize the initial state in which time courses were recorded. After 1 h of darkness, a single leaf was enclosed in the CIRAS-2 chamber enabling an initial reading of R_d, recorded for 3 min, and then exposed to a saturating light of 1200 μmol photon m^{-2} s^{-1} for 30 min to measure the induction gain. Tungsten-halogen lamps coupled to the chamber of CIRAS-2 were used as the light source.

Photosynthetic induction state (IS) was evaluated based on time courses of A as described in CHAZDON & PEARCY (1986a):

$$IS = (A_{60} - A_{low}) / (A_{max} - A_{low}) \times 100$$

where A_{60} represents the assimilation rate measured 60 sec after illumination with saturating PPFD, A_{low} is the rate when light is 0, and A_{max} is the steady-state light saturated assimilation rate. Photosynthetic induction curves were fitted using a sigmoidal model, as in ZIPPERLEN & PRESS (1997). Time estimates to attain 50% (T_{50}) and 90% (T_{90}) of A_{max} were obtained through the fitted sigmoidal function. Both hyperbolic and sigmoid curves were observed in the responses measured, however,

in order to calculate the parameters IS, R_d, A_{max}, T_{50} and T_{90}, only sigmoidal were considered. The induction curves allowed the calculation of the integrated variables A_i, g_{si} and ETR_i, as the integral of the induction time course.

Electron transport measurements: Apparent electron transport rate (ETR) was measured with a portable pulse-modulated chlorophyll fluorometer (FMS 2, Hansatech Instruments, UK) in the same healthy and fully expanded leaves used in gas exchange measurements. Both measurements were taken simultaneously through the FMS 2 fiber-optic cable placed in a grooved neck in the CIRAS-2 chamber. In order to evaluate photochemical performance under different light environments, the electron transport rate was calculated according to KRALL & EDWARDS (1992): ETR = $\Delta F/F_M'$ x PPFD x 0.5 x 0.84, where $\Delta F/F_M'$ is the effective quantum efficiency of PSII, 0.5 is the fraction of excitation energy assumed to be distributed to photosystem II (PSII), PPFD is the photosynthetic photon flux density at the time of the measurement and 0.84 is the fraction of total PPFD absorbed by leaves (DEMMIG & BJÖRKMAN, 1987). In order to obtain F_M' (maximum fluorescence in light), pulses of saturating actinic light were applied at 5 min intervals during the course of gas exchange measurements, allowing to estimate the PSII quantum yield: $(F_M' – F)/F_M'$, where F is the fluorescence measured briefly before the saturating light pulse (GENTY et al., 1989).

Chlorophyll content and specific leaf weight: Samples of the same leaf material used for gas exchange and chlorophyll fluorescence measurements were collected to chlorophyll (Chl) content determination. Leaf discs were collected from different leaves and assays were carried out immediately after its removal. Leaf samples were homogenized in 80% acetone and then centrifuged for 5 min at 3000 g. Spectrophotometer readings of the supernatant were obtained by the absorbance at 645 nm and 663 nm, being used to determine Chl *a* and *b* contents, respectively, as well as total Chl content on dry weight basis. The Chl concentration in the supernatant was determined according to equations described by PORRA et al. (1989).

In order to determine specific leaf weight (SLW, dry weight per unit leaf area, Kg m^{-2}), leaf area was measured with an leaf area meter (Li-3000A, Licor, Nebraska, USA) and dry leaf mass was obtained after drying at 60º C until constant weight.

Data analysis: Differences in mean values of the variables measured among the four species grown in the three light environments were analyzed by a two-way analysis of variance (ANOVA) and *a posteriori* Tukey's test, at 5% significance level using the software Statistica (v7, Statsoft Tulsa, OK, USA). Throughout the text every difference considered significant had a $P < 0.05$ (Tukey's test).

Principal component analysis (PCA) is a technique which enables the exploration of multivariate data sets through the reduction of *n* variables to lower dimensions, which are formed by principal components. These are independent axes composed of linear combinations of the original variables, as to maximize the explained variances. Thus, the first principal component (PC1) explains most of the variance in the observed data, followed by PC2 and so on, being an effective approach to identify groups formed by the combined effect of the evaluated variables (HAIR et al., 2006). The analysis was performed in the language for statistical computing R, by means of the function *prcomp* (R Development Core Team, 2009), on the normalized data of all replicates (variances scaled to 1) with the variables: A_{max}, A_i, SLW, A_{max}/SLW, initial, final and integrated g_s, ETR_i, IS, R_d, T_{50}, T_{90}, Chl *a*, Chl *b*, Chl *a*:*b*.

In order to assess acclimation capacity more quantitatively, a rough estimate of overall physiological change due to irradiation regime was defined based on the Euclidian distance between centroids in the PC1 *vs* PC2 coordinate plane. The Euclidian distance between two irradiation regime within a given species would not be a direct measure of acclimation, but instead would reflect overall physiological change between a pair of conditions, which can then be used to indicate acclimation in light of other results.

The shade adjustment coefficient, as proposed by Laisk et al. (2005) is an index used to characterize the extent of change in a given variable due to shade conditions being calculate as: SAC = 1 - (Shade/Sun). Where Shade and Sun are the mean values of a given variable in each respective condition. Higher absolute values

indicate greater extent of shade adjustment, being positive when Sun values were higher.

RESULTS

Chlorophyll content and specific leaf weight

Light availability affected total chlorophyll (Chl) content, which was at least two fold higher in leaves grown under 10% light than under full sun in all species studied (Table 1). Accordingly, the separate Chl *a* and Chl *b* content was also significantly higher under 10% light than under full sun in all species. Comparing among species, Chl *a* and *b* contents were significantly lower in *H. courbaril* under 10% light, while there were no significant differences among species under 50% light and full sun. The Chl *a:b* ratio was higher under full sun than under 10% light for all species, however this difference was not statistically significant in *C. pachystachya* and *H. courbaril*. There was no significant difference among species under 10% and 50% light, while under full *T. impetiginosa* showed the highest Chl *a:b* ratio, whereas *C. pachystachya* and *H. courbaril* showed the lowest values.

Specific leaf weight (SLW), an estimate of density and leaf thickness, was significantly influenced by light availability (Table 2). Leaves of all four species grown under full sun had significantly higher SLW than leaves grown under 10% and 50% light. *H. courbaril* presented the lowest differences in SLW among the light environments, having a significantly higher SLW than other species under 10% and 50% light. Under full sun conditions, SLW was significantly higher in *T. impetiginosa* and lower in *S. terebinthifolius*.

Table 1. Chlorophyll *a* and *b* content, *a:b* ratio in leaves of *S. terebinthifolius*, *C. pachystachya*, *T. impetiginosa* and *H. courbaril* grown in the 10% and 50% light environments and in the Full sun. Capital letters indicate difference between species in same light environment whereas small letters indicate statistical difference between light environments ($p<0.05$ by Tukey's test). Data represents mean values (n=6) ± SE.

Species	Light environment	Chl *a*	Chl *b*	Chl *a*:*b*
		($\mu g\ g^{-1}$ DM)		
S. terebinthifolius	10%	628.45 ± 80.94 ^{Aa}	453.51 ± 56.92 ^{Aa}	1.41 ± 0.12 ^{Ab}
	50%	308.96 ± 14.38 ^{Ab}	174.22 ± 22.13 ^{Ab}	2.06 ± 0.34 ^{Aab}
	Full sun	208.85 ± 19.54 ^{Ab}	73.75 ± 11.68 ^{Ac}	3.06 ± 0.40 ^{ABa}
C. pachystachya	10%	612.09 ± 25.29 ^{Aa}	457.14 ± 37.39 ^{Aa}	1.38 ± 0.09 ^{Aa}
	50%	338.19 ± 21.92 ^{Ab}	194.05 ± 12.57 ^{Ab}	1.77 ± 0.12 ^{Aa}
	Full sun	238.08 ± 10.26 ^{Ac}	123.13 ± 18.11 ^{Ab}	2.28 ± 0.37 ^{Ba}
T. impetiginosa	10%	621.48 ± 18.43 ^{Aa}	429.63 ± 30.40 ^{Aa}	1.49 ± 0.14 ^{Ab}
	50%	285.09 ± 8.01 ^{Ab}	134.43 ± 17.80 ^{Ab}	2.30 ± 0.24 ^{Ab}
	Full sun	188.66 ± 18.95 ^{Ab}	59.30 ± 14.54 ^{Ab}	3.81 ± 0.51 ^{Aa}
H. courbaril	10%	455.37 ± 54.39 ^{Ba}	319.69 ± 15.81 ^{Ba}	1.46 ± 0.22 ^{Aa}
	50%	255.80 ± 6.29 ^{Ab}	179.40 ± 23.41 ^{Ab}	1.54 ± 0.23 ^{Aa}
	Full sun	252.49 ± 8.08 ^{Ab}	125.28 ± 22.13 ^{Ab}	2.21 ± 0.30 ^{Ba}

Photosynthetic parameters and electron transport rate

Comparing the effect of light environment in dark respiration rates (R_d), both ES *S. terebinthifolius* and *C. pachystachya*, showed higher R_d in the full sun, whereas *T. impetiginosa* did not show significant difference between the 50% light environment and the full sun, and *H. courbaril* did not differ among light environments. R_d did not differ significantly among species under 10% light, being highest in *T. impetiginosa* and lowest in *H. courbaril* under 50% light, and lowest in *H. courbaril* under full sun (Figure 1).

Table 2. Mean (± SE, n=6) of specific leaf weigh (SLW), its ratio with the maximum assimilation rate A_{max}/SLW, assimilation rate (A_i) and stomatal conductance (g_{si}) integrated over the induction time course, stomatal conductance in the beginning (g_s initial) and end (g_s final) of the induction time course. Capital letters indicate difference between species in same light environment whereas small letters indicate statistical difference between light environments ($p < 0.05$ by Tukey's test).

Species	Light environment	SLW (Kg m^{-2})	A_{max}/SLW (μmol CO_2 g^{-1} s^{-1})	A_i (mmol CO_2 m^{-2} h^{-1})	g_{si} (mol H_2O m^{-2} h^{-1})	g_s initial (mmol H_2O m^{-2} s^{-1})	g_s final
S. terebinthifolius	10%	0.035 ± 0.003 ^{Bc}	0.24 ± 0.02 ^{Aa}	738.03 ± 48.55 ^{Aa}	14.90 ± 1.63 ^{Aa}	73.17 ± 16.20 ^{Aa}	136.67 ± 9.87 ^{Aa}
	50%	0.061 ± 0.004 ^{Bb}	0.19 ± 0.02 ^{Aab}	979.59 ± 106.30 ^{Aa}	15.86 ± 3.74 ^{Aa}	79.33 ± 40.77 ^{Aa}	141.83 ± 30.00 ^{Aa}
	Full sun	0.081 ± 0.003 ^{Ba}	0.13 ± 0.01 ^{Ab}	873.33 ± 61.36 ^{Ba}	15.29 ± 1.31 ^{Ba}	76.67 ± 11.64 ^{ABa}	135.17 ± 12.39 ^{Ba}
C. pachystachya	10%	0.036 ± 0.002 ^{Bc}	0.20 ± 0.01 ^{Aa}	550.49 ± 37.97 ^{Ab}	11.20 ± 1.24 ^{Ab}	32.67 ± 5.89 ^{Bb}	146.83 ± 12.99 ^{Ab}
	50%	0.061 ± 0.003 ^{Bb}	0.20 ± 0.03 ^{Aa}	743.19 ± 177.15 ^{Ab}	13.71 ± 2.57 ^{Ab}	33.67 ± 7.49 ^{Bb}	207.50 ± 30.86 ^{Ab}
	Full sun	0.093 ± 0.004 ^{ABa}	0.15 ± 0.02 ^{Aa}	1143.54 ± 74.50 ^{Aa}	28.47 ± 2.00 ^{Aa}	142.00 ± 32.08 ^{Aa}	313.83 ± 28.24 ^{Aa}
T. impetiginosa	10%	0.038 ± 0.002 ^{Bc}	0.16 ± 0.01 ^{ABa}	466.52 ± 31.20 ^{Ab}	8.47 ± 0.88 ^{Ab}	23.83 ± 6.63 ^{Bb}	117.33 ± 7.90 ^{Aa}
	50%	0.068 ± 0.002 ^{Bb}	0.15 ± 0.01 ^{ABa}	583.86 ± 49.79 ^{Bb}	9.96 ± 1.09 ^{Ab}	37.17 ± 7.05 ^{Bb}	151.67 ± 12.66 ^{Aa}
	Full sun	0.096 ± 0.003 ^{Aa}	0.12 ± 0.01 ^{Aa}	880.72 ± 89.55 ^{Ba}	18.49 ± 2.63 ^{Ba}	162.67 ± 33.70 ^{Aa}	200.67 ± 19.76 ^{Ba}
H. courbaril	10%	0.080 ± 0.010 ^{Ab}	0.11 ± 0.01 ^{Ba}	588.41 ± 93.72 ^{Aa}	8.37 ± 2.26 ^{Aa}	35.67 ± 18.95 ^{Ba}	136.67 ± 21.53 ^{Aa}
	50%	0.084 ± 0.009 ^{Aab}	0.11 ± 0.01 ^{Ba}	536.32 ± 77.02 ^{Ba}	8.12 ± 2.19 ^{Aa}	36.50 ± 18.52 ^{Ba}	125.17 ± 13.90 ^{Aa}
	Full sun	0.093 ± 0.001 ^{ABa}	0.10 ± 0.01 ^{Aa}	487.07 ± 64.91 ^{Ba}	7.28 ± 1.26 ^{Ca}	17.67 ± 3.34 ^{Ba}	123.17 ± 14.97 ^{Ba}

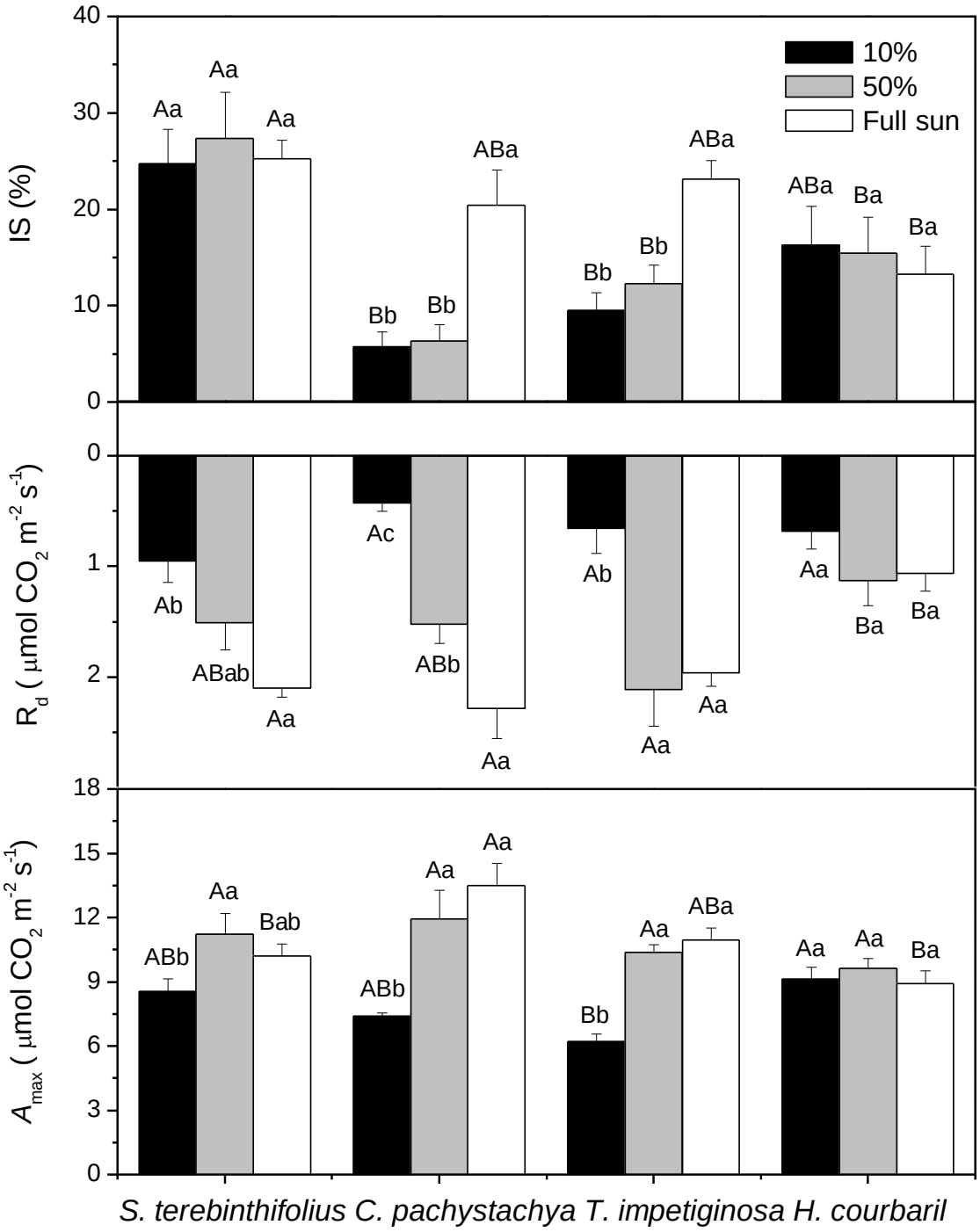

Figure 1. Photosynthetic induction state (IS), dark respiration (R_d) and maximum CO_2 assimilation (A_{max}), in leaves of *S. terebinthifolius*, *C. pachystachya*, *T. impetiginosa* and *H. courbaril* grown in the 10% and 50% light environments and in the Full sun. Capital letters indicate difference between species in same light environment whereas small letters indicate statistical difference between environments ($p<0.05$ by Tukey's test). Data represents mean values (n=6) ± SE.

Regarding the maximum assimilation rate (A_{max}), light environment had a clear significant effect only on the ES *C. pachystachya* and on the LS *T. impetiginosa*, which showed significantly higher values under full sun when compared to 10% light, but not to 50% light environment (Figure 1). The ES *S. terebinthifolius* and the LS *H. courbaril* showed no clear differences across light environments, sharing a similar response. Under 10% light, *H. courbaril* exhibited significantly higher assimilation rates than other species, while under 50% light there was no significant difference among species, and under full sun *C. pachystachya* had a significantly higher A_{max}.

The integrated assimilation rate (A_i), calculated over the whole induction time course, showed significant differences across light environments only in *C. pachystachya* and *T. impetiginosa*, being highest under full sun and having no significant difference under 10% and 50% light (Table 2). As in A_{max}, light environment had no significant difference in the A_i of *S. terebinthifolius* and *H. courbaril*. However, the response of both species were hardly the same given the significantly higher values shown by *S. terebinthifolius* under 50% light and, albeit not statistically significant, the almost 85% higher values under full sun. A_i was not significantly different among species under 10% light, being higher in both ES species under 50% light, and significant higher in *C. pachystachya* under full sun (Table 2).

The ratio A_{max}/SLW, indicating the photosynthetic efficiency as assimilation per unit of mass, show a trend to increase with shading, although significant difference across light environments was only detected in *S. terebinthifolius* (Table 2). Comparing among species, *H. courbaril* showed the significant lowest A_{max}/SLW under 10 and 50% light.

The induction state (IS) was clearly influenced by light environment on both the ES *C. pachystachya* and the LS *T. impetiginosa*, which showed significantly higher IS in the full sun, but no significant differences between 10% and 50% light (Figure 1). Interestingly, IS did not change significantly across light environments in both the ES *S. terebinthifolius* and the LS *H. courbaril*, although the ES had significantly higher values than the LS practically in all conditions. In fact, *S. terebinthifolius* presented significantly higher IS than other species in all three light environments studied. These comparisons can also be visualized through the induction courses (Figure 3).

There was a high correlation between assimilation (*A*) and electron transport rate (ETR) time courses, with R^2 values above 0.85 and $P < 0.01$ for all species in all light environments studied (data not shown). This is expected since, after light increases, the assimilation rate depends on ETR. Following exposure to high PPFD, ETR increased rapidly and reached steady-state values in about 10–15 min (Figure 2). In *S. terebinthifolius*, *C. pachystachya* and *T. impetiginosa* ETR was generally higher under full sun and lower in the 10% light environment, whereas in *H. courbaril* there were no clear differences among light environments. In fact, ETRi showed a gradient among light environments, being significantly under full sun and lowest in the 10% light environment in all species, except for *H. courbaril* which presented no significant differences (Figure 2).

Representative time courses of the photosynthetic induction curves of all four species studied are shown in Figure 3. In response to a sudden increase, from practically zero irradiance to saturating light, all four species in all three light environments showed typical induction profiles where photosynthesis gradually rose to a steady–state rate (Figure 3). As described by CHAZDON & PEARCY (1986b) and LEAKEY et al. (2005), the time-course of CO_2 assimilation and stomatal conductance appear nearly coincident, while intercellular CO_2 concentration (C_i) show an initial drop upon illumination and then remains relatively stable during the induction period (data not shown). The increase in photosynthesis was also accompanied by a respective increase in the stomatal conductance (g_s), although the integrated, initial and final g_s differed between species and light environments.

The ES *C. pachystachya* and the LS *T. impetiginosa* showed a similar variation in integrated, intial and final g_s among light environments, consisting in significantly lower g_s under 10% and 50% light and up to four-fold higher values under full sun (Table 2). The ES *S. terebinthifolius* and the LS *H. courbaril* showed similar profiles regarding these three g_s parameters, presenting indistinct rates among light environments, except for intial g_s in *H. courbaril* which decreased under full sun (Table 2). Comparing species, initial g_s was significantly higher in *S. terebinthifolius* under 10 % and 50% light and significantly lower in *H. courbaril* under full sun. Final g_s was significantly lower in *C. pachystachya* under 10% and 50% light, but significantly higher under full sun. Regarding g_{si}, *H. courbaril* showed significantly lower values under full sun than any other species.

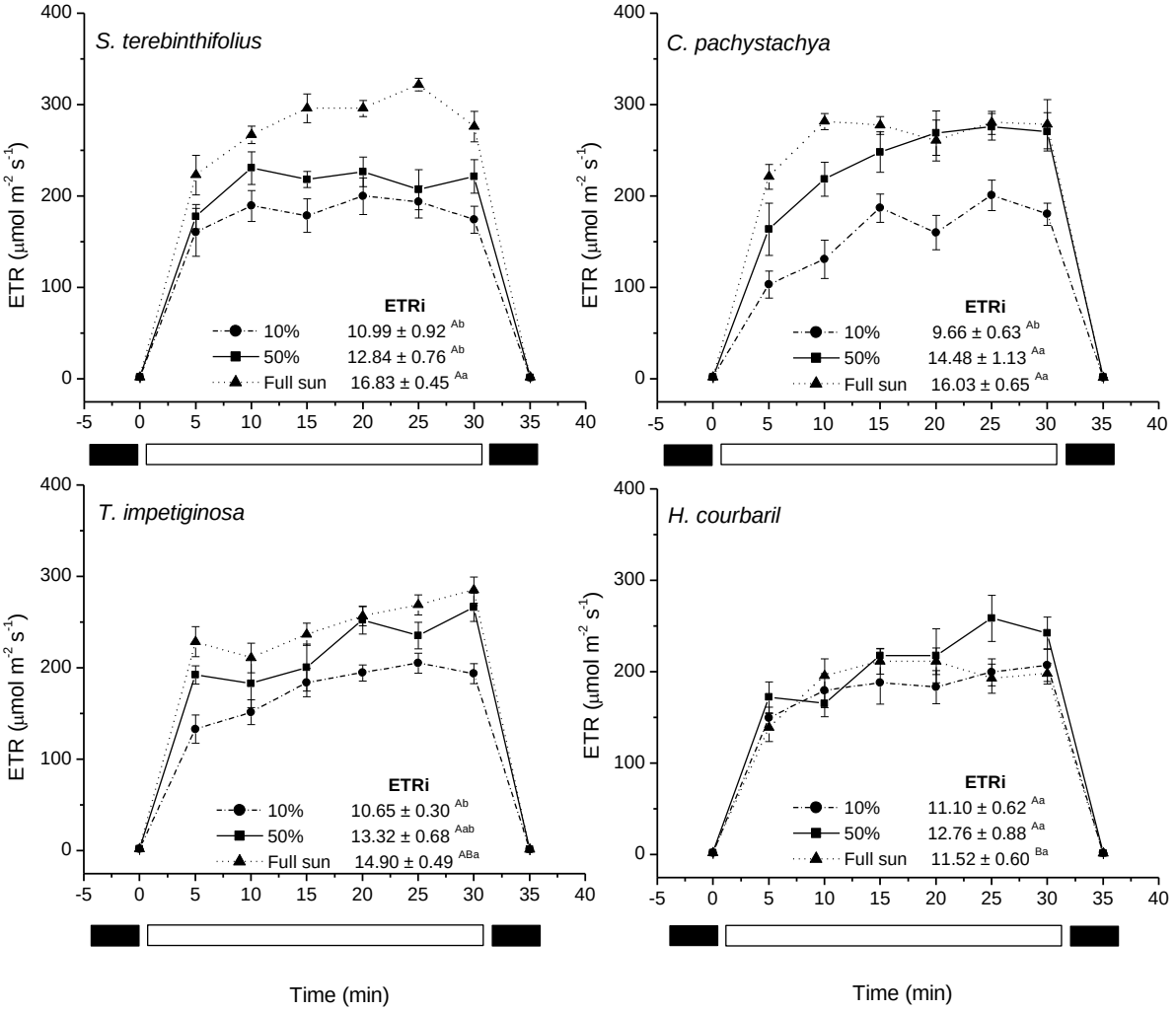

Figure 2. Time course of electron transport rate (ETR) during photosynthetic induction in leaves of *S. terebinthifolius*, *C. pachystachya*, *T. impetiginosa* and *H. courbaril* grown in the 10% (●) and 50% light environments (■) and in the Full sun (▲). Transversal black bars indicate darkness and white bars indicate exposure to light saturating. ETRi values are expressed in mmol m^{-2} h^{-1} and capital letters indicate difference between species in same light environment whereas small letters indicate statistical difference between environments ($p<0.05$ by Tukey's test). Data represents mean values (n=6) ± SE.

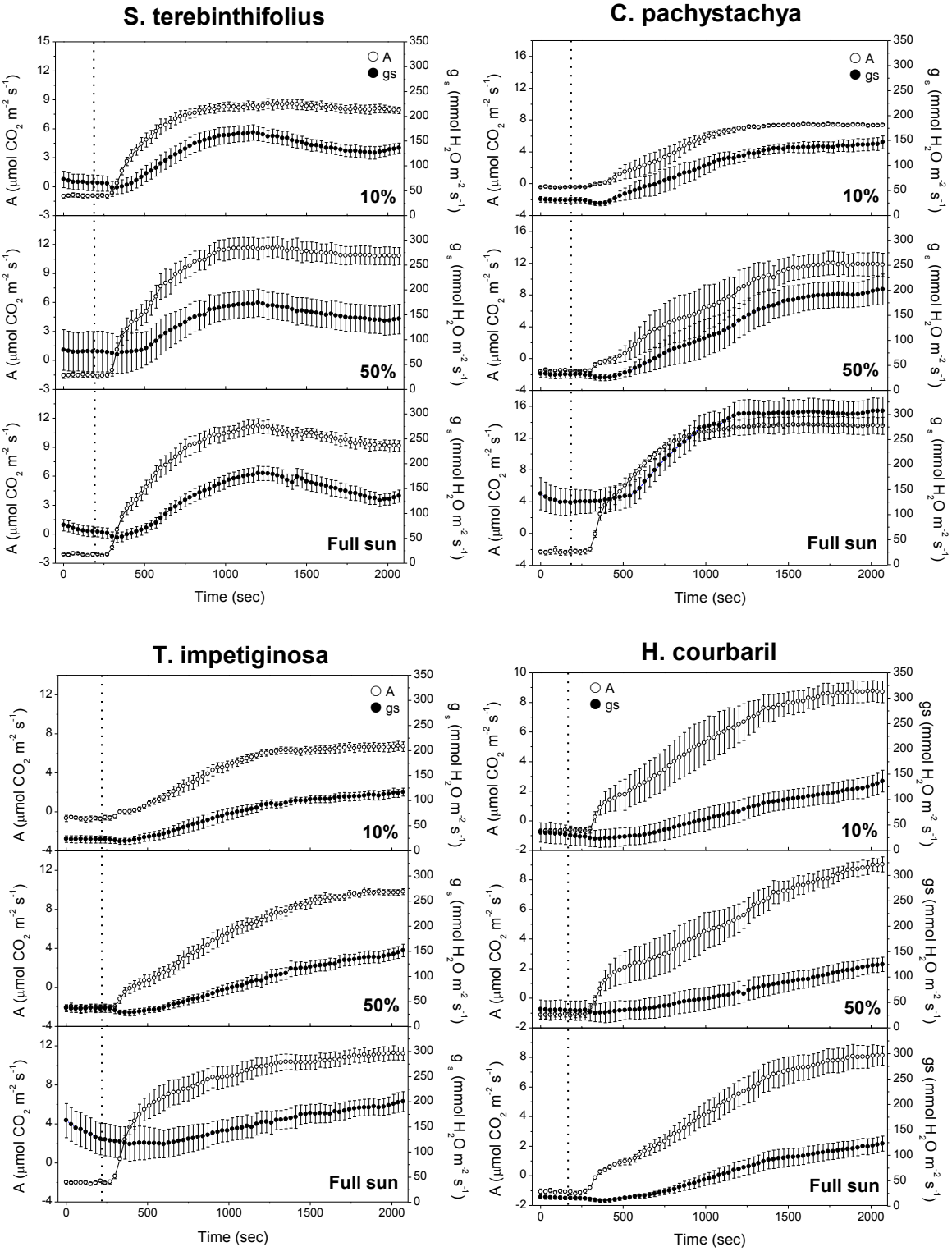

Figure 3. Time course of the carbon assimilation (*A*) and stomatal conductance (g_s) during photosynthetic induction of leaves of *S. terebinthifolius*, *C. pachystachya*, *T. impetiginosa* and *H. courbaril*. Each point is the mean ± SE of single measurements from six leaves, each on from a different plant. Vertical line represents the beginning of the saturating light.

Additionally, the importance of g_s in photosynthetic induction was evidenced by different stomatal responses to light onset, where hyperbolic responses were related to a higher relative induction gain when compared to sigmoidal responses (data not shown). This is in agreement with previous reports by VALLADARES et al. (1997).

The photosynthetic response of the fully non-induced leaves to a sudden increase in PPFD required an induction period of 10–28 min before A_{max} was reached (Figure 3), and Table 3 summarizes the time required to reach 50% and 90% of A_{max} (T_{50} and T_{90} respectively). While a significant trend to reduce induction time with increase in irradiation is verified in the ES *C. pachystachya* and LS *T. impetiginosa*, this is not the case of *H. courbaril* and *S. terebinthifolius* (Table 3). *S. terebinthifolius* showed significantly faster induction under 10% and 50% light when compared to other species, reaching T_{50} and T_{90} within about 4 min and 8-9 min of exposure to saturating light, respectively. This result can be also evidenced by the IS values of this species (Figure 1), indicating a higher capacity to respond to light increase. Under full sun conditions, *H. courbaril* showed significantly slower induction, being in accordance with other results shown and suggesting a limitation to exploit this environment.

Overall comparisons

The principal component analysis performed on the variables A_{max}, A_i, SLW, A_{max}/SLW, initial, final and integrated g_s, ETR_i, IS, R_d, T_{50}, T_{90} of all replicates of all species and environments evaluated, explained a total of 63.5% of all the variance contained in the data, considering only the first two principal components (Figure 4). PC1 accounted for 41.1% of the variance being composed mainly by combinations of the variables (ranked in order of importance): g_{si}, ETR_i, A_i, R_d, Chl *b* content, A_{max}, Chl *a* content, initial and final g_s. PC2 accounted for 22.4% of the total data variance, being composed mainly by the variables (ranked in order of importance): T_{90}, A_{max}/SLW, T_{50}, SLW, Chl *b* content and Chl *a* content. The analysis provided a clear overall separation of the different irradiance regimes, evidencing the overall effect of light environment of the physiology of different species. According to the previously

Table 3: Mean (± SE) time (s) required to reach 50% (T_{50}) and 90% (T_{90}) steady-state net photosynthetic rate in leaves of *S. therebinthifolius*, *C. pachystachya*, *T. impetiginosa* and *H. courbaril* grown in the 10% and 50% light environments and in the Full sun. Capital letters indicate difference between species in same light environment whereas small letters indicate statistical difference between light environments ($p<0.05$ by Tukey's test). Data represents mean values (n=6) ± SE.

Species	Light environment	T_{50} (s)	T_{90} (s)
S. terebinthifolius	10%	218,82 ± 34,28 ^{Ba}	448,34 ± 38,86 ^{Ba}
	50%	253,69 ± 52,05 ^{Ba}	492,92 ± 42,29 ^{Ba}
	Full sun	219,60 ± 31,66 ^{Ba}	551,63 ± 57,59 ^{Ba}
C. pachystachya	10%	529,27 ± 95,66 ^{Aab}	694,28 ± 106,45 ^{ABab}
	50%	589,52 ± 152,15 ^{Aa}	819,07 ± 116,27 ^{Aa}
	Full sun	249,16 ± 49,11 ^{Bb}	561,07 ± 74,24 ^{Bb}
T. impetiginosa	10%	583,41 ± 71,79 ^{Aa}	851,00 ± 79,28 ^{Ab}
	50%	811,10 ± 66,94 ^{Aa}	1349,21 ± 151,24 ^{Aa}
	Full sun	336,10 ± 114,01 ^{Bb}	919,71 ± 261,46 ^{Bb}
H. courbaril	10%	678,32 ± 163,57 ^{Aa}	1119,69 ± 211,86 ^{Aa}
	50%	715,24 ± 224,85 ^{Aa}	1168,60 ± 214,37 ^{Aa}
	Full sun	797,95 ± 92,79 ^{Aa}	1521,41 ± 115,59 ^{Aa}

described results, *H. courbaril* present all replicates within a close vicinity of the coordinate plane, indicating a lower response to light treatments. In a lesser extent, this is also true for *S. terebinthifolius* which apparently show lower distance between replicates than *C. pachystachya* and *T. impetiginosa*.

In order to quantitatively assess these interpretations, the Euclidian distance between the centroids of each light environment, within a given species, was calculated as a mean to indicate overall change (Table 4). For the sake of simplicity, only distances between full sun and 10% light environment were shown, however the other pair wise distances are qualitatively similar. Accordingly, the largest distances between centroids are associated with *C. pachystachya* followed by *T. impetiginosa*,

which indeed showed a large response to light conditions. *S. terebinthifolius* had even lower distances between centroids corresponding to distinct light environments, but still which exceeded considerably the distances observed in *H. courbaril*, which were undoubtedly the shortest.

The shade adjustment coefficient (SAC), proposed by Laisk et al. (2005), sheds an additional light in the overall photosynthetic responses of the studies species to contrasting light (Table 4). The differences between full sun and 10% light which were considered significant in the Tukey's test were marked in bold face, guiding the interpretation of the significance of the change in a given variable. Ubiquitous responses are found in the top of the table, where all species showed a significant increase in Chl *a* and *b* contents under 10% light (SAC < 0), and a decrease in SLW (SAC > 0). Then, agreeing with the suggested limitation in photosynthetic acclimation of *H. courbaril* to high irradiance conditions, a series of variables that showed increase in full sun conditions in the remaining species, had no significant change in the LS *H. courbaril i.e.* SLW, albeit significant it was low, Chl *a:b* ratio, A_{max}, R_d, ETR_i and A_i. According to the observed gradient-like response in the ES *C. pachystachya* and the LS *T. impetiginosa*, SAC values evidence the significant decreases in IS, g_{si}, initial and final g_s, and increase in T_{50} and T_{90}, from full sun to 10% light. Finally, *S. terebinthifolius* was the only species that significantly changed A_{max}/SLW, increasing it under shade conditions.

Thus, the number and nature of the variables significantly changed under contrasting light regimes, indicated by the SAC, which are coherent with the Euclidian distances extracted from the PCA. Both consistently indicate that physiological changes were greatest in the ES *C. pachystachya*, followed by the LS *T. impetiginosa*, the ES *S. terebinthifolius*, and the LS *H. courbaril*, which showed the lowest response to changes in irradiance. It is crucial to bear in mind that the magnitude of overall change in physiological variables does not translate directly to acclimation capacity since, as discussed below, it depends on the specific ecophysiological meaning of the observed responses.

Table 4. Shade adjustment coefficient for the variables indicated in the rows (*see Material and Methods for abbreviations*), was calculated as SAC = 1 - (Shade/Sun), where Sun and Shade represent the mean values of a given variable measured under full sun and 10% light, respectively. Negative values of SAC indicate higher mean under 10% light, whereas the absolute values grows with higher change between light environments. Bold face letters indicate significant differences detected with Tukey's *post-hoc* test at 0.05% significance distances are the Euclidian distances between full sun and 10% light within a given species, calculated based on the mean values of the two main principal components.

Variables	*C. pachystachya*	*T. impetiginosa*	*S. terebinthifolius*	*H. courbaril*
Distance	100	90.80	73.08	22.53
Chl *b*	-**2.51**	-**6.24**	-**5.15**	**-1.55**
Chl	-**1.62**	-**2.29**	-**2.01**	**-0.80**
SLW	**0.59**	**0.58**	**0.54**	**0.09**
Chl *a:b*	**0.32**	**0.61**	**0.54**	0.34
A_{max}	**0.45**	**0.43**	**0.16**	-0.02
R_d	**0.81**	**0.62**	**0.55**	0.36
ETR_i	**0.40**	**0.29**	**0.35**	0.04
A_i	**0.52**	**0.47**	**0.15**	-0.21
IS	**0.72**	**0.59**	0.02	-0.22
T_{50}	-**0.41**	-**0.58**	0.18	-0.02
T_{90}	-**0.27**	-**0.18**	0.20	0.02
g_s initial	**0.77**	**0.85**	0.05	-1.02
g_{si}	**0.61**	**0.54**	0.03	-0.15
g_s final	**0.53**	0.42	-0.01	-0.11
A_{max}/SLW	-0.30	-0.34	-**0.86**	-0.14

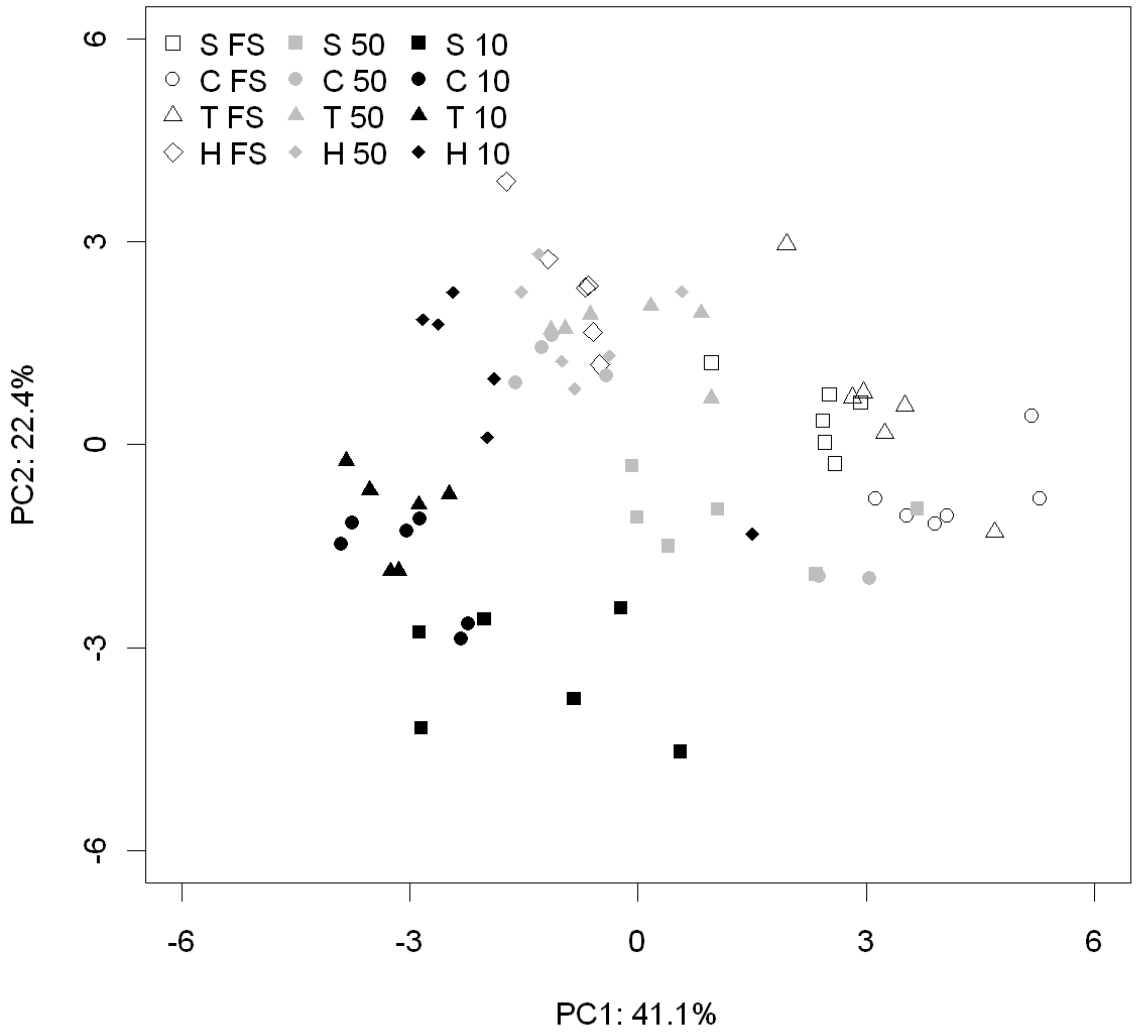

Figure 4. First and second principal components explaining a total of 63.5% of the variance present on data, comprised of the variables A_{max}, A_i, SLW, A_{max}/SLW, initial, final and integrated g_s, ETR_i, IS, Rd, T_{50}, T_{90}, Chl *a*, Chl *b*, Chl *a*:*b* (*see Material and Methods for abbreviations*). Points are replicates of measurements of each species and light environment studied, plotted into the PC1 *vs* PC2 coordinate plane. S is *S. terebinthifolius*, C is *C. pachystachya*, T is *T. impetiginosa* and H is *H. courbaril*, while 10 and 50 represent 10% and 50% light respectively, and FS the full conditions.

DISCUSSION

According to PEARCY (1999) one of the most universal responses to increased light availability is the plasticity in leaf photosynthetic properties that results in development of “sun” leaves, having higher photosynthetic capacity per unit area (A_{max}), greater leaf thickness and greater leaf mass per unit area, referred by MURCHIE & HORTON (1997) as leaf level acclimation. In fact, the results reported herein show that light conditions had large effects on the accumulation of leaf mass per area, since all species presented significantly higher SLW, albeit small in *H. courbaril*, under full sun (Table 2) while, curiously, not all species presented higher A_{max} or A_i (Figure 1, Table 2). This suggests that higher investment in leaf tissues under high light conditions is not necessarily related to a proportional increase in A_{max}. Higher SLW in high light grown leaves was also described by KRALL et al. (1995), MURCHIE & HORTON (1997) and PORTES et al. (2006). The latter study showed higher SLW both in the ES *Bauhinia forficata* and the LS *E. leiocarpa*, when grown in the gap as compared to understory, showing significant differences between ecological groups.

The A_{max}/SLW trended to be higher under shade conditions, reflecting the higher carbon assimilation efficiency per mass of leaf tissue, which is mainly due to a lower SLW under low light. However, *S. terebinthifolius* was the only species to show a large and significant difference in this parameter, which is not solely due to SLW since, in other species, even larger SLW decreases did not lead to increase in A_{max}/SLW (Table 4). In addition, when compared to the other species, the small change in SLW in *H. courbaril* probably indicate a low photosynthetic acclimation potential to high irradiances, also supported by the lack of change in A_{max} even in full sun conditions, maintaining relatively low values.

An increase in growth irradiance is commonly observed to result in an increase in the CO_2 assimilation capacity by Calvin cycle enzymes, which is matched by modulation of the number of electron transport components per molecule of Chl, allowing a higher rate of electron flow (DE LA TORRE & BURKEY, 1990). The rate of regeneration of ribulose-1,5-bisphosphate (RuBP) is determined by ETR (EVANS, 1996), and the activity of Rubisco is highly correlated with ETR (KRALL et al., 1995). Indeed our results indicate a correspondence in A_{max} and ETR, generally higher in

plants grown in the full sun and lower under 10% light, where A_{max} is lowest (Figure 1,2) as indicated by the high correlation value between A-ETR courses (R^2 values above 0.85 and $P < 0.01$ in all treatments). Accordingly, ETR_i was significantly higher under full sun and lowest under 10% light in all species, except for *H. courbaril* which presented no significant differences (Figure 2). This constitutes yet another evidence of the low photosynthetic acclimation capacity of *H. courbaril* to high light.

The significant accumulation of both Chl *a* and *b* under 10% light, in all four species (Table 1), is probably related to an increased allocation of resources in light-harvesting functions, rather than in electron transport and CO_2 fixation capacity (BJÖRKMAN, 1981). Accordingly, acclimation to shade conditions has been shown to result in a decrease of Chl *a:b* ratio, reflecting changes in the concentration of light-harvesting Chl complexes relative to reaction centres (ANDERSON & OSMOND, 1987). Considering ecological groups, KRAUSE et al. (2001) verified that leaves of ES responded to prolonged sun exposure in wider gaps with a significant increase in Chl *a:b* ratio (above 3.0), which was not observed in the LS. We observed that only the ES *S. terebinthifolius* and mainly the LS *T. impetiginosa* presented Chl a*:b* ratios above 3.0, not supporting the apparent difference between ecological groups.

In order to grow in low light environments such as the forest understory, where the carbon gain is low (STRAUSS-DEBENEDETTI & BAZZAZ, 1996; CHAZDON et al., 1996), plants must minimize carbon loss through reduction of both respiration and tissues construction cost (GIVNISH, 1988). In fact, all species studied showed significantly lower R_d under 10% light than in the other environments (Figure 1,3), being in agreement with previous reports (FREDEEN & FIELD, 1991; HAN et al., 1999). Some studies also described that ES usually show higher R_d than LS (BAZZAZ & PICKETT, 1980; CHAZDON et al., 1996) however, no difference in R_d was detected among species, except for *H. courbaril* which showed lower rates than other species under full sun and under 50% light. These results indicate that the response in R_d is not necessarily related to ecological group.

In tropical forests, ES are known for having much higher CO_2 assimilation capacity than LS (POORTER, 1999). Our results conflict with this classical prediction since no significative differences in A_{max} or A_i was strictly related to ecological groups. Interestingly, while *C. pachystachya* (ES) and *T. impetiginosa* (LS) showed a

marked increasing trend in A_{max}, A_i and IS towards the highest irradiance, both *S. terebinthifolius* and *H. courbaril* maintained similar values among light environments (Figure 1). This is intriguing since high values of IS are unexpected for *S. terebinthifolius*, mainly under 10% light, indicating a high photosynthetic acclimation to low light conditions which was not verified in the other ES (*C. pachystachya*). Conversely, in *H. courbaril*, the lack of an increasing trend with light intensity in A_{max}, A_i and IS rather indicates a low acclimation capacity to high light, given their significantly smaller values under 10% and 50% light.

Agreeing with the results with *S. terebinthifolius* and *H. courbaril*, photosynthetic induction responses were indistinguishable among plants of the same species grown in forest gaps or in the understory in a rainforest field study in Panama (KURSAR & COLEY, 1993). Another interesting result is the increasing A_{max}, A_i and IS with increasing irradiance observed in the *T. impetiginosa* (LS), similar to the response of ES *C. pachystachya* (ES), which is surprising for its successional status being probably related to a potential to occupy gap-like niches. Probably, high IS observed in *T. impetiginosa* and in *C. pachystachya* under full sun is related with the high initial, final and integrated g_s detected in both species, also enabling the decrease in T_{50} and T_{90} (Figure 3, Table 3). This indicates a possible stomatal limitation in *S. terebinthifolius* and *H. courbaril* which could be underlying the lack of change observed in A_{max} and IS throughout light environments.

Stomata can exert significant influence over photosynthetic induction and lightfleck use efficiency (KIRSCHBAUM & PEARCY, 1988; TINOCO-OJANGUREN & PEARCY, 1992, 1993) thus, the maintenance of high g_s is critical to the light utilization, being essential to achieving a positive carbon balance in understorey species, as demonstrated by LEAKEY et al. (2005) in dipterocarp seedlings. This is further illustrated in time courses of photosynthetic induction, which show either a hyperbolic or sigmoidal shape, depending on initial g_s. Although only sigmoidal responses were considered in the analysis presented herein, hyperbolic induction profiles were observed in association with high g_s. This is expected since without stomatal limitations, photosynthetic induction would be mainly constrained by biochemical limitations (KIRSCHBAUM & PEARCY, 1988).

As earlier described with other variables, no difference in T_{50} and T_{90} was observed among light environments in *S. terebinthifolius* and *H. courbaril* (Table 2). In

fact, previous studies have found that the induction times of some tropical species are not affected by light environment (CHAZDON & PEARCY, 1986a; KURSAR & COLEY, 1993; ZIPPERLEN & PRESS, 1997; RIJKERS et al., 2000). Furthermore, our results did not indicate the expected faster induction times in LS when grown in the shade (hypothesis *ii*), on the contrary the LS *T. impetiginosa* presented significantly higher induction times than the ES *S. terebinthifolius* under 10% and 50% light (TINOCO-OJANGUREN & PEARCY, 1992; KURSAR & COLEY, 1993; POORTER & OBERBAUER, 1993; ÖGREN & SUNDIN, 1996; VALLADARES et al., 1997; NAUMBURG & ELLSWORTH, 2000). Induction times in ES were consistent with previous works that showed shade grown pioneer seedlings can have slower induction (TINOCO-OJANGUREN & PEARCY, 1992), as *C. pachystachya*, or no difference in induction time (*S. terebinthifolius*) (POORTER & OBERBAUER, 1993) compared with sun grown plants.

The ecophysiological relevance of the changes in physiology discussed above, taken together with the results from SAC and PCA, allow for a more singularized interpretation of the photosynthetic responses (Figure 4, Table 4). The most ubiquitous response, considering differences from the full sun condition, was an increase in Chl *a* and *b* contents (SAC < 0), and a decrease in SLW (SAC > 0) under 10% light. Although SLW was significantly different between full sun and 10% light in the LS *H. courbaril*, the relative change was small. This is consistent with the apparent low photosynthetic acclimation to high light of this species, having no significant differences between light conditions in many variables, with significantly lower ETR_i, A_{max}, A_i and IS, as well as higher induction times when compared to other species under full sun. Although the ES *S. terebinthifolius* also showed similar physiological responses across light environments, this lack of change does not indicate low acclimation capacity to high light. Rather, high relative values of A_{max}/SLW, IS and low induction times under 10% and 50% light suggest a high photosynthetic acclimation capacity, unexpected given its successional status. High IS and low induction times are associated with the ability to exploit sunflecks, usually attributed to LS species (CHAZDON, 1988). Another type of response was displayed by both the ES *C. pachystachya* and the LS *T. impetiginosa*, basically consisting in a low photosynthetic acclimation to low light. This is supported by lower values of A_{max}, A_i and IS as well as higher induction times under 10% light relative to other species.

The fact that the LS *T. impetiginosa* showed such a resource-limited response, suggesting a high light optimal growth condition is also unexpected.

FREDEEN & FIELD (1996) and STRAUSS-DEBENEDETTI & BAZZAZ (1996) state that ES typically have greater ability to acclimate photosynthesis to low light than do LS to high light, which would be due to a higher variability in early as compared to late successional habitats (BAZZAZ, 1979; BAZZAZ & PICKETT, 1980). The results reported in the present study do not support this prediction (hypothesis *i*) given that, although the ES *S. terebinthifolius* truly showed acclimation of the photosynthetic induction response to low light, the LS *T. impetiginosa* showed a large photosynthetic acclimation to high light. Likewise, a number of studies have reported increases in assimilation rates among LS when grown under high irradiances (FETCHER et al., 1987; PEARCY, 1987; WALTERS & FIELD, 1987). This apparently unexpected acclimation capacity may be, in fact, required since some LS reach the canopy only after repeated gap openings occur, suggesting that these species must acclimate to long-term environmental heterogeneity (PEARCY, 1987). In addition, hypothesis *ii)* was also not supported considering that the ES *S. terebinthifolius* showed higher light utilization efficiency under 10% light than the LS *T. impetiginosa*.

In a comparative study WRIGHT et al. (2003) verified few strictly shade-tolerant or extremely light demanding species, illustrating that most species have intermediate light requirements and lifestyles, revealed by an overlap of light preferences. In agreement with this intermediary light requirement, we verified that all species studied herein presented high photosynthetic rates in the 50% light environment, many times with no significant difference in relation to full sun conditions (Figure 1). Taken together, these results indicate that shade tolerance or light requirement *per se* are not appropriate criteria to distinguish species in ecological groups, supporting WRIGHT´s et al. (2003) findings.

Plants are highly plastic (SULTAN, 2000) and variation in the light environment can lead to plasticity in physiology (STRAUSS-DEBENEDETTI & BAZZAZ, 1996; VALLADARES et al., 2000). Some traits are highly plastic due to unavoidable constraints imposed by the biochemistry, physiology or developmental biology of the organism (SULTAN, 1995). Plasticity in the photosynthetic apparatus is fundamental given its essential role in the survival in heterogeneous and variable environments (BRADSHAW, 1965; PINTADO et al., 1997). Thus, it is expected that both light-

demanding and shade-tolerant species express plasticity, which has been supported by evidences indicating that adjustments in the photosynthetic apparatus are not necessarily related to successional status or light requirement (TURNBULL, 1991; POPMA et al., 1992). Accordingly, FETCHER et al. (1987) could not correlate successional status with photosynthetic acclimation potential in six rainforest tree species, and similar photosynthetic acclimation to light was observed in a gap specialist and a generalist *Piper* species (WALTERS & FIELD, 1987).

CONCLUSION

The results reported herein do not support the hypothesis *i)* that ES have higher acclimation capacity than LS, hypothesis, since the LS *T. impetiginosa* presented high acclimation to high light while the ES *C. pachystachya* showed low acclimation to low light. Hypothesis *ii)*, that LS would show higher light utilization efficiency under low light, was also not supported since the ES *S. terebinthifolius* showed high A_{max}, A_i and IS and the lowest induction times under low light. Despite the low number of species studied, we observed that major representatives of Brazilian tropical forests successional groups do not support hypotheses that have been prevalent for over 30 years.

Three distinct acclimation responses were detected: *i*) the ES *S. terebinthifolius* had a light-demanding profile but with high acclimation capacity to low light, *ii*) the LS *H. courbaril* had a shade-tolerant profile but with low acclimation capacity to high light, *iii*) the ES *C. pachystachya* and the LS *T. impetiginosa* had a light-demanding profile, but with low acclimation capacity to low light. This is compatible with the high heterogeneity in tropical forest environments, since even stereotypical habitats as gap and understory present variability in light conditions. Light availability is conditioned by factors as size and height of surrounding trees in gap environments, and canopy height and density, in the understory. In addition it is important to consider that the establishment of these species depends on other characteristics not considered in this study, such as resource allocation pattern, total leaf area, crown architecture and other anatomical, morphological, physiological and ecological traits. Thus, although the different acclimation capacities observed

indicates the potential to occupy different light environments, the actual occurrence in distinct habitats is further conditioned by trade-offs not reflected in the leaf level light acclimation.

Finally, it is possible to conclude that shade tolerance and light preference are not necessarily linked to successional status and should not be used as sole determinant criteria in the classification of tropical forest species into ecological groups.

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Capítulo II

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Water deficit affects photosynthetic induction in *Bauhinia forficata* Link (Fabaceae) and *Esenbeckia leiocarpa* Engl. (Rutaceae) growing in understorey and gap conditions

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Abstract - In tropical forests, light is considered the most limiting resource for plant growth and reproduction. Besides light, water deficit leads invariably to a decrease in photosynthesis. Thus, despite of the unquestionable role of light in CO_2 assimilation (*A*), it is expected that water deficit affects and limits the light utilization by plants. In this study, we investigated how water deficit influenced the photosynthetic induction of the early successional tree *Bauhinia forficata* (Fabaceae) and the late successional *Esenbeckia leiocarpa* (Rutaceae) in the understorey and in the forest gap. Field measurements were carried out in June and August 2006 in plants of approximately two-years-old. In August, the plants were subjected to a period of 45 d without rain characterizing a water deficit situation. Leaf water potential was significantly lower in August, both in forest gap and understorey (-2.5 and -3.8 MPa, respectively), than in June (-0.6 and -1.6 MPa, respectively). In June, both species presented a rapid increase in *A* after saturating light pulse under gap conditions. However, in the understorey the increase in *A* was slower in *B. forficata* than in *E. leiocarpa*. In August water deficit limited the increase in A_{max} in both species, indicating that potential utilization of increasing irradiance was reduced by water deficit conditions. The constrain in A_{max} was less pronounced in the understorey where plants at least reached the irradiance compensation point, whereas carbon gain by photosynthesis of the plants grown in the gap did not compensate the carbon loss by respiration.

Key words: drought, ecophysiology, gas exchange, light utilization, sunflecks, tropical forest succession

Resumo – (Déficit hídrico afeta a indução fotossintética em *Bauhinia forficata* Link (Fabaceae) e *Esenbeckia leiocarpa* Engl. (Rutaceae) crescendo em condições de sub-bosque e de clareira). Em florestas tropicais, a luz é considerada o recurso mais limitante para o crescimento e reprodução das plantas. Além da luz, o déficit hídrico leva invariavelmente à diminuição da fotossíntese. Assim, apesar do indiscutível papel da luz na assimilação de CO_2 (*A*), espera-se que o déficit hídrico afete e limite a utilização da luz pelas plantas. Neste estudo, investigou-se como o déficit hídrico influenciou a indução fotossintética de uma espécie pioneira arbórea, *Bauhinia forficata* (Fabaceae), e de uma secundária, *Esenbeckia leiocarpa* (Rutaceae), no sub-bosque e na clareira. As medidas foram feitas em junho e agosto de 2006 em plantas com aproximadamente dois anos. Em agosto, as plantas foram sujeitas a um período de 45 d sem chuva, caracterizando uma situação de déficit hídrico. O potencial hídrico foliar foi significativamente menor em agosto, na clareira e no sub-bosque (-2,5 e –3,8 MPa, respectivamente), do que em junho (-0,6 and –1,6 MPa, respectivamente). Em junho, ambas as espécies apresentaram um rápido aumento em *A* após o pulso de luz saturante na clareira. Contudo, no sub-bosque, o aumento em *A* foi mais lento em *B. forficata* do que em *E. leiocarpa*. Em agosto, o déficit hídrico limitou o aumento em A_{max} em ambas as espécies, indicando que a utilização potencial do aumento da irradiância foi reduzida por condições de déficit hídrico. A restrição em A_{max} foi menos pronunciada no sub-bosque, onde as plantas ao menos atingiram a assimilação de compensação, enquanto o ganho de carbono pela fotossíntese das plantas desenvolvidas na clareira não compensou a perda de carbono pela respiração.

Palavras-chave: ecofisiologia, seca, sucessão florestal tropical, “sunflecks”, trocas gasosas, utilização da luz

INTRODUCTION

Considering all environmental factors affecting plants, light is perhaps the most spatially and temporally heterogeneous. This heterogeneity takes on special importance in tropical forests because light is considered the most limiting resource for plant growth and reproduction. Accordingly, the life cycle and physiological responses of many trees and understorey species have been shown to be closely related to changes in light availability (BAZZAZ & PICKETT, 1980; DENSLOW, 1980; DENSLOW, 1987). Light acclimation is the process that allows environmentally induced changes in the photosynthetic utilization of light, depending upon the light regime under which leaves develop (BJÖRKMAN, 1981). Comparisons between low- and high-light specialists suggest that these two groups of plant species generally exhibit different capacities for light acclimation (BJÖRKMAN, 1981; STRAUSS-DEBENEDETTI & BAZZAZ, 1996). Early-successional species generally exhibit a high degree of plasticity in photosynthetic capacity compared to species of later forest successional stages (STRAUSS-DEBENEDETTI & BAZZAZ, 1996). However, both light-demanding and shade-tolerant species are capable of phenotypic plasticity, indicating that adjustments are not necessarily related to the species successional status (TURNBULL, 1991; POPMA et al., 1992). Phenotypic plasticity may be essential for survival in heterogeneous and variable environments, especially for sessile photosynthetic organisms (BRADSHAW, 1965; SULTAN, 1992; PINTADO et al., 1997).

Changes in the light environment experienced by forest plants during their lifetime may range from sunflecks, lasting from seconds to minutes, to more sustained changes occurring when gaps are formed or canopies develop (PEARCY & SIMS, 1994). Sunflecks are generally more limited as a light resource in shade than in sunny microsites in plant canopies, since they contribute with 60–90% of total daily photosynthetic photon flux density (PPFD) received by plants in the understorey of tropical rain forests, driving up to 65% of total daily carbon gain (PEARCY, 1983; PEARCY & CALKIN, 1983; CHAZDON, 1988; PFITSCH & PEARCY, 1989). In this variable and constraining understorey light environment, plants depend on sunflecks to maintain a positive carbon balance (CHAZDON, 1988). Sunfleck utilization

requires quick activation of the plant's photosynthetic system in order to exploit the brief light pulses. Photosynthetic induction response dependent on several regulatory mechanisms working at different time scales (PEARCY, 1999). The light-dependent stomatal opening process is relatively slow, whereas light-dependent activation of photosynthetic enzymes and build–up of the Calvin cycle metabolite pool can occur within a few minutes (EDWARDS & WALKER, 1983; PEARCY, 1999). The degree to which a high state of induction can be maintained during variable irradiance partially determines the species capacity to exploit sunflecks within plant canopies (PEARCY, 1990).

Besides light, water availability is one of the most important constraints for plant productivity, mostly affecting the growth of leaves and roots, stomatal conductance, photosynthesis and dry matter accumulation (BLUM, 1997). Water deficit leads invariably to a decrease in photosynthetic rate, although levels of tolerance can vary for different plant species (KAISER, 1987; CHAVES, 1991; LARCHER, 1995; CHAVES et al., 2002). Stomatal closure influences photosynthesis reduction as a consequence of reduced leaf water potential induced by drought (CHAVES, 1991; SANTOS et al., 2004, 2006). Also, a decrease in stomatal conductance is a common response to soil and leaf water limitations (TARDIEU & SIMONNEAU, 1998). Thus, despite of the basic and unquestionable role of light to CO_2 assimilation, it is expected that water deficit affects and limits the utilization of this resource by plants.

According to the spatial and temporal plant distribution in the forest mosaic, woody species may be broadly classified into two groups: (*i*) an early successional group with light-demanding species (pioneer and early secondary species); and (*ii*) a late successional group with shade-tolerant species (late secondary species) (PICKETT et al., 1987). Therefore, we hypothesize that late successional species growing in low-light environments, where plants have low carbon gain (CHAZDON et al., 1996; STRAUSS-DEBENEDETTI & BAZZAZ, 1996), must minimize carbon loss reducing both respiration and tissues construction cost (GIVNISH, 1988) and maximize light utilization by improving their photosynthetic induction. Furthermore, we expected that, even under water deficit, late successional species would maintain a higher state of photosynthetic induction than pioneer ones, since the latter species

often show higher transpiratory rates to support high levels of CO_2 assimilation (BAZZAZ & PICKETT, 1980; SOUZA et al., 2004).

Thus, in order to test these hypotheses, the aim of this study was to investigate water deficit influence on photosynthetic induction in two tree species of different successional groups, growing in the understorey and in the forest gap.

MATERIAL AND METHODS

Plants characterization and study site: In this study we analyzed plants of approximately two-years-old of different ecological groups, *Bauhinia forficata* Link (Fabaceae) and *Esenbeckia leiocarpa* Engl. (Rutaceae). The former species is an early successional and the latter is a late successional species (LORENZI, 1992). Three saplings of each species were planted directly in the soil in the understorey and forest gap environment, without addition of fertilizers or extra water supply, developing under naturally changing environment.

The gap studied herein presents an area of 34.5 m^2, which corresponds to a small gap with canopy openness around 10% following the classification proposed by MARTINS & RODRIGUES (2002). The study site is situated in a fragment of semi-deciduous seasonal forest with 5.5 ha located in Narandiba (22º24'24''S; 51º31'29''W, 354 a.s.l.), São Paulo State, Brazil. The climate is Aw type, defined as tropical with wet summer, according to the Köppen classification. The region has a mean annual temperature of 23ºC, mean rainfall of 1223 mm and a mean annual potential evaporative demand of 1170 mm (EMBRAPA, 2003). The mean incident daily PPFD, from 0800 to 1600h, was measured each hour intervals using a quantum sensor attached to the leaf chamber of the infra-red gas analyzer device (CIRAS-2, PPSystems, Hertfordshire, UK) (Figure 1). In the forest gap maximum PPFD was around 1600 μmol m^{-2} s^{-1} and, in the understorey, it did not exceed 25 μmol m^{-2} s^{-1}. Air vapor pressure deficit (VPD_{air}) under gap conditions reaches 2.8 and 4.0 kPa in June and August, respectively, whereas in the understorey maximum VPD_{air} was 1.5 kPa in June and 2.8 kPa in August. These values were observed around midday, when air temperatures were high (Figure 1).

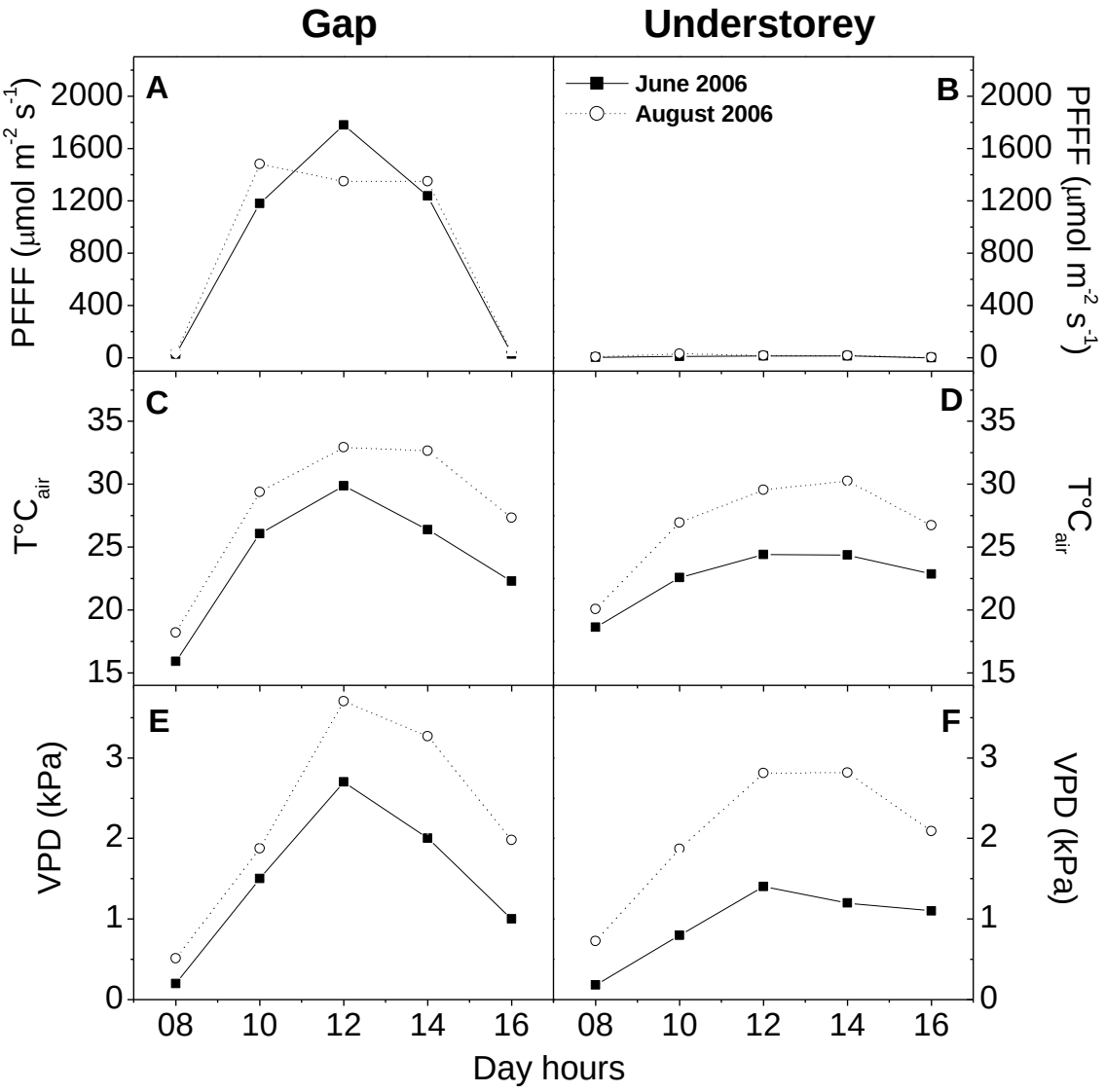

Figure 1. Daily courses of photosynthetic photon flux density (PPFD), air temperature (T_{air}) and vapor pressure deficit (VPD) in the gap (A, C, E) and in the understorey (B, D, F) in June (squares) and August (circles) 2006.

Table 1. Soil analysis of nutrients and organic matter (O.M.) in both gap and understorey in August 2006.

	pH	Al	Ca	Mg	K	P	S	Mn	Fe	O.M.
		($mmol_c\ dm^{-3}$)				($mg\ dm^{-3}$)				($g\ dm^{-3}$)
Gap	3.8	10	1	1	0.3	8	9.6	4.6	70.1	17
Understorey	4.0	8	4	2	1.1	8	8.7	9.6	87.5	19

The soil nutrient analysis of the gap and understorey (Table 1) indicates a typical impoverished soil of tropical forest regions (RIDDOCH et al., 1991). Remarkable differences between gap and understorey environments were not detected. The experiment was carried out without any kind of nutrient supplies as the objective of the study was to simulate conditions closer as possible of natural environment.

The field measurements were carried out in June and August 2006. Environmental conditions, especially water availability, were different in these evaluation dates. Rainfall data obtained from a meteorological station at the study site are presented in Figure 2. Rainfall values were 21 and 18 mm in June and August, respectively. Despite the small difference between these values, in August the measurements were carried out after a period of 45 d without rain.

Plant growth was evaluated taking into account primary and secondary growth measured as plant height and stem diameter at 5 cm height, respectively. In order to determine specific leaf weight (SLW), leaf area was measured with an area meter (Li-3000A, Licor, Nebraska, USA) and leaf dry mass was obtained after drying at 60 ºC until constant weight. Leaf nutrient contents (N, P and other elements) were evaluated according to MALAVOLTA et al. (1997). Leaf N content was determined by Kjehldal method (*i.e.*, digestion in concentrated sulfuric acid, followed by distillation

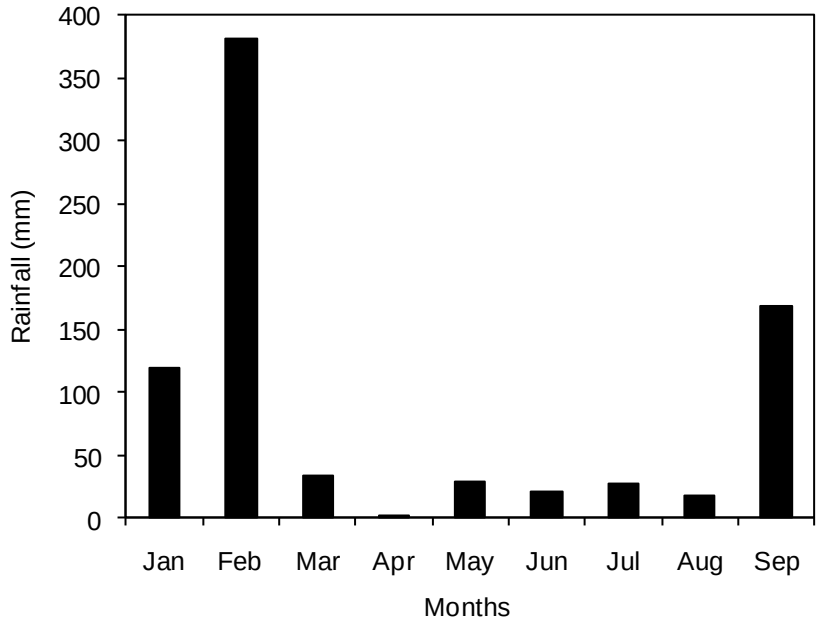

Figure 2. Rainfall of January to September 2006 at the study site, a forest fragment located in Narandiba, southeastern Brazil.

and titration), and the other elements by atomic absorption spectrophotometry. All measurements were taken in five healthy and fully developed leaves of each species in both light environments in August 2006. The leaves used to these measurements were not necessarily the same leaves used in leaf water potential or photosynthetic induction measurements.

Leaves used for measuring photosynthetic induction responses were darkened for 10 min inside the sample chamber of above–mentioned infrared gas analyzer covered with a black cloth, reducing incident irradiance on the sampled leaf to zero (enabling an initial reading of dark respiration). After this period, leaves were exposed to saturating PPFD (1000 μmol m^{-2} s^{-1}). The light intensity was determined previously by SOUZA et al. (unpublished data) through light response curves.

Gas exchange measurements: In both environments leaf gas exchange measurements were carried out from 0900 to 1600 h in healthy and fully developed leaves, from the upper exposed parts of the shoots. Three plants per species (one

leaf per plant) were evaluated in each environmental condition. All measurements were recorded in days with no or few clouds. Measurements of net CO_2 assimilation (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and dark respiration (R_d) were recorded using the CIRAS-2, at 10 s intervals.

Photosynthetic induction state (IS) was calculated as described in CHAZDON & PEARCY (1986a) as follows,

$$IS\ (\%) = 100\ (A - A_{low})\ (A_{max} - A_{low})^{-1}$$

in which A is the transient CO_2 assimilation rate at the time of calculation, A_{low} is the steady-state assimilation rate in low light and A_{max} is the steady-state light saturated assimilation rate. Photosynthetic induction curves were fitted using a sigmoidal model following ZIPPERLEN & PRESS (1997).

Leaf water potential measurements: Leaf water potential (Ψ_w) was measured using a Scholander pressure chamber (model PMS-1000, PMS Instruments, Oregon, USA). The measurements were performed at pre-dawn, before the first sunbeams reached the forest gap.

Data analysis: Differences in mean values of Ψ_w, maximum assimilation, R_d and IS between the two species growing in two contrasting forest light environments were analyzed by two-way analysis of variance (ANOVA) and the mean values compared by *a posteriori* Tukey's test, at 0.05 significance level. Data transformation was unnecessary since they were normally distributed and homoscedastic.

RESULTS

Leaf N and P concentrations did not show any significant difference ($P < 0.05$) between the two species, even when comparing gap and understorey environments. However, SLW was higher ($P < 0.05$) under gap than understorey conditions for both species. The non-pioneer species *E. leiocarpa* showed higher SLW in both environments than that of the pioneer one *B. forficata* (Table 2).

Table 2. Specific leaf weight (SLW), phosphorus (P) and nitrogen (N) concentrations in leaves of *B. forficata* and *E. leiocarpa* in both understorey and forest gap. Capital letters indicates significant differences between species in the same environment, and small letters refer to statistical differences between environments in the same species ($P < 0.05$, Tukey's test). Data are the mean ($n = 5$) ± SE.

		Parameters		
		SLW (Kg m^{-2})	P (g Kg^{-1})	N (g Kg^{-1})
Bauhinia forficata	Gap	0.136 ± 0.007 ^{Bb}	1.23 ± 0.18 ^{ns}	31.87 ± 1.24 ^{ns}
	Understorey	0.040 ± 0.006 ^{Ba}	1.70 ± 0.25 ^{ns}	32.70 ± 1.45 ^{ns}
Esenbeckia leiocarpa	Gap	0.328 ± 0.064 ^{Aa}	2.20 ± 0.36 ^{ns}	31.37 ± 1.45 ^{ns}
	Understorey	0.131 ± 0.007 ^{Ab}	2.21 ± 0.10 ^{ns}	28.47 ± 1.12 ^{ns}

Bauhinia forficata showed higher height and diameter than *E. leiocarpa* in both environments, and the former species presented higher height and diameter in the gap than in the understorey (Table 3). The differences between environments were smaller in *E. leiocarpa* for both parameters. These results make evident the expected growth differences. Through the growth parameters analyzed is clear to verify that the pioneer species showed a higher growth when comparing with the non-pioneer species, mostly in the gap (Table 3).

Table 3. Growth parameters, height and diameter, in *Bauhinia forficata* and *Esenbeckia leiocarpa* grown in gap and understorey environments. Capital letters indicates significant differences between species in the same environment, and small letters refer to statistical differences between environments in the same species ($P < 0.05$, Tukey's test). Data are the mean ($n = 5$) ± SE.

	Height (cm)		Diameter (mm)	
	Gap	Understorey	Gap	Understorey
Bauhinia forficata	121.0 ± 13.7 ^{Aa}	105.5 ± 0.4 ^{Aa}	13.8 ± 0.7 ^{Aa}	9.0 ± 0.2 ^{Ab}
Esenbeckia leiocarpa	64.3 ± 1.6 ^{Ba}	65.3 ± 3.0 ^{Ba}	7.8 ± 0.4 ^{Ba}	7.5 ± 0.4 ^{Aa}

In August, when plants were exposed to a long rainless period, Ψ_w was significantly lower ($P < 0.05$) than in June in both forest gap and understorey (Table 4). In the forest gap, Ψ_w was –0.6 and –2.5 MPa in June and August, respectively. In the understorey Ψ_w was considerably lower, –1.6 in June and –3.8 MPa in August. Therefore, the low Ψ_w obtained in August clearly characterize a high state of leaf water deficit.

Table 4. Leaf water potential (Ψ_w) of *Bauhinia forficata* and *Esenbeckia leiocarpa* in both understorey and forest gap in June and August 2006. Capital letters indicates significant differences between light environments, and small letters refer to statistical differences between months ($P < 0.05$, Tukey's test). Data are the mean ($n = 3$) ± SE.

Leaf water potential (-MPa)			
		Gap	Understorey
Bauhinia forficata	June	0.55 ± 0.08 ^{Bb}	1.25 ± 0.13 ^{Ab}
	August	2.47 ± 0.22 ^{Ba}	3.30 ± 0.06 ^{Aa}
Esenbeckia leiocarpa	June	0.70 ± 0.11 ^{Bb}	1.75 ± 0.16 ^{Ab}
	August	2.67 ± 0.12 ^{Ba}	4.17 ± 0.33 ^{Aa}

In June, *B. forficata* and *E. leiocarpa* presented a rapid increase in *A* after saturating light pulse in the forest gap (Figure 3). This result indicates that irradiance activates the photosynthetic apparatus, supporting high photosynthetic induction in these plants (Figure 5). In the understorey, the slower increase in *A* of *B. forficata* indicates that in shade conditions this species takes more time to fully activate the photosynthetic apparatus. The *A* reached in the steady-state for *B. forficata* in both environments was very similar. However, *E. leiocarpa* presented lower A_{max} in the understorey than in the forest gap. There were no significant differences ($P > 0.05$) between IS reached by *E. leiocarpa* and *B. forficata* in both environments. On the other hand, IS was higher in the forest gap than in the understorey for both species (Figure 5).

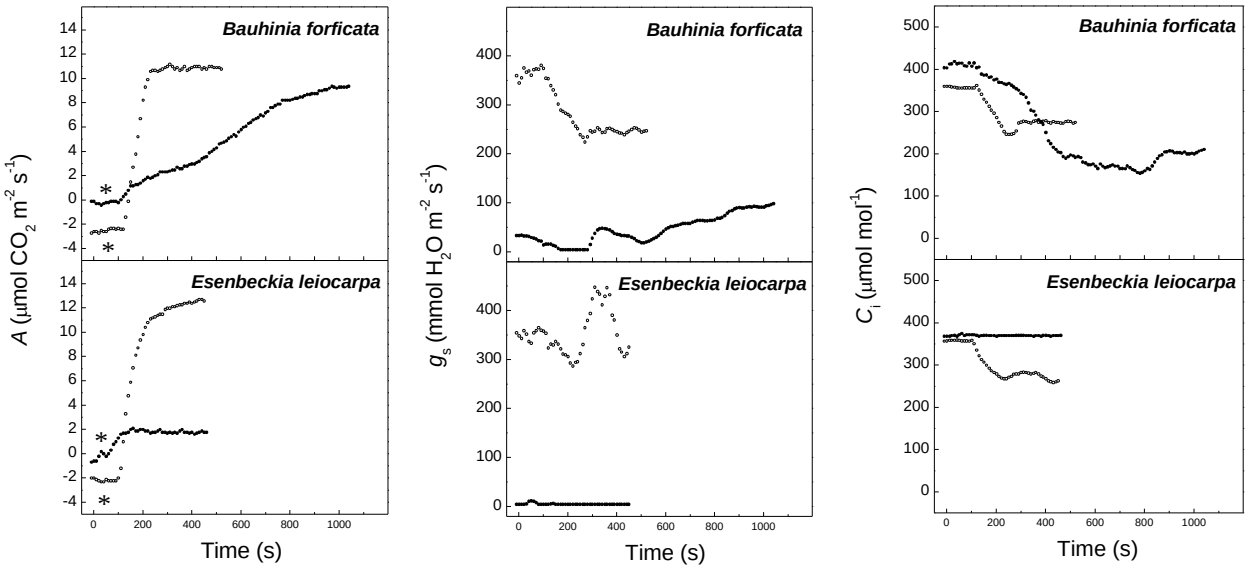

Figure 3. Time course of the net CO_2 assimilation rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in leaves of *Bauhinia forficata* and *Esenbeckia leiocarpa* in the understorey (closed symbols) and in the forest gap (open symbols) in June. Asterisks indicate the saturating light pulse after 10 min of darkness.

For both species, g_s was greater in the forest gap than in the understorey (Figure 3). In the understorey, as A increased slowly in *B. forficata* an increase in g_s and a decrease in C_i occurred. This result indicated that CO_2 was consumed to a greater extent when g_s increased, thus supporting the increase in A. In both environments, C_i was high before the saturating light pulse, decreasing afterwards (Figure 3). In the forest gap, as A increased, g_s and C_i decreased. In the understorey, *E. leiocarpa* presented low g_s and the increase in A, although small, was not accompanied by a decrease in C_i, as observed in the forest gap and also in *B. forficata*. In plants with no limiting Ψ_w (June), the C_i reduction after saturating light pulse indicates that the carbon had been used in photosynthesis.

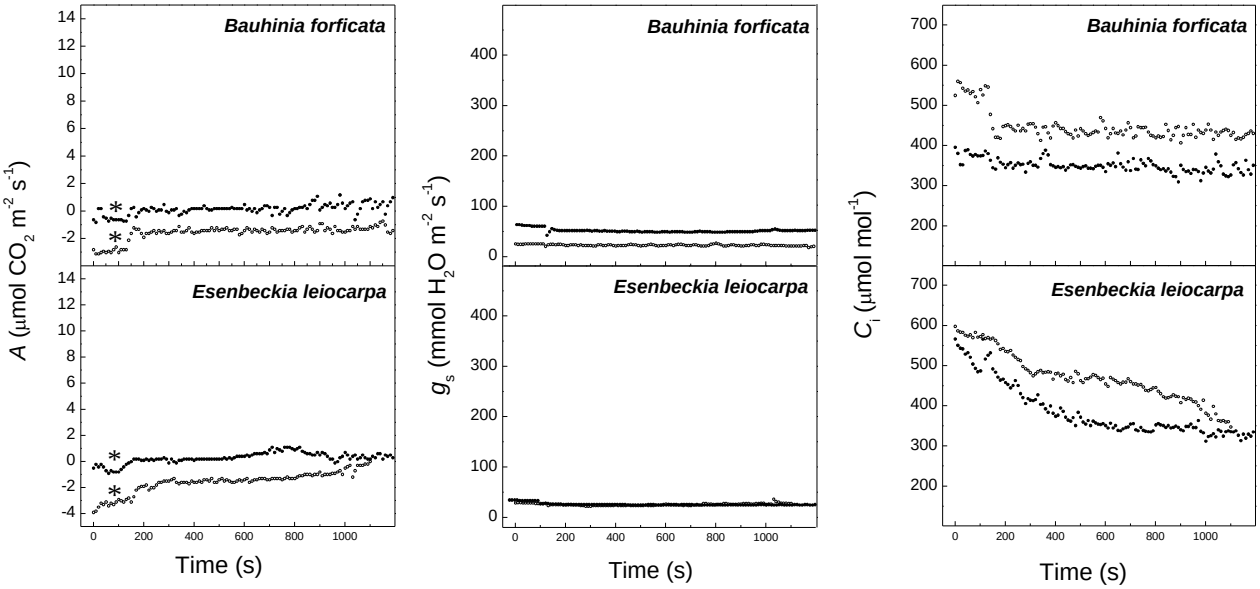

Figure 4. Time course of the net CO_2 assimilation rate (A) stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in leaves of *Bauhinia forficata* and *Esenbeckia leiocarpa* in the understorey (closed symbols) and in the forest gap (open symbols) in August. Asterisks indicate the saturating light pulse after 10 min the darkness.

The fast response in A of *E. leiocarpa* in the forest gap, after 10 min of darkness, indicates the potentiality of this species in readily exploiting irradiance increases. This result indicates that this species is capable of quickly activating its photosynthetic apparatus in order to maximize the utilization of irradiance increases, since it is a late successional species, typical of understorey.

The highest A_{max} were observed in June (Figure 5). In both months, we did not observe significant differences ($P > 0.05$) between species in the same environment. In June, *E. leiocarpa* presented higher A_{max} in the forest gap than in the understorey, and *B. forficata* did not present significant differences between environments. Despite low A_{max} under water deficit (August), *B. forficata* presented higher IS in the forest gap. Nevertheless, *E. leiocarpa* did not present significant differences between environments. High values of IS presented by plants under water deficit (Figure 5) are related to the fact that A_{max} has been quickly reached (Figure 4). This is due to the calculation of IS, which is based on the relationship between A_{max}, the steady-

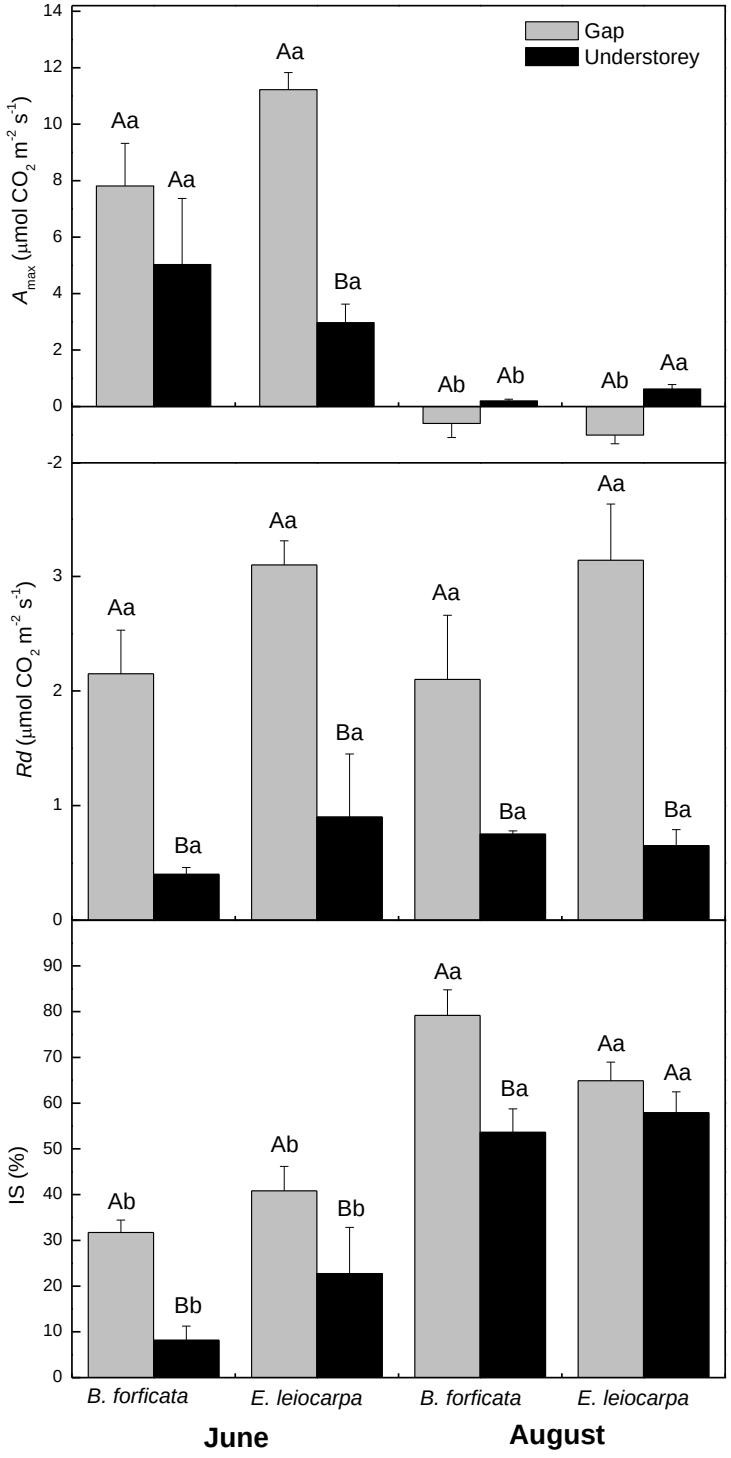

Figure 5. Maximum net CO_2 assimilation rate (A_{max}), dark respiration (R_d) and photosynthetic induction (IS) in leaves of *Bauhinia forficata* and *Esenbeckia leiocarpa* submitted to 10 min of darkness in both forest gap and understorey, in June and August 2006. Capital letters represent differences between environments whereas small letters represents mean statistical differences between months ($P < 0.05$, Tukey's test). Data are the mean ($n = 3$) ± SE.

state light saturated assimilation rate, and A_{low}, the net assimilation rate 60 s after saturating light pulse. However, it is important to notice that in both environments *A* was very low and virtually near to the compensation point. In June, the highest IS was observed in the forest gap for both species (Figure 5).

It is possible to observe that in August the water deficit clearly limited the increase in *A* after the saturating light pulse, however, there was a slight increase in *A* in both species and environments (Figure 4). After the saturating light pulse *E. leiocarpa*, in the gap, presented a decrease in R_d from –4 to close to 0 μmol CO_2 m^{-2} s^{-1}, and *B. forficata* from –2.5 to –0.5 μmol CO_2 m^{-2} s^{-1}, showing that, although positive values of *A* have not been reached, a positive response of the photosynthetic activity took place. Under water deficit, both species presented very low g_s in the understorey and gap environments. Moreover, after the saturating light pulse there was a decrease in C_i in both species and environments, even without a significant increase in *A*.

Dark respiration was higher in the forest gap in both months for both species (Figure 5). Significant differences ($P < 0.05$) between species in the same environment were not observed.

DISCUSSION

Values of leaf Ψ_w as measured in August in both environments has been considered very low (SOUZA et al., 2004), which indicates an intense water deficit. Despite the higher evaporative demand in the gap, Ψ_w was even lower in the understorey and is probably related to a higher plant covering density (Table 4). Moreover, the leaf transpiration surface and the surface exposed to direct radiation are both much higher in the canopy than in the gap. Although forest gap presents higher temperatures due to higher incident irradiance and more exposed soil, the transpiratory surface in contact with atmosphere is much lower, thus, total evapotranspiration is lower than in the surrounding forest composed by trees with dense canopies with a very high gas-exchange surface (LARCHER, 1995). Another factor that decreases water availability to younger plants in the understorey, which

have a shallower root system, is the occurrence of tall adult tree species with deep and well–established root system. Thereby, the effects on plant performance under water deficit could be more critical in the shade than in the open. Similar results were reported by VALLADARES & PEARCY (2002) who attributed the greater soil moisture depletion (dry season) in the understorey due to greater competition for water. Similarly, ABRAMS & MOSTOLLER (1995) found that shaded understorey leaves of all species studied were more susceptible to drought than sun leaves.

In the understorey, *E. leiocarpa* presented a greater potential for sunfleck utilization than *B. forficata*, since it responded faster to increases in irradiance. In fact, some studies of lightfleck utilization have suggested that shade leaves may be capable of using sunflecks more efficiently than sun leaves (CHAZDON & PEARCY, 1986b; KÜPPERS & SCHNEIDER, 1993; TANG et al., 1994). This capacity could be related to a significant efficiency in increasing photosynthetic capacity exhibited by shade species in response to increasing light availability (CHOW et al., 1988; TURNBULL, 1991; THOMPSON et al., 1992). VALLADARES et al. (1997) demonstrated that understorey species showed the rapid induction, since IS was significantly higher, and higher lightfleck-use efficiency for short lightflecks compared to species found in clearings or small gaps.

Simulation studies indicated that under natural sunfleck regimes induction might reduce daily carbon gain of *Alocasia macrorrhiza* in the understorey by up to 25% over the expected if there was no induction requirement (PEARCY et al., 1994). KIRSHBAUM & PEARCY (1988) verified that in environments with fluctuating PPFD, the fast-inducing component is an important factor in determining the leaf potential for photosynthetic carbon gain in *A. macrorrhiza*. Induction appeared to be less limiting for sunfleck use in the understorey shrub *Piper aequale* than in the pioneer species *P. auritum* when both were grown in the shade (TINOCO-OJANGUREN & PEARCY, 1992).

CHEN & KLINKA (1997) obtained similar results to the present study in *Pseudotsuga menziesii*, considered a late successional species, which showed a higher increase in photosynthetic rates in open–grown than understorey grown branches, after an increase in PPFD from 50 to 500 μmol m^{-2} s^{-1}. However, those authors did not verify any difference in R_d between understorey and open–grown

branches. HAN et al. (1999) observed that in *Fagus crenata, Daphniphylum humile* and *Acer rufinerve* seedlings, both R_d and A_{max} were higher in the gap than in the understorey. RIJKERS et al. (2000) studied photosynthetic induction in saplings of three shade–tolerant tree species, comparing understorey and gap habitats. Their results showed that A_{max} in *Dicorynia guianensis* was similar between forest environments whereas for the other two species, *Pourouma bicolor* spp *digitata* and *Vouacapoua americana*, it was two-fold higher in the gaps than in the understorey.

Several authors also showed that respiration is higher in forest gap than in understorey environments (*e.g.*, RAMOS & GRACE, 1990; FREDEEN & FIELD, 1991). In this study, water deficit did not promote an increase in R_d, however R_d may also be affected by plant developmental stage, temperature, nitrogen content (AMTHOR 2000; LEE et al., 2005), and seasonal environmental changes (LEE et al., 2005; MIRANDA et al., 2005). Some studies describe that pioneer species usually show higher leaf respiration than late successional ones (BAZZAZ & PICKETT, 1980; CHAZDON et al., 1996), but in the present report, significant differences in R_d between both species were generally not observed (Figure 5).

Combined shade and drought imposes special constraints, because mechanisms for capture of above–ground resources such as increased investment in leaf area restrict investment for the capture of below–ground resources (SACK et al., 2003). Probably the only way to avoid this conflict is to develop a reduced resource demand, which is characteristic of the stress–tolerator syndrome (GRUBB, 1998). A decrease in R_d might be a mechanism that reduces this demand for resources.

The potential utilization of increases in irradiance was reduced under water–deficit conditions, since there was no substantial increase in A_{max}. After saturating light A increased slightly but did not exceed the light compensation point in both species in the forest gap. Accordingly, R_d was higher for both species in this environment, which presented a lower Ψ_w. In the understorey, the saturating light allowed both species to reach the compensation point. Moreover, the reduction in R_d in the understorey could contribute to carbon economy. To maintain a positive carbon balance, assimilation rates must exceed respiration rates. Since R_d for both species was higher in the forest gap, plant susceptibility to water deficit effects in this environment could be higher.

There are evidences that the decrease in *A* found in drought–stressed leaves cannot be simply reversed by increasing the external CO_2 supply, showing that drought stress must also affect mesophyll metabolism (LAWLOR, 2002; CORNIC & FRESNEAU, 2002). Water deficit may cause damages to the biochemical CO_2 fixation, decreasing both activation and carboxylase activity of Rubisco, primarily due to the action of inhibitors (MEDRANO et al., 2002; PARRY et al., 2002). Moreover, reduction in the Rubisco efficiency may be caused by an increase in the mesophyll resistance due to stomatal closure, constraining CO_2 uptake into chloroplasts and increasing the oxygenase action of Rubisco with consequent increase in photorespiration. Considering that the regeneration of RuBP, activation of Rubisco and stomatal opening are limiting in the different phases of photosynthetic induction, plants under water deficit presents low photosynthetic induction possibly due to the involvement of these factors in the induction process.

STRAUSS-DEBENEDETTI & BAZZAZ (1996) state that differences in photosynthetic characteristics are generally viewed as being adaptative in nature, although they may instead simply reflect the constraints imposed by resource limitation. As plasticity addresses the expression of variable phenotypes under different environments (BRADSHAW, 1965), since both light-demanding and shade-tolerant species are capable of phenotypic plasticity, it is possible to conclude that adjustments are not necessarily related to the successional status of species (TURNBULL, 1991; POPMA et al., 1992).

Considering the initial hypothesis, our results showed that there were no significant differences in photosynthetic induction between species in the same environment. Under water deficit both species presented limitation in A_{max}, and steady-state CO_2 assimilation (Figure 4) just remains around the compensation point in the understorey (Figure 5). Moreover, the hypothesis that late successional species under water deficit could maintain a state of photosynthetic induction higher than pioneers, which often shows higher transpiration rates, was not supported since there was no significant difference between species.

Besides the restricted number of species representing different functional groups, which does not allow an ecophysiological generalization about ecological groups, we conclude that the physiological responses between species did not differ

under normal or water-deficit conditions. Thus, growth environments rather than successional status promoted differences in photosynthetic light utilization. It is important to consider that the gap where the experiments were carried out is a small one, with canopy openness near 10%. According to CHAZDON et al. (1996), the differences between pioneer and secondary species trend to be lower under small-gap conditions. Thus, it is likely that late successional species would have attained a suitable acclimation state in the small gap considered herein.

Although light has unquestionable importance to tropical forests development, this study showed that water deficit affected significantly the photosynthetic light utilization and consequently CO_2 assimilation. Water deficit was a strong constraining resource on A_{max}, mainly in the forest gap. Even though the IS trended to be higher in August, A_{max} was low and very close to the compensation point. The constrain in A_{max} was less pronounced in the understorey where plants at least reached the compensation point, while carbon gain by photosynthesis of the plants grown in the gap did not compensate the carbon loss by respiration (Figure 4). The fact that the plants had been near to the compensation point in the understorey indicated that, despite of low *A*, they reduced the reserve consumption by respiration. Ecologically, this carbon economy could improve plant survival under adverse environmental conditions, such as low-water availability.

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Capítulo III

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Time-course of photosynthetic induction in four tropical woody species grown in contrasting irradiance habitats

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Abstract - We investigated the photosynthetic induction time-course in species of different ecological groups grown in contrasting forest irradiance environments, gap and understorey, exposed to different darkness times in order to verify the plant capacity to exploit irradiance heterogeneity. Photosynthetic induction was studied in leaves of *Bauhinia forficata* and *Guazuma ulmifolia* (early succession, ES-species), and *Esenbeckia leiocarpa* and *Hymenaea courbaril* (late succession, LS-species). T_{50} and T_{90} (time estimates to attain 50% and 90% of maximum net photosynthetic rate, respectively) varied according to the time of previous exposure to darkness and growth irradiance. In both darkness times of 10 and 30 min, T_{50} was lower in the LS- than ES-species. These results, jointly with significant higher induction state of the leaves (IS) after 10 min of darkness, suggest that the LS-species has a higher potential to sunfleck utilization compared to ES-species, both grown in the understorey. After 10 and 30 min of darkness the differences between ecological groups were not clearly detected in the gap for T_{50} and T_{90}, indicating that eco-physiological characteristics of each ecological group did not influence the induction time of the species evaluated herein. Thus the capacity to show phenotypic plasticity is not exclusive to an ecological group, but it is rather a more intrinsic feature related to the differential capacity of individuals.

Key words: Brazilian semi-deciduous seasonal forest, plant eco-physiology; sunfleck utilization; tropical forest succession

Resumo – (Cursos de indução fotossintética em quatro espécies tropicais arbóreas crescidas em habitats com irradiâncias contrastantes). Nós investigamos o curso da indução fotossintética em espécies de diferentes grupos ecológicos crescidas em ambientes florestais com irradiâncias distintas, clareira e sub-bosque, e expostas a diferentes tempos de permanência no escuro, com o objetivo de verificar a capacidade das plantas em explorar a heterogeneidade luminosa do ambiente. A indução fotossintética foi estudada em folhas de *Bauhinia forficata* e *Guazuma ulmifolia* (espécies iniciais da sucessão florestal, espécie-ES), além de *Esenbeckia leiocarpa* e *Hymenaea courbaril* (espécies mais tardias da sucessão florestal, espécies-LS). T_{50} e T_{90} (estimativas de tempo para atingir 50 e 90% da taxa máxima de assimilação líquida, respectivamente) variaram com o tempo prévio de exposição ao escuro e com o ambiente luminoso no qual a espécie estava exposta. Em ambos os tempos de permanência no escuro, 10 e 30 min, o T_{50} foi menor nas espécies-LS do que nas espécies-ES. Esses resultados, conjuntamente com o estado de indução fotossintético significativamente maior após 10 min de escuro, sugerem que as espécies-LS apresentam maior potencial de utilização de sunflecks comparado a espécies-ES, quando ambas foram crescidas no sub-bosque. Após 10 e 30 min de escuro, as diferenças entre grupos ecológicos não foram claramente detectadas na clareira pelo T_{50} e T_{90}, indicando que as características fisiológicas de cada grupo ecológico não influenciaram o tempo de indução das espécies avaliadas no presente estudo. Assim, a capacidade de expressar plasticidade fenotípica não é exclusiva de um determinado grupo ecológico, estando mais relacionada a características intrínsecas das espécies e na capacidade diferencial dos indivíduos.

Palavras-chave: Florestas estacionais semi-deciduais brasileiras, ecofisiologia vegetal, utilização de *sunfleck*, sucessão florestal tropical

INTRODUCTION

In tropical forests, the formation and closure of canopy openings of different dimensions create a remarkably heterogeneous irradiance environment (CHAZDON & FETCHER, 1984; KIRA & YODA, 1989). As a consequence, leaves are exposed to highly fluctuating irradiance changing over time that ranges from seconds to minutes or even longer. When irradiance increases after a period of low values, the corresponding increase in photosynthetic CO_2 fixation is not instantaneous and shows a time delay before the maximum rate of assimilation is achieved (OSTERHOUT & HASS, 1919; RABINOWITCH, 1956; WALKER, 1981). This delay is associated with the process of photosynthetic induction (IS), which involves activation and synthesis of various biochemical components, and with stomata movements (PEARCY, 1990). Thus, IS response is dependent on several regulatory mechanisms, each working at a different time scale (PEARCY, 1989, 1990). In general, the IS response to irradiance increase can be separated into two phases: an initial fast-induction phase which requires 1−2 min for completion and involves irradiance activation of some Calvin cycle enzymes and build-up of metabolic pools, particularly ribulose-1,5-bisphosphate (RuBP) regeneration (KIRSCHBAUM & PEARCY, 1988a; SASSENRATH-COLE & PEARCY, 1992), and a slow-induction phase, lasting 5−30 min or more, in which RuBP carboxylase/oxygenase (RuBPCO) is activated and stomata open (KIRSCHBAUM & PEARCY, 1988b; PEARCY, 1990). RuBPCO activation is a two-step process, involving the initial activation of activase and the subsequent activation of RuBPCO (LAN et al., 1992). The ability of a plant to utilize variable irradiance regimes depends on the photosynthetic induction state of the leaf (CHAZDON & PEARCY, 1986a; PFITSCH & PEARCY, 1989) and its capacity for post-irradiation CO_2 fixation (PEARCY et al., 1985; CHAZDON, 1988), which determines the readiness of a leaf to respond to an irradiance increase.

Understanding the dynamics of photosynthetic responses to variable irradiance is of fundamental importance for explaining the ecological distribution of species and natural succession (KÜPPERS & SCHNEIDER, 1993). The degree to which a high state of photosynthetic induction can be maintained during variable irradiance partially determines the capacity of a species to exploit sunflecks within

plant canopies (PEARCY, 1990). These periods of high irradiance can last for a few seconds to several minutes, and can contribute from 10−80 % of daily photon flux density (PFD) received by a plant (CHAZDON, 1988). Photosynthesis during sunflecks may contribute to 30−60 % of the daily carbon gain, indicating that plants depend on sunflecks to maintain a positive carbon balance (CHAZDON, 1988). Previous works have shown that understorey plants typically exhibit photosynthetic adaptation and acclimation that allows maximization of carbon gain under such dynamic irradiance regimes (CHAZDON & PEARCY, 1986a,b; ÖGREN & SUNDIN, 1996; VALLADARES et al., 1997).

Plant growth is not determined only by photosynthesis, but the photosynthetic utilization of photon energy plays a major role in the rain forest understorey where irradiance is frequently the most limiting environmental factor (KÖRNER, 1991; KÜPPERS & SCHNEIDER, 1993; FETCHER et al., 1994). Differences in photosynthetic characteristics between shade tolerant and irradiance demanding species may allow for differential utilization of the patchy radiation resource in rain forest environments (PRESS et al., 1996).

Variability in the physical environment, especially air and soil temperature, soil moisture, and irradiance in early succession (ES) habitats is higher than in late succession (LS) habitats (BAZZAZ, 1979). These and other differences between habitats have selected species with specific adaptations to each environment. The degree of flexibility of different species to acclimate to environmental extremes (as temperature, water, and photon availability) must itself be related to the level of environmental variation that is characteristic of the habitat in which the species is normally found (BAZZAZ & CARLSON, 1982). Thus, ES-species might have higher physiological flexibility relative to that of species found in LS-habitats, since the former species typically inhabit environments with higher abiotic variability (BAZZAZ, 1979).

Based on this hypothesis we expect that ES-species would show higher CO_2 assimilation under full sunlight but not in shade. Moreover, in the understorey, it is expected that the LS-species present faster IS than the ES-ones. We analyzed the time-course of IS of four species from different ecological groups of the tropical forest succession grown in contrasting irradiance environments, with the objective of

investigating the IS response in order to verify the plant capacity to exploit irradiance heterogeneity.

MATERIALS AND METHODS

Study site and plants: The study was carried out on a fragment of semi-deciduous seasonal forest with 5.5 ha located in Narandiba, São Paulo, Brazil (22º24'24''S; 51º31'29''W, altitude of 354 m). Semi-deciduous seasonal forest is typical Brazilian vegetation conditioned by a duple climatic seasonality, wet and dry season, with 20−50 % of leaf loss in the dry period. The climate is Aw type, defined as tropical with wet summer and dry winter, according to the Köppen classification. The region has a mean annual temperature of 23 ºC, mean rainfall of 1 223 mm, and a mean annual potential evaporative demand of 1 170 mm (EMBRAPA, 2007). Irradiance in the forest gap, from 08:00 to 16:00 h, was approximately 1 600 μmol(photon) m^{-2} s^{-1} and in the understorey did not exceed 25 μmol (photon) m^{-2} s^{-1}. We studied two ES-species, *Bauhinia forficata* Link (Caesalpinoideae) and *Guazuma ulmifolia* Lam. (Sterculiaceae), and two LS-species, *Esenbeckia leiocarpa* Engl. (Rutaceae) and *Hymenaea courbaril* L. (Caesalpinoideae) (LORENZI, 1992), with approximately two years old. Ten saplings of each species were planted directly in the soil of the understorey and forest gap environments, without additional use of fertilizer or irrigation, and grown in these sites for about 1 year before the measurements. The gap studied herein presents an area of 34.5 m^2, which corresponds to a small gap with canopy openness around 10 % following the classification proposed by MARTINS & RODRIGUES (2002).

All measurements were taken in three healthy and fully developed leaves in three different individuals of each species in both forest environments. IS measurements were taken in leaves subjected to 10 or 30 min of darkness. These leaves were darkened inside the sample chamber of the open system portable infrared gas analyzer (*CIRAS-2*, *PPSystems*, UK) covered with a black cloth, reducing incident irradiance on the sampled leaf to zero (enabling an initial reading of dark respiration). After this period, leaves were exposed to a pulse of saturating PFD

[1200 µmol (photon) m^{-2} s^{-1}]. The light intensity was determined previously by SOUZA et al. (unpublished) through PFD-response curves, which indicated that this irradiance did not cause photoinhibition in these plants species.

Gas exchange: In both forest environments leaf gas exchange measurements were carried out from 09:00 to 16:00 h in healthy and fully developed leaves, from the sun exposed parts of the shoots. The measurements were recorded in three plants per species (one leaf per plant), in each environmental condition, in days with no or few clouds. Measurements of net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and dark respiration rate (R_D) were recorded using the *CIRAS-2*, at 10-s intervals, during 1 000 and 1 800 s for plants exposed to 10 and 30 min of darkness, respectively.

IS [%] was calculated as described in CHAZDON & PEARCY (1986b):

$$IS\ [\%] = 100\ (P_{sat} - P_{low})\ (P_{max} - P_{low})^{-1}$$

where P_{sat} is the measured net photosynthetic rate 1 min after saturating irradiation, P_{low} is net photosynthetic rate under low irradiance, and P_{max} is the steady-state irradiance saturated photosynthesis. Photosynthetic induction curves were fitted using a sigmoid model, following ZIPPERLEN & PRESS (1997).

Time estimates to attain 50 (T_{50}) and 90 (T_{90}) % of P_{max} during measurements of transient photosynthesis in saturating irradiance were obtained by fitting a sigmoid function to the induction response curves.

Data analysis: Differences in mean IS, P_{max}, and R_D among the four species growing in two contrasting forest irradiances were analyzed by a 4×2 factorial analysis of variance (two-way ANOVA). The mean values were compared by *a posteriori* Tukey test and considered significantly different at $P < 0.05$.

RESULTS

- *Photosynthetic induction after 10 min of darkness*: In the gap, the four species analyzed showed similar final P_N after 10 min of darkness (Figure 1). Accordingly, Figure 2*A* indicates that there are no significant differences ($P > 0.05$) in P_{max} among species in the gap. Moreover, differences in IS were not significant ($p>0.05$) (Figure 2), indicating that photosynthetic induction did not differ between the functional groups in the gap. ES-species *B. forficata* and *G. ulmifolia* attained significantly greater P_{max} ($P < 0.05$) than LS-species *E. leiocarpa* and *H. courbaril* in the understorey (Figures 1, 2), although IS was significantly higher ($P < 0.05$) in LS-species (Figure 2). Thus, following 10 min of darkness in the understorey, ES-species attained a more elevated P_{max}, however, LS-species showed faster photosynthetic induction. Differences in P_{max} between forest environments, gap and understorey, were significant ($P < 0.05$) only in LS-species (Figure 2).

The g_s was higher in the gap than in the understorey for all species, although it did not show a sigmoidal response as P_N in both forest environments (Figure 1). Species presented a steady pattern of g_s in the gap, except *G. ulmifolia*, while in the understorey there was a trend to increase throughout the IS time course in ES-species (Figure 1).

In general, C_i did not show marked differences between forest environments and had the tendency to decrease in the IS time course when exposed to continuous saturating irradiance after 10 min of darkness in all species in both forest environments (Figure 1). Initial C_i was similar in all species studied (about 340−380 μmol mol^{-1}).

For all species studied, R_D was significantly higher ($P < 0.05$) in the gap than in the understorey, even though in both forest environments there were no significant differences ($P > 0.05$) among species (Figure 2).

Both estimates of T_{50} and T_{90} were higher in the understorey than in the gap for all species (Table 1), since plants required *ca*. 3−4 min to attain 50 % of P_{max} in the gap, whereas in the understorey they required *ca*. 4−10 min. In the gap, in general, both ES- and LS-species presented similar T_{50}, whereas in the understorey

the LS-species presented lower T_{50}. With regard to T_{90} in the gap, *G. ulmifolia* presented higher T_{90} than the other three species. In the understorey, *H. courbaril* presented lower T_{90} than the other species, indicating a faster IS response (Table 1).

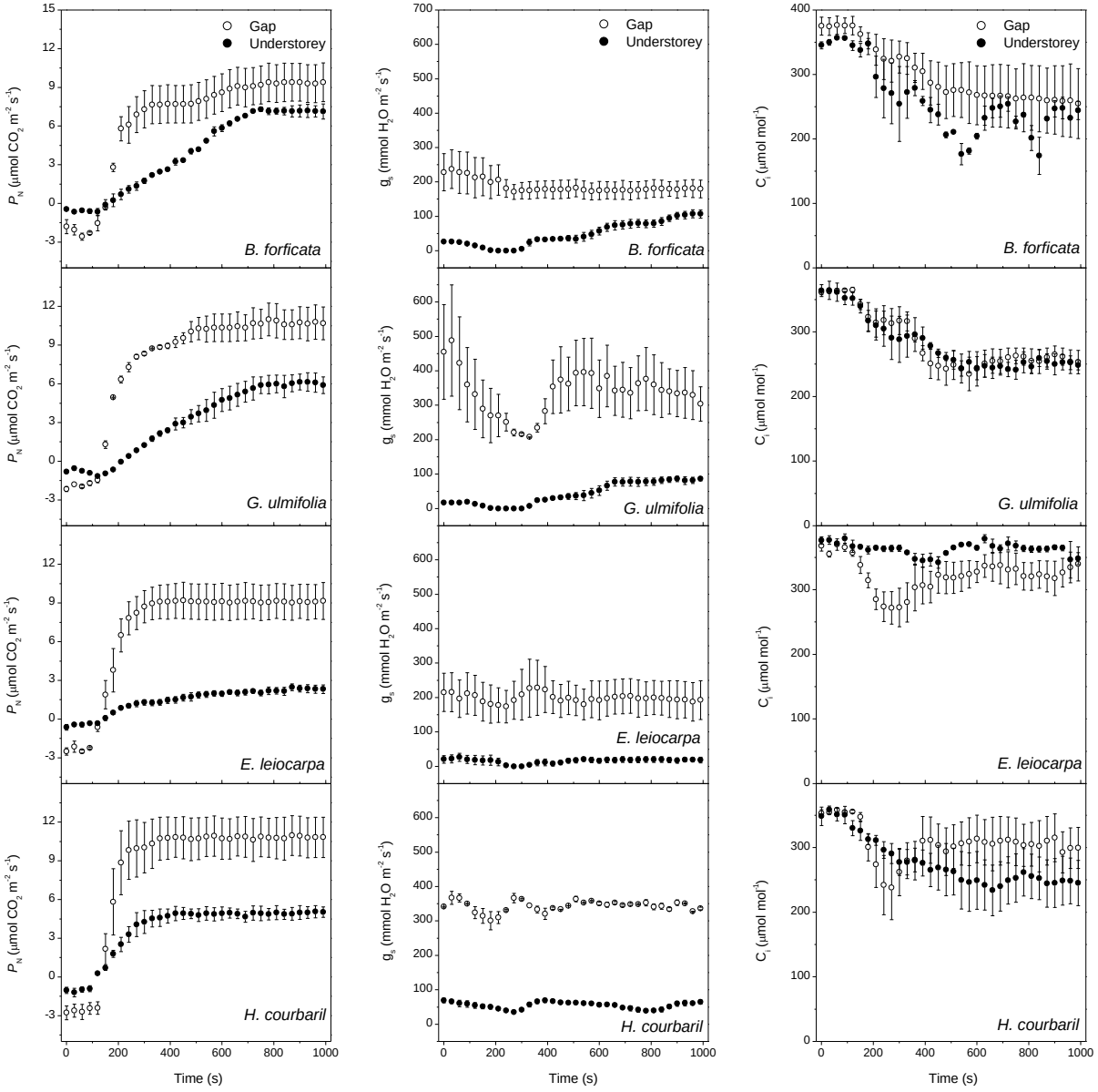

Figure 1. Time course of the net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in leaves of *Bauhinia forficata*, *Guazuma ulmifolia*, *Esenbeckia leiocarpa*, and *Hymenaea courbaril* grown in the forest gap (○) and in the understorey (●) after darkness time of 10 min (n = 3). Means of three replicates (n = 3); bars represent ± standard error.

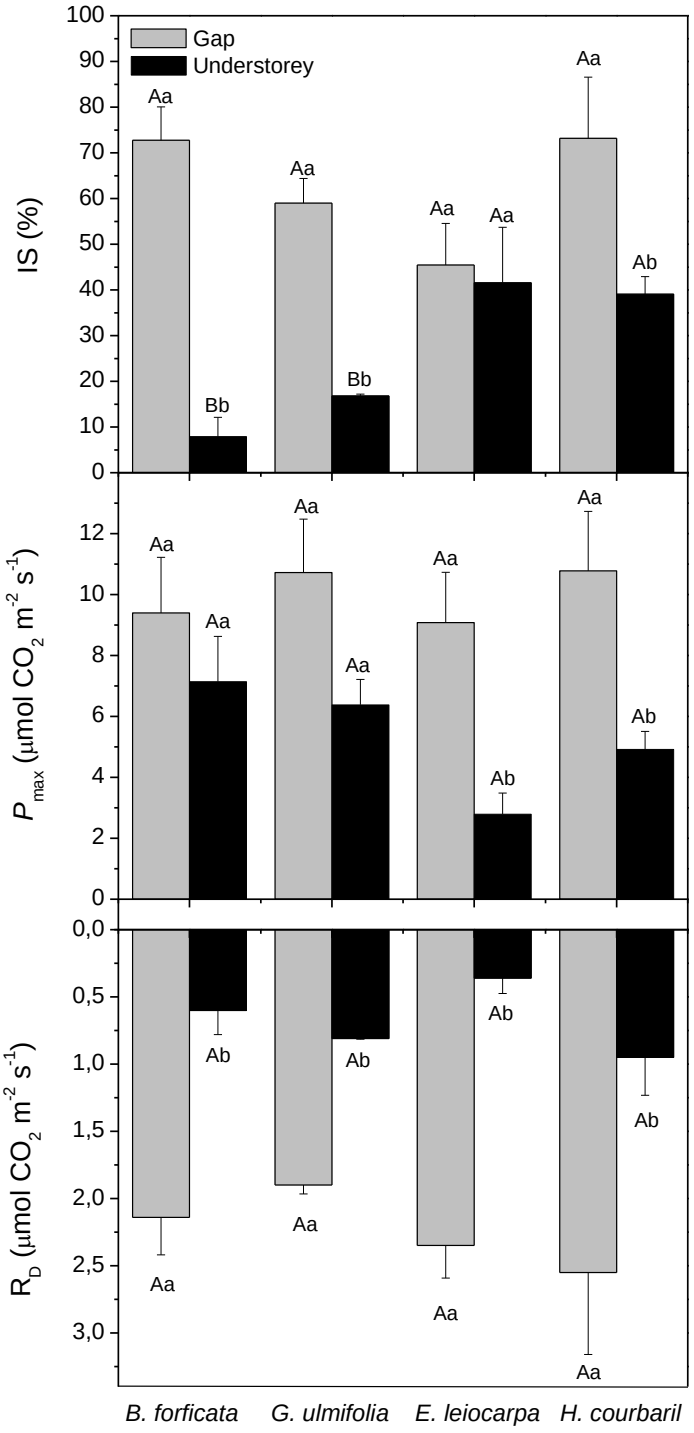

Figure 2. Photosynthetic induction state (IS), maximum net photosynthetic rate (P_{max}), and dark respiration rate (R_D) in leaves of *Bauhinia forficata*, *Guazuma ulmifolia*, *Esenbeckia leiocarpa*, and *Hymenaea courbaril* grown in the forest gap and in the understorey submitted to 10 min of darkness. Capital letters mean difference between species whereas small *letters* mean statistical difference between environments ($P < 0.05$). Means of three replicates ($n = 3$); bars represent ± standard error.

Table 1. Time estimate (s) to attain 50% (T_{50}) and 90% (T_{90}) of maximum net photosynthetic rate in leaves of *Guazuma ulmifolia*, *Bauhinia forficata*, *Esenbeckia leiocarpa*, and *Hymenaea courbaril* grown in the forest gap (Gap) and understorey (Us) following 10 and 30 min at darkness. Means of three replicates (*n*=3) ± standard error.

Species	Environment	Darkness time	T_{50}	T_{90}
B. forficata	Gap	10 min	196.13 ± 5.55	227.27 ± 8.69
		30 min	404.89 ± 38.12	601.94 ± 77.77
	Us	10 min	615.07 ± 84.79	904.44 ± 160.32
		30 min	531.95 ± 34.04	765.06 ± 47.27
G. ulmifolia	Gap	10 min	244.54 ± 39.96	359.78 ± 95.78
		30 min	541.44 ± 147.93	798.95 ± 224.00
	Us	10 min	544.20 ± 35.95	797.96 ± 88.23
		30 min	569.91 ± 47.25	740.88 ± 44.59
E. leiocarpa	Gap	10 min	202.8 ± 8.44	252.41 ± 12.04
		30 min	377.71 ± 9.35	537.21 ± 2.68
	Us	10 min	437.39 ± 151.43	755.03 ± 251.60
		30 min	464.88 ± 66.43	1028.37 ± 236.74
H. courbaril	Gap	10 min	199.67 ± 20.23	240.39 ± 34.73
		30 min	400.76 ± 63.02	633.94 ± 112.37
	Us	10 min	237.26 ± 23.38	317.81 ± 40.86
		30 min	474.15 ± 90.18	1040.53 ± 190.71

- *Photosynthetic induction after 30 min of darkness*: All the species studied attained greater P_N in the gap than in the understorey after 30 min of darkness (Figure 3), although differences in P_{max} between forest environments were not significant (P > 0.05) in all the species (Figure 4). Moreover, significant differences in P_{max} among species in both forest environments were not detected (P > 0.05) (Figure 4). Considering IS, there were no significant differences (P > 0.05) in both forest environments among species, but regarding forest environments all species presented significantly higher IS (P < 0.05) in the gap, except *E. leiocarpa* which did not show significant differences (P > 0.05) (Figure 4). All together these results indicate that after 30 min of darkness there were practically no significant differences in P_{max} and IS between ecological groups or forest environments when exposed to continuous saturating irradiance. Significant differences in R_D among species in both forest environments were not detected (P > 0.05), however between forest environments all species presented R_D significantly higher (P < 0.05) in the gap than in the understorey.

All species studied presented higher g_s in the gap than in the understorey (Figure 3). The ES-species in both forest environments exhibited a sigmoid IS response for g_s when exposed to continuous saturating irradiance after 30 min of darkness. *E. leiocarpa* practically did not show alterations in g_s in both forest environments and *H. courbaril* presented a slow increase in g_s in the IS time course in both forest environments (Figure 3).

After the saturating irradiance, all species studied exhibited a decrease in C_i in both forest environments (Figure 3). Initial C_i was similar in all species studied (*ca*. 370 μmol mol^{-1}), except in *G. ulmifolia* in the gap which presented elevated higher C_i (*ca*. 430 μmol mol^{-1}).

Plants required *ca*. 6−10 min to attain 50 % of P_{max} in the gap, whereas *ca*. 7−9 min in the understorey (Table 1). *B. forficata, E. leiocarpa*, and *H. courbaril* exhibited similar T_{50} in the gap, whereas in the understorey T_{50} was lower in LS-species than in the ES-ones (Table 1). However, pioneer species exhibited lower T_{90} when compared to LS-species.

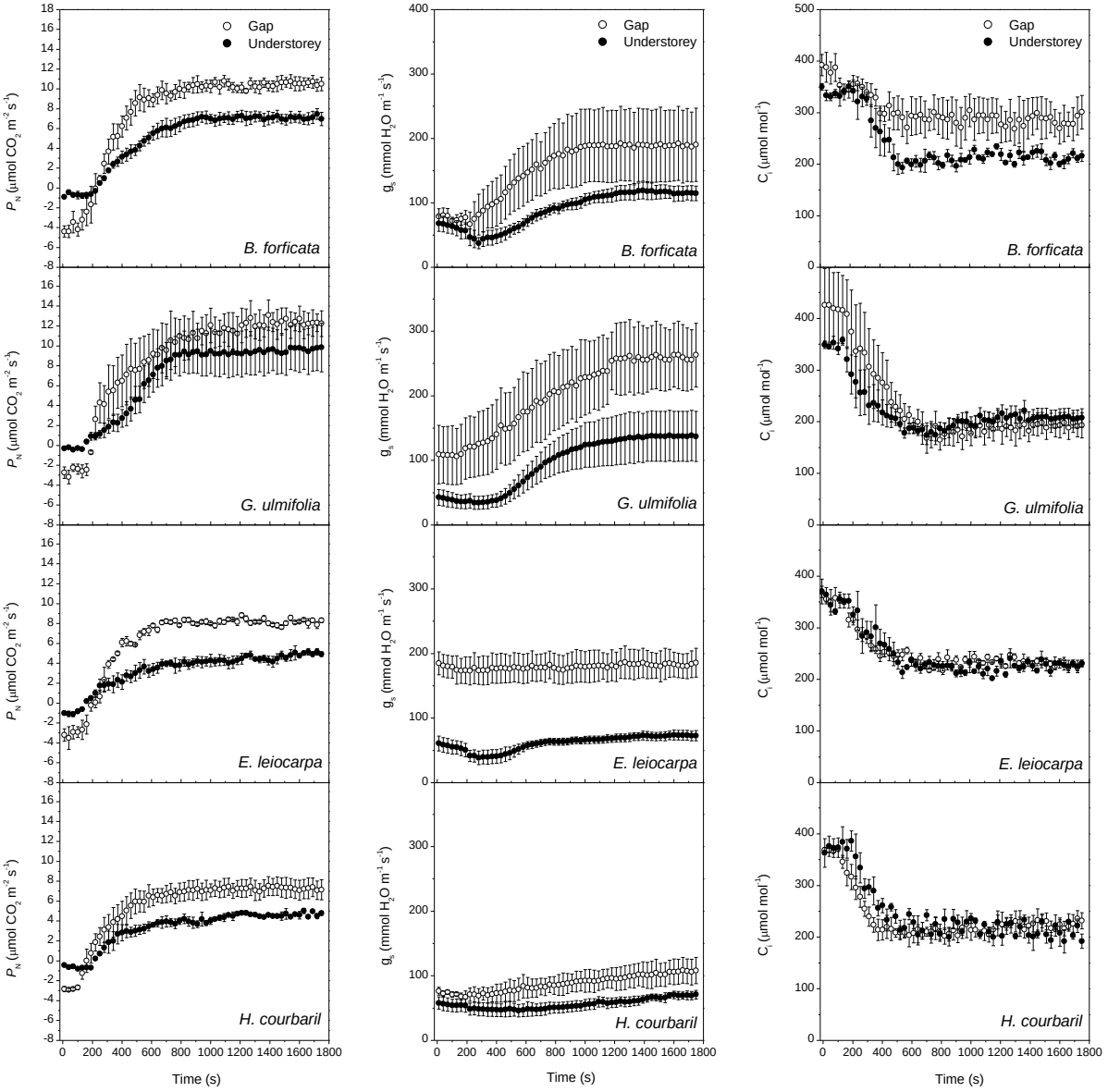

Figure 3. Time course of net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in leaves of *Bauhinia forficata*, *Guazuma ulmifolia*, *Esenbeckia leiocarpa*, and *Hymenaea courbaril* grown in the forest gap (○) and in the understorey (●) after darkness time of 30 min (n = 3). Means of three replicates (n = 3); bars represent ± standard error.

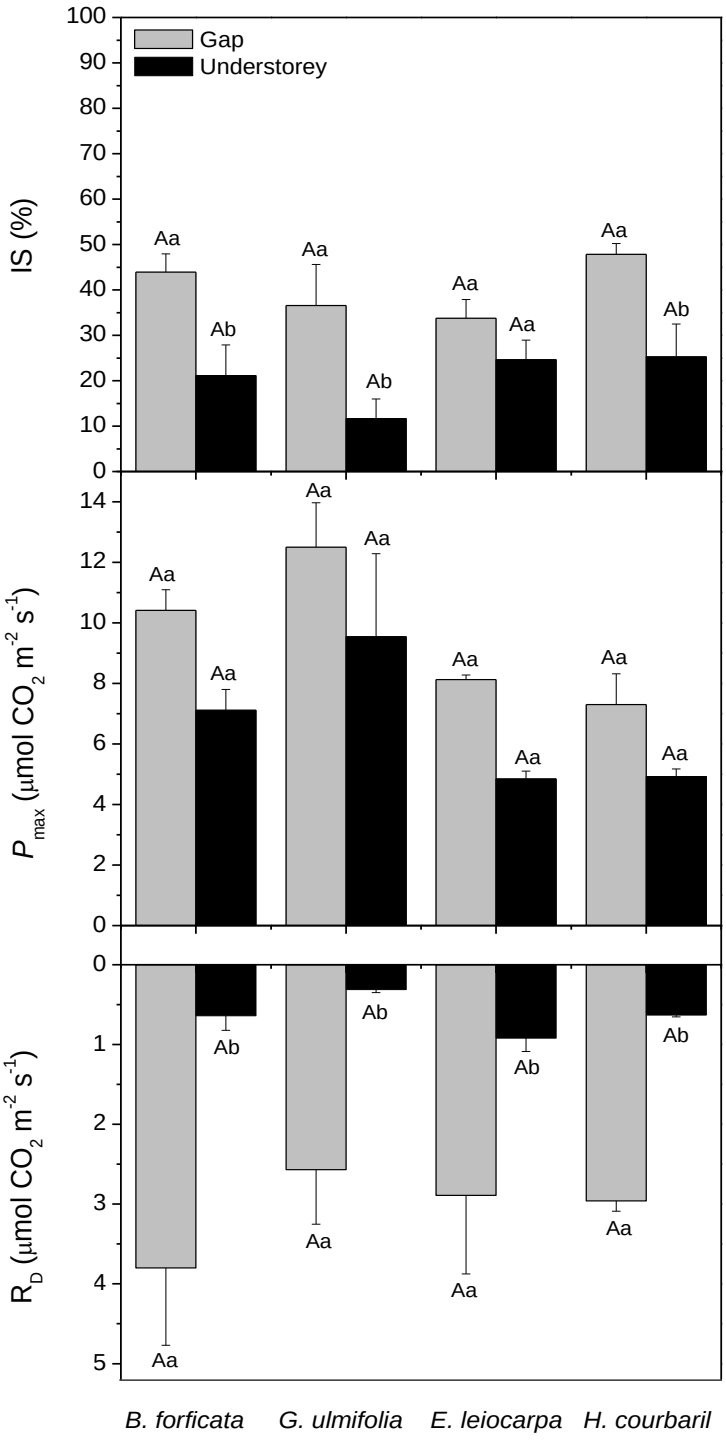

Figure 4. Photosynthetic induction state (IS), maximum net photosynthetic rate (P_{max}), and dark respiration rate (R_D) in leaves of *Bauhinia forficata*, *Guazuma ulmifolia*, *Esenbeckia leiocarpa*, and *Hymenaea courbaril* grown in the forest gap and in the understorey submitted to 30 min of darkness. *Capital letters* mean difference between species whereas *small letters* mean statistical difference between environments ($P < 0.05$). Means of three replicates ($n = 3$); bars represent ± standard error.

DISCUSSION

Previous investigations have revealed that stomata can exercise significant influence over photosynthetic induction and light-fleck use efficiency (KIRSCHBAUM & PEARCY, 1988b; TINOCO-OJANGUREN & PEARCY, 1992, 1993a). According to CHAZDON & PEARCY (1986b), the time-course of P_N and g_s appear nearly coincident, while C_i show an initial drop upon irradiation and then remains relatively stable during the IS period. In our experiments, C_i followed the description of CHAZDON & PEARCY (1986b), although a coordinated response between P_N and stomatal opening was not always observed in either darkness times or forest environments (Figures 1, 3). This coordinated response was only detected in ES-species after 30 min of darkness in both forest environments (Figure 3).

Previous studies reported that increases in P_N after 10−12 min of the irradiance increase have generally been credited solely to subsequent increases in g_s (TINOCO-OJANGUREN & PEARCY, 1993b; PEARCY et al., 1994). We verified an increase in P_N besides of a non-simultaneous increase in g_s, mainly after 10 min of darkness (Figure 1). These results in the photosynthetic induction course indicate that P_N has a certain degree of independence of increases in g_s, since IS responses were not limited by stomata behaviour. Moreover, before the saturating irradiance pulse, C_i was above 350 μmol mol^{-1} in all species in both forest environments and darkness periods (Figures 1, 3). The occurrence of a g_s-independent IS is probably due to the high initial C_i, possibly derived from respiration, which together with the current g_s should have been sufficient to support CO_2 assimilation after darkness period. Furthermore, several authors have reported that non-uniform stomata opening (*i.e*. stomata patchiness) in response to a sudden increase in irradiance might cause differences in biochemical activation throughout the leaf (KIRSCHBAUM & PEARCY, 1988b; TINOCO-OJANGUREN & PEARCY, 1993a; KÜPPERS et al., 1999; ALLEN & PEARCY, 2000).

In order to grow in low irradiance environments such as the forest understorey, where plants have low carbon gain (CHAZDON et al., 1996; STRAUSS-DEBENEDETTI & BAZZAZ, 1996), plants must minimize carbon loss trough reduction of both respiration and tissue construction costs (GIVNISH, 1988), attaining

a positive leaf carbon balance. Our data are in accordance with those previously reported since all species studied showed significant higher R_D in the forest gap than in the understorey (Figures 2, 4). Several authors also verified that respiration is higher in forest gap than in understorey, since higher irradiance usually implies higher metabolic rates (RAMOS & GRACE, 1990; FREDEEN & FIELD, 1991; HAN et al., 1999). Moreover, some studies also describe that ES-species usually show higher leaf respiration than the LS-ones (BAZZAZ & PICKETT, 1980; CHAZDON et al., 1996), but in our report significant differences were not detected in R_D between species in both forest environments (Figures 2, 4).

The higher standard error in the graphs of P_N, g_s, and C_i in both darkness times, principally for ES-species in the gap, indicates the greater variability between samples in this environment (Figures 1, 3). Since all gas exchange parameters showed higher standard error in the gap, we suggest that under this environment plants showed a higher variability of response compared to understorey. In this latter, more constant environment, plants possibly have a more stable metabolism, implying a smaller standard error. Accordingly, BAZZAZ (1979) described that it is generally assumed that variability in the physical environment especially in air and soil temperature, soil moisture, and irradiance in ES-habitats is higher than in LS-habitats.

We found that T_{50} and T_{90} varied according to the time of previous exposure to darkness mainly in the gap, where the difference between darkness times was higher (Table 1). Such results indicate that the rate at which IS is recovered depends on the previous exposure to darkness and irradiance growth environment. As previously reported, the time required to reach full photosynthetic induction depends in part on the length of the period and PFD of low irradiance (PONS et al., 1992; WHITEHEAD & TESKEY, 1995). The IS state of a leaf is determined by the immediate past irradiance (PEARCY & SEEMANN, 1990) and declines when an induced leaf is shaded longer than a minute or so, and can be increased by exposure of a shaded leaf to a series of sunflecks (PEARCY, 1989).

Differences in T_{50} and T_{90} among plants exposed to 10 and 30 min of darkness were higher in the gap than in the understorey (Table 1). This result indicates that when plants remain over a 10 or 30 min dark period, their photosynthetic apparatus

demands more time to induce in the gap, whereas in the understorey estimates of T_{50} and T_{90} indicated that the photosynthetic apparatus remains induced for longer time suggesting a more rapid activation of RuBPCO, requiring less time to attain T_{50} and T_{90}. RIJKERS et al. (2000) suggested that leaves of understorey saplings of *Pourouma bicolor*, which required the least time to reach 75% of biochemical induction, presented a more rapid activation of RuBPCO. However, in several studies RuBPCO activity did not vary among and within species (SEEMANN & KOBZA, 1988; TINOCO-OJANGUREN & PEARCY, 1993a).

After 10 and 30 min of darkness the differences between ecological groups were not clearly detected in the gap for T_{50} and T_{90}, indicating that eco-physiological characteristics of each ecological group did not influence the IS time of the species evaluated herein. On the other hand, after both darkness times, the LS-species showed lower T_{50} in the understorey indicating a faster IS. After 30 min of darkness, the ES-species showed lower T_{90} than the LS-species in the understorey, possibly related to the required eco-physiological characteristics of their typical growth environment. Therefore, although LS-species recovered the IS more rapidly, P_{max} was attained faster by ES-species (Table 1). Conversely, previous studies showed that T_{90} was short in shade-tolerant species (KURSAR & COLEY, 1993; CHEN & KLINKA, 1997; VALLADARES et al., 1997; NAUMBURG & ELLSWORTH, 2000). Nevertheless, for some other species such as *Alocasia macrorrhiza*, *Toona australis*, *Shorea leprosula*, *Dryobalanops lanceolata*, *P. bicolor,* and *Dicorynia guianensis* in tropical environments, IS times were not affected by irradiance (CHAZDON & PEARCY, 1986b; KURSAR & COLEY, 1993; ZIPPERLEN & PRESS, 1997; RIJKERS et al., 2000).

After 10 min of darkness, the LS-species in the understorey showed significantly higher IS than the ES-ones (Figure 2) while after 30 min, LS presented a trend to show higher IS although the differences were not significant (Figure 4). These results of IS jointly to lower T_{50} in LS-species indicate that, after a period of darkness, this ecological group has a higher potential to exploit sunflecks compared to ES-species when both are grown in the understorey. Since sunflecks occur in short intervals, parameters such as IS and T_{50} can indicate the capacity to promptly respond to irradiance increase. Probably shade leaves maintained a higher IS longer at lower PFDs than sun leaves. Some comparative studies have suggested the latter

may be capable of using sunflecks more efficiently than the previous (CHAZDON & PEARCY, 1986a; CHOW et al., 1988; KÜPPERS & SCHNEIDER, 1993; TANG et al., 1994; YANHONG et al., 1994; ÖGREN & SUNDIN, 1996). In agreement with this assumption, POORTER & OBERBAUER (1993) and KÜPPERS et al. (1996) reported that LS-species maintained a higher IS status longer than the ES-ones. In *A. macrorrhiza*, RuBPCO is 50 % deactivated after 30 min in low irradiance, whereas in *T. australis*, the shade-intolerant species, it is deactivated more rapidly (CHAZDON & PEARCY, 1986a). VALLADARES et al. (1997) demonstrated that understorey species showed rapid induction, since IS was significantly higher, and higher light-fleck use efficiency for short light-flecks compared to species found in clearings or small gaps. Photosynthetic utilization of sunflecks requires a quick, dynamic physiological response, which is dependent on several regulatory factors, each working at a different time scale and exhibiting remarkable variation among species and individuals grown under different irradiance (PEARCY, 1990). Due to the differences in responses between plants exposed to 10 and 30 min of darkness it was possible to verify in our results that the species' capacity declines after a long period of darkness, possibly due to the inactivation of the photosynthetic apparatus. After 30 min of darkness differences between species were not as marked, indicating that the photosynthetic apparatus of species from both ecological groups deactivates and requires induction to utilize photon energy efficiently.

Our results are in agreement with those reported by VALLADARES et al. (1997), who found that P_{max} and g_s were lower in understorey species than in species growing in small gaps or clearings. However, some shade-tolerant species show a significant efficiency to increase photosynthetic capacity in response to increase in irradiance availability (CHOW et al., 1988; TURNBULL, 1991; THOMPSON et al., 1992). Our results show that LS-species presented elevated P_{max} in the gap and ES-ones presented elevated P_{max} in the understorey (Fig. 2 and 4), indicating high photosynthetic plasticity in species of both groups. STRAUSS-DEBENEDETTI & BAZZAZ (1996) state that differences in photosynthetic characteristics are generally viewed as being adaptive in nature, even if they may only reflect the constraints imposed by resource limitation. As plasticity addresses the expression of variable phenotypes under different environments (BRADSHAW, 1965), since both light-demanding and shade-tolerant species are capable of phenotypic plasticity, we

conclude that adjustments are not necessarily related to the succession status of the species (TURNBULL, 1991; POPMA, et al., 1992).

Corroborating our initial hypothesis, the ES-species presented higher P_N in the gap than in the understorey, suitably with their eco-physiological characteristics, although these species had presented a high performance also in the understorey after 10 or 30 min of darkness. As expected, the LS-species presented a faster photosynthetic induction than the ES-ones in the understorey, also in agreement with their eco-physiological features. We found that, after 10 min of darkness, plants in the understorey presented a higher potential in sunflecks utilization than after 30 min, above all the LS-species. Probably after a longer darkness period the photosynthetic apparatus is partially deactivated, which tended to be higher in ES-species.

The IS time course and other parameters evaluated in the present study evidences that plant responses can be modulated by their growth environment, indicating that the physiological and metabolic state of plant species are conditioned to environmental physical factors. Species occupy distinct niches based on their abilities to respond to different environment, and the capacity to show phenotypic plasticity is not exclusive to an ecological group. It is rather a more intrinsic feature related to the differential capacity of individuals. Thus, the influence of the species ecological group derives from some distinctive characteristics of their typical habitat, which partially determines their performance when faced to adverse conditions.

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Capítulo IV

Exposure time to moderately high temperature affects photosynthetic response to water deficit in *Eucalyptus globulus* Labill.

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Abstract - The co–occurrence of drought and heat stress in the field is frequent and the plant response to a combination of these abiotic stresses is singular, remaining largely unknown. Understanding physiological responses to a combined stress is of particular importance in face of the imminent global climate changes, which is predicted to entail not only higher temperatures, but also increase in drought, imposing restrictions to the productivity of economically important species as *Eucalyptus globulus*. This forest species is critically affected by changes in temperature and water availability, since its high productivity implies in high rates of water use. In order to evaluate the photosynthetic responses of *E. globulus* to these environmental conditions, young plants were grown at 25^{o} C and 35^{o} C under different irrigation regimes: full irrigation – FI, moderate drought – MD, and severe drought – SD. Plants grown at 25^{o} C FI and MD were also subjected to a short–term exposure to 35^{o} C. Several physiological variables, derived mainly from leaf gas exchange and chlorophyll *a* fluorescence measurements, were compared in order to test the hypotheses that: *i) E. globulus* shows different responses to short or long–term exposure to 35^{o} C; *ii)* the effect of both stresses combined will not be simply the sum of each condition applied separately. Our results showed that plants respond differently to water deficit according to the thermal regime. The combination of water deficit and a long–term exposure to 35^{o} C caused a down–acclimation of the photosynthetic capacity, while plants under the short–term treatment showed a higher performance, even when compared to the 25^{o} C control condition, supporting the hypothesis *i*. Thus, the short–term exposure to 35^{o} C induced an increase in tolerance to water deficit. Since the response to a combination of heat and drought differed from the superposition of individual responses, as observed mainly at 35^{o} C, the hypothesis *ii* was also supported by our results.

Key words: *Eucalyptus globulus*, water deficit, heat stress, photosynthesis, acclimation

Resumo – (O tempo de exposição a temperaturas moderadamente altas afeta a resposta fotossintética à deficiência hídrica em *Eucalyptus globulus* Labill.) A co–ocorrência de seca e estresse térmico é freqüente no campo, sendo que a resposta das plantas à combinação de tais estresses abióticos é singular e permanece relativamente desconhecida. O entendimento das respostas fisiológicas a estresses combinados é de particular importância considerando a previsão de iminentes mudanças climáticas globais, uma vez que não está previsto somente o aumento na temperatura, mas também o aumento da seca que impõem restrições à produtividade de espécies economicamente importantes como *Eucalyptus globulus.* Essa espécie florestal é criticamente afetada por mudanças na temperatura e disponibilidade hídrica, uma vez que sua alta produtividade implica em taxas de consumo de água elevadas. Com o objetivo de avaliar as respostas fotossintéticas de *E. globulus* às referidas condições ambientais, plantas jovens foram crescidas a 25^{o} C e 35^{o} C sob diferentes regimes de irrigação: 100% de irrigação – FI, seca moderada – MD e seca severa – SD. Plantas crescidas a 25^{o} C FI e MD foram também sujeitas a uma curta exposição a 35^{o} C. Diversas variáveis fisiológicas, extraídas principalmente das medidas de trocas gasosas foliares e fluorescência da clorofila *a*, foram comparadas a fim de testar a hipótese de que: *i) E. globulus* apresenta respostas diferentes a uma exposição curta e prolongada a 35^{o} C; *ii)* o efeito de ambos os estresses combinados não é simplesmente a soma de cada condição aplicada separadamente. Nossos resultados mostraram que as plantas respondem diferentemente à deficiência hídrica de acordo com o regime térmico. A combinação de deficiência hídrica e uma exposição prolongada a 35^{o} C causaram a *down-acclimatation* na capacidade fotossintética, enquanto plantas expostas a 35^{o} C por um curto período mostraram um aumento em seu desempenho fotossintético, quando comparadas à condição controle a 25^{o} C, suportando a hipótese *i*. Assim, a curta exposição a 35^{o} C induziu o aumento na tolerância das plantas à deficiência hídrica. Uma vez que a resposta à combinação de calor e seca diferiu das respostas obtidas quando cada estresse foi aplicado separadamente, conforme observado principalmente a 35^{o} C, a hipótese *ii* também foi suportada pelos resultados obtidos.

Palavras-chave: *Eucalyptus globulus*, deficiência hídrica, estresse térmico, fotossíntese, aclimatação

INTRODUCTION

Generally, in the field, several abiotic stresses occur simultaneously, being especially frequent the co–occurrence of drought and heat stress. Increase in leaf temperature is also a consequence of drought since plants decrease transpirational cooling due to stomatal closure (SHARKEY, 2005). Although both stress types are associated, molecular and metabolic studies suggest that the plant response to a combination of drought and heat is unique and cannot be directly extrapolated from the individual response to each stress (RIZHSKY et al., 2002, 2004).

Given that photosynthesis is a central process to cellular metabolism, involving large fluxes of carbon, nitrogen and energy (LAWLOR, 2001) and being integrated with respiration, electron transport and ATP synthesis in the mitochondria (ATKIN & MACHEREL, 2009), it is considerably vulnerable to water deficit and temperature. Indeed photosynthesis, together with cell growth, is among the main processes to be affected by drought (CHAVES, 1991). Direct effects are decreased CO_2 availability caused by diffusion limitations through the stomata and mesophyll (CHAVES et al., 2003, 2009; FLEXAS et al., 2004, 2007) and alterations in the photosynthetic metabolism (LAWLOR & CORNIC, 2002; LAWLOR & TEZARA, 2009). Moreover, under drought, the balance between energy harvesting and metabolism may be disturbed, leading to a decrease in the efficiency of the photochemical phase of photosynthesis, occurring in parallel with an increase in energy dissipation in the chloroplast (DEMMIG–ADAMS & ADAMS, 1992). Additionally, when drought becomes severe oxidative stress may arise due to the generation of reactive oxygen species (REDDY et al., 2004).

Regarding the effects of high temperature, photochemical reactions in the thylakoid lamellae and carbon metabolism in the stroma of the chloroplasts have been suggested to be the primary sites of injury (WISE et al., 2004). Direct injuries due to high temperatures include protein denaturation and aggregation, as well as an increase in the fluidity of membrane lipids and electrolyte leakage (WAHID et al., 2007). Plant metabolism can undergo acclimation after a prolonged exposure to a different growth temperature, which could eventually result in metabolic homeostasis, for example, with the maintenance of similar photosynthetic and respiratory rates (STITT & HURRY, 2002; ATKIN & TJOELKER, 2003). Furthermore, an indication of

thermal acclimation is the deviation of the temperature optimum towards the new growth temperature (BERRY & BJÖRKMAN, 1980), as reported in *Eucalyptus globulus* by BATTAGLIA et al. (1996).

Thus far, most studies on plant physiology have focused on the impact of a single environmental stress, *e.g.* either water deficit or heat shock (REDDY et al., 2004; CAMEJO et al., 2005), whereas the combination of stress types has received less attention (RIZHSKY et al., 2004). The effects of the combination of drought and heat stress were previously studied on the growth and productivity of maize, barley, sorghum and different grasses (MITTLER, 2006) but, to our knowledge, there was no attempt to evaluate the combined effect of water stress and moderately high temperature in tree species, as *Eucalyptus globulus*. *E. globulus* is a forest species with considerable economical importance, once it is the foremost pulpwood eucalypt species planted in temperate regions (DOUGHTY, 2000) and its productivity is particularly sensitive to drought, given its high water demand (WHITEHEAD & BEADLE, 2004).

Understanding how this plant species respond to increases in water deficit and elevated temperature is particularly important in face of the imminent global climate changes, given that the predicted global warming, in the next century, by an average of 2–4^{o} C in conjunction with changes in precipitation will likely lead to droughts and supra–optimal temperatures in many areas of the globe (IPCC, 2007). Drought may induce large–scale declines in tree growth in temperate forests, once the productivity of forest ecosystems is severely constrained by water availability (BRÉDA et al., 2006). In addition, temperature–mediated changes in leaf photosynthesis and respiration are now accepted as important components of the biosphere's response to global climate change (ATKIN & TJOELKER, 2003). Thus, the present study has the aim to evaluate the effects of water deficit on the photosynthetic capacity in plants of *E. globulus* under different thermal regimes.

In order to evaluate the photosynthetic responses of *E. globulus* to these environmental conditions, young plants were grown at 25^{o} C and 35^{o} C under different irrigation regimes: full irrigation – FI, moderate drought – MD, and severe drought – SD. Plants grown at 25^{o} C FI and MD were also subjected to a short–term exposure to 35^{o} C. Several physiological variables, derived mainly from leaf gas exchange and chlorophyll *a* fluorescence measurements, were compared in order to

test the hypotheses that: *i) E. globulus* show different responses to short or long–term exposure to 35^{o} C; *ii)* the effect of both stresses combined will not be simply the sum of each condition applied separately (MITTLER, 2006). We will show a marked difference between the responses to long and short–term exposure to 35^{o} C, in different irrigation treatments, where the short–term treatment induced a lower down–acclimation of the photosynthetic capacity than the long–term and, furthermore, promoted a potential increase in tolerance to water deficit.

MATERIAL AND METHODS

- *Plant material and growth conditions*: Seeds of *Eucalyptus globulus* Labill. were germinated in pots in a fully sunlit greenhouse where they remained for about 1 month. Seedlings with one or two pairs of true leaves were then transferred to 3 L plastic pots containing a 2:1 (v/v) soil and sand mixture and placed in a controlled growth chamber (FITOCLIMA 10000EHHF, Aralab) set to maintain temperature at 25/18^{o} C (day/night cycle), 800 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) provided with fluorescent tubes, 60% of relative humidity and a photoperiod of 16 h. Healthy 5 months–old plants were then transferred to another controlled growth chamber (FITOCLIMA 700EDTU, Aralab) with identical conditions, where they remained for 1 month to ensure acclimation before experiments started. Pots were irrigated daily to full soil capacity and a standard Hoagland's solution was applied every ten days in order to prevent deficiency in essential nutrients until the beginning of the experiments.

- *Experimental set up:* Experiments were performed in leaves grown under two diurnal thermal conditions: *i)* 25/18^{o} C, and *ii)* 35/18^{o} C (day/night cycle). In each thermal treatment, six plants were randomly selected for three irrigation treatments: *i)* irrigated to field capacity (full irrigation – FI), *ii)* irrigated to 25% of the field capacity (moderate drought treatment – MD), and *iii)* irrigated to 12.5% of the field capacity (severe drought treatment – SD). Measurements were performed two weeks after the beginning of the irrigation treatments, and pots were frequently rotated to minimize microclimatic effects inside the growth chamber. Additionally, a heat shock

experiment was performed, consisting in exposing plants grown at 25^{o} C to an abrupt increase in diurnal temperature to 35^{o} C, remaining under such condition for four days. This treatment, designated “35^{o} C Short–term”, was performed under FI and MD conditions where the water deficit was applied one week before the heat shock.

- *Leaf gas exchange and chlorophyll a fluorescence measurements:* Light (A_N–PPFD) and CO_2 (A_N–C_i) response curves were performed, where leaf gas exchange parameters were determined simultaneously with chlorophyll fluorescence measurements using an open infrared gas–exchange analyzer (Li–6400, Li–Cor Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (Li–6400-40; Li–Cor Inc.) that provides LED–based fluorescence and irradiation. Measurements were taken between 8:00–12:00 h in young fully expanded leaves from the light exposed parts of the shoots acclimated to the above mentioned conditions. Measurements were performed with a 2 cm^2 Li–6400 leaf chamber in one attached leaf in each of the six plants evaluated per irrigation and thermal treatment. Net CO_2 assimilation (A_N – μmol CO_2 m^{-2} s^{-1}) and transpiration (E – mmol H_2O m^{-2} s^{-1}) rates, stomatal conductance (g_s – mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i – μmol CO_2 mol^{-1}) were calculated using the LI–6400 data analysis program which uses VON CAEMMERER & FARQUHAR’s (1981) general gas exchange formula. Environmental conditions in the leaf chambers were controlled with the LI–6400, maintaining conditions practically identical to the growth chamber conditions (described above). During all measurements vapour pressure deficit (VPD) was maintained around 1.0–1.5 kPa using a dew point generator (Li–610, Licor) attached to the LI–6400.

Light response curves (A_N–PPFD) were obtained by gradually varying PPFD from 1400 to 25 μmol photon m^{-2} s^{-1} (in ten levels) under a constant CO_2 concentration of 380 μmol mol^{-1} and block temperature at 25° C or 35° C, according to thermal treatment. PPFD was provided by the Li–6400–40 light source, with 10% blue light to maximize stomatal aperture. Measurements were recorded with 4–5 min intervals between each reading and were logged when the total coefficient of variation (CV) was ≤1 %, indicating stability of the reading. CO_2 response curves (A_N–C_i) were performed at 1000 μmol photon m^{-2} s^{-1} of PPFD with CO_2 concentration varying from 100 to 1500 μmol mol^{-1}. Measurements were also recorded when *A*

values were stable (CV ≤1%), 4–5 min after each change in chamber CO_2 concentration.

• *Model and derived parameters:* Light and CO_2 response curves were fitted using the following equation (PRADO & MORAES, 1997)

$$A = A_{max} (1 - \mathbf{e}^{-k(X-CP)}),$$

where A is the net CO_2 assimilation, A_{max} is the maximum CO_2 assimilation, **e** is the Euler's number, k is a constant related to the convexity of the curve, X is PPFD or C_i and CP is the light (LCP) or CO_2 compensation point (CCP). Light and CO_2 saturation points, LSP and CSP respectively, were estimated calculating the values in which A reached 90% of A_{max}, while apparent quantum (α) and carboxylation (ε) efficiencies were estimated using the initial linear slope of A_N–PPFD and A_N–C_i curves respectively.

Relative stomatal limitation of photosynthesis (L_s) was calculated from A_N–C_i curves as proposed by FARQUHAR & SHARKEY (1982):

$$L_s = [(A'–A)/A']*100$$

where A' is the CO_2 assimilation when C_i equals the atmospheric concentration (380 μmol mol^{-1}) and A is the CO_2 assimilation when CO_2 concentration in the sample chamber (C_e) equals the atmospheric concentration. The maximum rate of Rubisco carboxylation (V_{cmax} – μmol CO_2 m^{-2} s^{-1}) and maximum electron transport rate at saturating light (J_{max} – μmol CO_2 m^{-2} s^{-1}) were determined on a C_i basis from A_N–C_i curves by fitting the model of FARQUHAR et al. (1980) with modifications by SHARKEY (1985) to A_N –C_i response curves, as described by MAROCO et al. (2002).

Mesophyll conductance (g_m – mol CO_2 m^{-2} s^{-1} bar^{-1}) was estimated with the method of HARLEY et al. (1992) using the following equation:

$$g_m = A_N/(C_i - (\Gamma^*(J + 8(A_N + R_d)))/(J - 4(A_N + R_d)))$$

where J is the photosynthetic electron transport rate and Γ^* is the CO_2 compensation point in the absence of R_d, which is the mitochondrial respiration occurring during the day, estimated using the LAISK (1977) method. The mean values of g_m presented correspond to C_e concentration around 380 μmol mol^{-1}.

Intrinsic water use efficiency (WUE_i – μmol CO_2 mol H_2O^{-1}) was calculated with A_N/g_s ratio from A_N–PPFD response curves. Leaf dark respiration (R_d – μmol CO_2 m^{-2} s^{-1}) was calculated with *A* values at PPFD = 0 measured in light response curves of leaves adapted to 30 min of darkness.

• *Chlorophyll a fluorescence parameters:* Maximal (F_M) and basal (F_0) fluorescence yields were measured in the same dark–adapted leaves used to estimated R_d, whereas steady–state (F_S) and maximal (F'_M) fluorescence were measured in light–adapted leaves during the A_N–PPFD curves (VAN KOOTEN & SNEL, 1990). Thus, variable fluorescence yield was determined in both dark–adapted ($F_V = F_M - F_0$) and light–adapted ($\Delta F = F'_M - F_S$) states. The fluorescence parameters calculated were: potential (F_V/F_M) and effective ($\Phi PSII = \Delta F/F'_M$) quantum efficiency of the photosystem II (PSII), photochemical {$qP = [(F'_M - F_S)/(F'_M - F'_0)]$} and non photochemical {$NPQ = [(F_M - F'_M)/F'_M]$} fluorescence quenching, PSII antennae efficiency {$F'_V/F'_M = [(F'_M - F_0)/ F'_M]$}, and apparent electron transport rate [$ETR = (PPFD \times (\Delta F/F'_M \times 0.5 \times 0.84)]$ (BILGER et al., 1995; MAXWELL & JOHNSON, 2000). F'_M was obtained with a pulse of saturating actinic light applied simultaneously to the gas exchange measurements, where F_S is the steady-state fluorescence measured briefly before the saturating light pulse allowing estimation of the PSII quantum yield (GENTY et al., 1989). F_0' is the basal fluorescence yield after photosystem I excitation by far–red light. For the calculation of ETR, 0.5 was used as the fraction of excitation energy distributed to PSII, and 0.84 as the fraction of light absorption (DEMMIG & BJÖRKMAN, 1987).

- *Leaf water potential* (Ψ_w): Pre–dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential were measured at 5:00 and 13:00 h, respectively, with a Scholander–type pressure chamber (PMS Instruments Co., Corvallis, OR, USA) in six fully expanded leaves of six different plants per treatment (n=6). The leaf water potential was recorded in the same days where gas exchange measurements were performed.

- *Statistical analysis*: The experiment was arranged in a randomized design with 6 replicates. All data were subjected to a two–way analyses of variance (ANOVA) followed by a post–hoc Tukey's test at the 0.05 significance level in order to assess the effects of thermal and irrigation treatments and their interaction on the different dependent variables evaluated. Statistical data analyses were performed with Statistica (v7, Statsoft Tulsa, OK, USA). Throughout the text every difference considered significant had a $p < 0.05$ (Tukey's test).

Principal component analysis (PCA) is a technique which enables the exploration of multivariate data sets through the reduction of n variables to lower dimensions, which are formed by principal components. These are independent axes composed of linear combinations of the original variables, as to maximize the explained variances. Thus, the first principal component (PC1) explains most of the variance in the observed data, followed by PC2 and so on, being an effective approach to identify groups formed by the combined effect of the evaluated variables (HAIR et al., 2006). The analysis was performed in the language for statistical computing R, by means of the function *prcomp* (R Development Core Team, 2009), on the normalized data of all replicates (variances scaled to 1) with the variables: C_i, A_N, g_s, g_m, V_{cmax}, J_{max}, F_0, F_M, F_V/F_M, F_V/F_0, L_s, R_d, $A_{max\ light}$, LCP, α, LSP, $A_{max\ CO2}$, CCP, ε, CSP and WUE_i.

RESULTS

The reduction in water availability resulted in a decrease in leaf water potential (Ψ_w) according to drought severity, both in predawn (Ψ_{pd}) and midday (Ψ_{md}), being significantly the lowest under SD at 25° C and 35° C and in the MD at 35° C Short–term (Figure 1). The decrease in Ψ_w and the increase in temperature constrained the photosynthetic apparatus of *E. globulus*, an effect derived from photochemical, biochemical and CO_2 diffusion limitations.

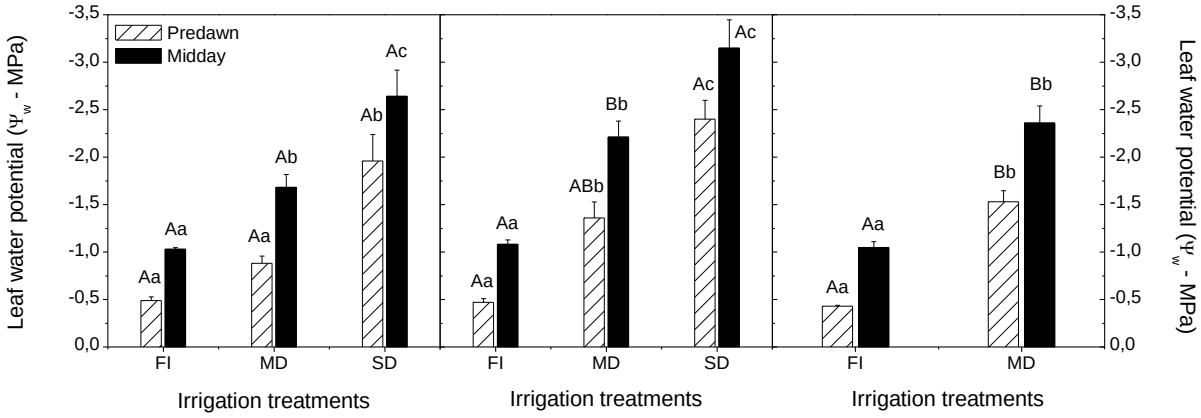

Figure 1. Leaf water potential (Ψ_w) at predawn (Ψ_{pd}) and midday (Ψ_{md}) measured in *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short-term under different irrigation treatments: full irrigation (FI), moderate drought (MD) and severe drought (SD). Capital letters indicate significant differences among thermal treatments and different lower-case letters express significant differences among irrigation treatments (Tukey's test, $p < 0.05$). Data represent means ± SE (n=6).

Photochemical limitations

The effects of water deficit in the photochemical apparatus were different in the distinct thermal treatments, as evidenced by the fluorescence parameters measured in light and dark–adapted leaves (Table 1, Figure 2). Fluorescence parameters in dark–adapted leaves indicated that the long–term exposure to 35^{o} C disturbed the photochemical apparatus mainly in the response to water deficit, given the significantly higher increase in basal (F_0) and maximal (F_M) fluorescence yields observed from FI to MD and SD. Curiously, at 35^{o} C Short–term, F_0 decreased under MD and F_M was similar under both irrigation regimes, indicating that the photochemical apparatus was practically unaffected by a short exposure to 35^{o} C. The potential quantum efficiency (F_V/F_M) and the variable to basal fluorescence ratio (F_V/F_0) generally decreased with reduced water availability, showing a prominent trend to decrease at 35^{o} C, whereas at 35^{o} C Short–term both parameters showed a non–significant increase under MD (Table 1).

Fluorescence parameters in light–adapted leaves were measured concomitantly with light response curves (A_N–PPFD), thus their courses are presented as a function of PPFD (Figure 2). Effective quantum efficiency (ΦPSII) and photochemical quenching (qP) showed similar courses, both being considerably reduced under MD and SD at 25^{o} C and 35^{o} C, whereas a lower decrease due to water deficit is verifiable at 35^{o} C Short–term. Electron transport rate (ETR) was more affected by the long–term exposure to 35^{o} C since it markedly decreased from FI to MD and SD, when compared to 25^{o} C. Water deficit had a remarkably lower effect in ETR at 35^{o} C Short–term, even when compared to 25^{o} C, showing the highest values under MD. Non–photochemical quenching (NPQ) was higher at 25^{o} C than at 35^{o} C in all irrigation treatments, also showing a pronounced difference between water deficit and FI treatments which was not verifiable in both 35^{o} C treatments (Figure 2).

Table 1. Chlorophyll *a* fluorescence parameters in dark–adapted leaves of *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short-term and exposed to different irrigation regimes: full irrigation (FI), moderate drought (MD) and severe drought (SD). Data represent means ± SE (n=6). Different capital letters express significant difference for thermal treatments and different lower-case letters express significant differences for irrigation treatments (Tukey's test, $p < 0.05$). F_0 = basal fluorescence yield, F_M = maximal fluorescence yield, F_V/F_M = potential quantum efficiency, F_V/F_0 = variable to basal fluorescence ratio.

Parameters	25° C			35° C			35° C Short-term	
	FI	MD	SD	FI	MD	SD	FI	MD
F_0	398.47 ± 11.52 ^{Aa}	416.95 ± 9.12 ^{Ba}	435.88 ± 17.13 ^{Ba}	437.70 ± 12.24 ^{Ab}	565.75 ± 52.89 ^{Aab}	651.32 ± 78.04 ^{Aa}	475.3 ± 18.78 ^{Aa}	451.1 ± 15.68 ^{ABa}
F_M	2120.35 ± 28.73 ^{Aa}	2177.55 ± 33.51 ^{Aa}	2149.34 ± 47.98 ^{Aa}	2020.75 ± 35.27 ^{Aa}	2211.97 ± 62.47 ^{Aab}	2325.47 ± 100.42 ^{Ab}	2120.58 ± 11.83 ^{Aa}	2124.48 ± 29.8 ^{Aa}
F_V/F_M	0.812 ± 0.01 ^{Aa}	0.808 ± 0.01 ^{Aa}	0.797 ± 0.01 ^{Aa}	0.783 ± 0.01 ^{Aa}	0.745 ± 0.02 ^{Aa}	0.715 ± 0.04 ^{Bb}	0.776 ± 0.01 ^{Aa}	0.788 ± 0.01 ^{Aa}
F_V/F_0	4.352 ± 0.22 ^{Aa}	4.230 ± 0.10 ^{Aa}	3.951 ± 0.15 ^{Aa}	3.628 ± 0.11 ^{Aa}	3.037 ± 0.31 ^{Aa}	2.759 ± 0.54 ^{Aa}	3.49 ± 0.18 ^{Aa}	3.725 ± 0.16 ^{Aa}

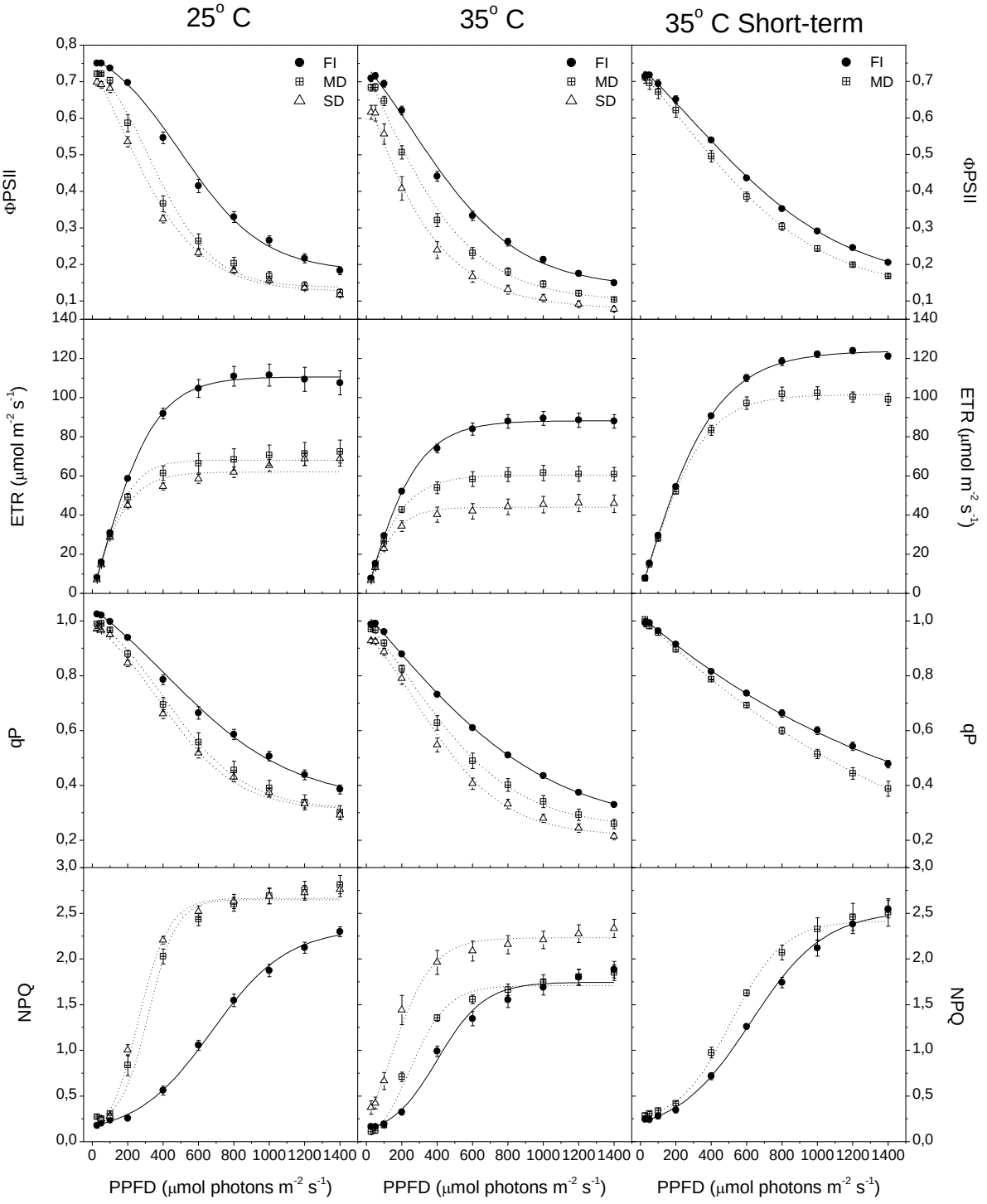

Figure 2. Effective quantum efficiency of PSII (ΦPSII), electron transport rate (ETR), photochemical quenching (qP) and non-photochemical quenching (NPQ) light response curves in leaves of *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short-term under different irrigation treatments: full irrigation (FI), moderate drought (MD) and severe drought (SD). Data represent means ± SE (n=6). PPFD is the photosynthetic photon flux density.

Biochemical and CO_2 diffusion limitations

The exposure time to 35° C lead to different photosynthetic responses in fully irrigated plants given that, comparing to 25° C FI, $A_{max\ light}$ was significantly lower at 35° C while at 35° C Short–term no significant difference was detected (Table 2). Such response can also be verified through the A_N–PPFD curves, which showed similar courses in plants under FI at 25° C and 35° C Short–term, while at 35° C the CO_2 assimilation course was lower possibly related with the lower stomatal conductance (g_s) observed in this treatment (Figure 3). Interestingly, the effects of water deficit on the photosynthetic efficiency were significantly less pronounced at 35° C Short–term, showing higher $A_{max\ light}$ under MD compared to the other thermal treatments (Table 2). Values of $A_{max\ light}$ are clearly related with their respective g_s, as can be seen in the reduction in A_N from FI to water deficit treatments, between 25° C and 35° C under FI, and in the maintenance of a higher A_N and g_s at 35° C Short–term under MD (Figure 3).

As with CO_2 assimilation rates and g_s, irrigation treatments affected intrinsic water use efficiency (WUE_i), showing significantly higher values under water deficit in all thermal treatments (Figure 3). Despite of the non–significant differences in FI among the thermal treatments, there was a trend to increase in WUE_i under water deficit at 35° C which is not pronounced at 35° C Short–term, suggesting that the stomatal control under drought is influenced by the exposure time to 35° C. Dark respiration (R_d) increased with the temperature and decreased with the water deficit in all irrigation treatments (Table 2).

Parameters derived from A_N–PPFD curves showed marked differences among the thermal treatments (Table 2). Light compensation point (LCP) was significantly higher in all irrigation treatments at 35° C and 35° C Short–term compared to 25° C. Moreover, while LCP increased under MD and SD at 35° C when compared to FI, at 35° C Short–term it decreased. Light saturation point (LSP) decreased significantly with water deficit at 25° C and 35° C Short–term, while at 35° C it was significantly higher under SD. The apparent quantum efficiency (α) showed a similar response in all thermal treatments, consisting in a significant decrease with the increase of water deficit severity. However, there was a lower decrease in α under a short–term

Table 2. Parameters extracted from A_N–PPFD and A_N–C_i curves performed in leaves of *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short–term under: full irrigation (FI), moderate drought (MD) and severe drought (SD). Data represent means ± SE (n=6). Different capital letters express significant differences among thermal treatments and different lower-case letters express significant differences among irrigation treatments (Tukey's test, $p < 0.05$). $A_{max\ light}$ = maximum CO_2 assimilation rate measured in the light response curves (μmol CO_2 m^{-2} s^{-1}), LCP = light compensation point (μmol photons m^{-2} s^{-1}), LSP = light saturation point (μmol photons m^{-2} s^{-1}), α = apparent quantum efficiency (μmol CO_2 μmol $photon^{-1}$), R_d = dark respiration (μmol CO_2 m^{-2} s^{-1}), $A_{max\ CO2}$ = maximum CO_2 assimilation rate measured in the CO_2 response curves (μmol CO_2 m^{-2} s^{-1}), CCP = CO_2 compensation point (μmol mol^{-1}), CSP = CO_2 saturation point (μmol mol^{-1}), ε = apparent carboxylation efficiency (μmol CO_2 m^{-2} s^{-1} Pa^{-1}), L_s = Stomatal limitation of photosynthesis (%).

Parameters	25° C			35° C			35° C Short-term	
	FI	MD	SD	FI	MD	SD	FI	MD
$A_{max\ light}$	16.11 ± 0.78 ^{Aa}	5.13 ± 0.47 ^{Bb}	3.97 ± 0.49 ^{Ab}	10.08 ± 0.57 ^{Ba}	4.89 ± 0.42 ^{Bb}	3.54 ± 0.42 ^{Ab}	15.19 ± 1.23 ^{Aa}	9.66 ± 0.88 ^{Ab}
LCP	30.45 ± 0.91 ^{Ba}	30.03 ± 1.72 ^{Ba}	42.87 ± 4.08 ^{Ba}	58.72 ± 2.98 ^{ABb}	70.12 ± 4.09 ^{Ab}	139.07 ± 30.19 ^{Aa}	71.66 ± 9.55 ^{Aa}	51.93 ± 2.46 ^{ABa}
LSP	600.61 ± 32.02 ^{ABa}	316.18 ± 17.14 ^{Bb}	348.41 ± 10.37 ^{Bb}	570.72 ± 42.83 ^{Bb}	538.39 ± 21.04 ^{Ab}	800.38 ± 51.45 ^{Aa}	713.33 ± 18.37 ^{Aa}	542.99 ± 32.27 ^{Ab}
α	0.051 ± 0.001 ^{Aa}	0.023 ± 0.002 ^{Bb}	0.015 ± 0.002 ^{Ac}	0.041 ± 0.002 ^{Ba}	0.023 ± 0.002 ^{Bb}	0.010 ± 0.001 ^{Ac}	0.05 ± 0.002 ^{ABa}	0.04 ± 0.002 ^{Ab}
R_d	-1.77 ± 0.13 ^{Ba}	-1.23 ± 0.15 ^{Ba}	-1.31 ± 0.08 ^{Aa}	-3.41 ± 0.20 ^{Aa}	-2.39 ± 0.26 ^{ABb}	-2.26 ± 0.05 ^{Ab}	-4.18 ± 0.48 ^{Aa}	-2.62 ± 0.18 ^{Ab}
$A_{max\ CO2}$	19.90 ± 0.25 ^{Aa}	17.86 ± 0.29 ^{Aa}	14.75 ± 2.52 ^{Aa}	20.92 ± 0.46 ^{Aa}	15.76 ± 1.91 ^{Aab}	11.85 ± 3.36 ^{Ab}	25.11 ± 0.87 ^{Aa}	16.78 ± 0.78 ^{Ab}
CCP	81.66 ± 2.23 ^{Ba}	93.26 ± 4.16 ^{Aa}	96.65 ± 6.76 ^{Aa}	103.91 ± 3.45 ^{Aa}	104.06 ± 6.58 ^{Aa}	119.79 ± 4.89 ^{Aa}	77.37 ± 2.85 ^{Ba}	89.29 ± 6.53 ^{Aa}
CSP	519.67 ± 17.25 ^{Aa}	663.54 ± 54.21 ^{Aa}	551.69 ± 95.13 ^{Aa}	700.83 ± 34.88 ^{Aa}	632.76 ± 59.90 ^{Aa}	519.64 ± 79.80 ^{Aa}	623.26 ± 24.77 ^{Aa}	495.52 ± 72.22 ^{Aa}
ε	0.06 ± 0.001 ^{Aa}	0.05 ± 0.005 ^{Aa}	0.07 ± 0.001 ^{Aa}	0.07 ± 0.005 ^{Aa}	0.07 ± 0.022 ^{Aa}	0.06 ± 0.011 ^{Aa}	0.07 ± 0.004 ^{Aa}	0.08 ± 0.011 ^{Aa}
L_S	6.00 ± 0.45 ^{Ab}	31.46 ± 1.88 ^{Aa}	38.74 ± 3.52 ^{Ba}	15.67 ± 1.31 ^{Ac}	32.52 ± 6.13 ^{Ab}	58.03 ± 3.89 ^{Aa}	9.31 ± 1.40 ^{Ab}	33.85 ± 8.50 ^{Aa}

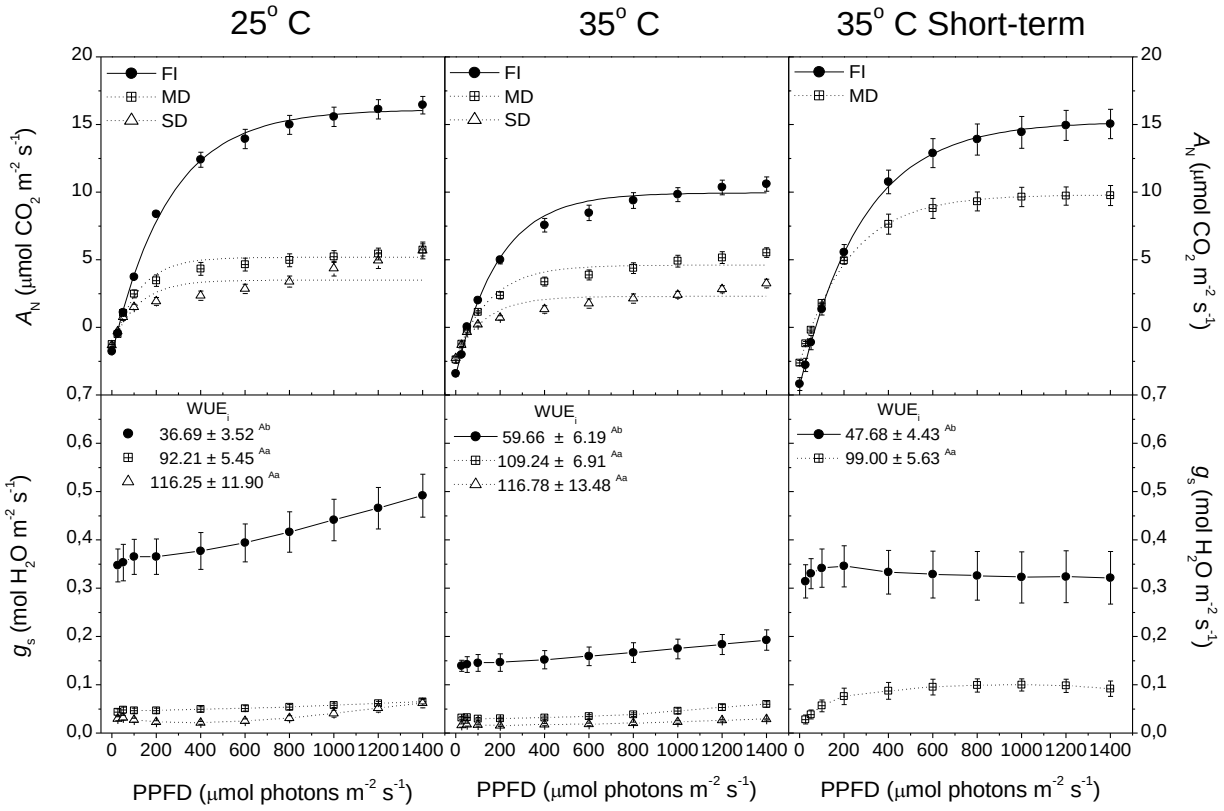

Figure 3. Net CO_2 assimilation (A_N) and stomatal conductance (g_s) light response curves, together with intrinsic water use efficiency (WUE_i – μmol CO_2 mol H_2O^{-1}) in leaves of *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short-term under different irrigation treatments: full irrigation (FI), moderate drought (MD) and severe drought (SD). PPFD is the photosynthetic photon flux density. Data represent means ± SE (n=6). Different capital letters express significant differences among thermal treatments and different lower-case letters express significant differences among irrigation treatments (Tukey's test, $p < 0.05$).

exposure to 35^{o} C, which showed the highest values under MD compared to other thermal treatments.

Parameters derived from CO_2 response curves (A_N–C_i) were mostly affected by water deficit (Table 2). Maximum assimilation rate ($A_{max\ CO_2}$) decreased with water deficit but no significant difference was detected among thermal treatments. Whereas the CO_2 compensation point (CCP) increased with water deficit in all thermal treatments, showing a trend to be higher at 35^{o} C, CO_2 saturation point (CSP) did not show a consistent trend at 25^{o} C, while at 35^{o} C and 35^{o} C Short–term it decreased with water deficit severity. The apparent carboxilation efficiency (ε) did not show a clear variation pattern comparing irrigation treatments in all thermal treatments studied (Table 2).

The relationship between net CO_2 assimilation (A_N) and intercellular CO_2 concentration (C_i), mesophyll conductance (g_m) and stomatal conductance (g_s), maximum electron transport rate (J_{max}) and Rubisco maximum carboxylation rate (V_{cmax}) extracted from A_N–C_i curves are presented in Figure 4, and such parameters showed distinct responses to water deficit in the different thermal treatments. The decrease in A_N with water deficit at 25^{o} C and 35^{o} C is probably related with the decrease in g_s and g_m, which caused a substantial decrease in C_i in the leaf mesophyll, together with the decrease in V_{cmax} and J_{max} which occurred mainly at 35^{o} C. However, the high A_N rates observed at 35^{o} C Short–term is probably related with the maintenance of a high g_m, which would have supported a high C_i even under a low g_s. Moreover, the maintenance of a high V_{cmax} and J_{max} can also have contributed to the high A_N at 35^{o} C Short–term (Figure 4).

In agreement with the progressive decrease in g_s with water deficit increase, the stomatal limitation of photosynthesis (L_s) increased significantly in response to drought in all thermal treatments studied, being even higher at 35^{o} C which reached more than 3–fold higher values under SD compared to FI (Table 2).

The principal component analyses (PCA) graph shows the representation of all thermal and irrigation treatments on the two major principal components, presenting some degree of separation among them (Figure 5). The first principal component (PC1) accounted for 49.3% of total data variance, while the second principal component (PC2) accounted for 16.8%, jointly explaining 66.1% of the data.

Irrigation regimes presented a clear separation within thermal treatments, mainly along PC1, where the most significant variables composing the first principal, in order of importance, were: α, A_N, $A_{max\ light}$, L_s and J_{max}. Thermal treatments were most distinguishable along PC2, where the most significant photosynthetic parameters were: LSP, LCP, F_V/F_0, F_V/F_M and R_d.

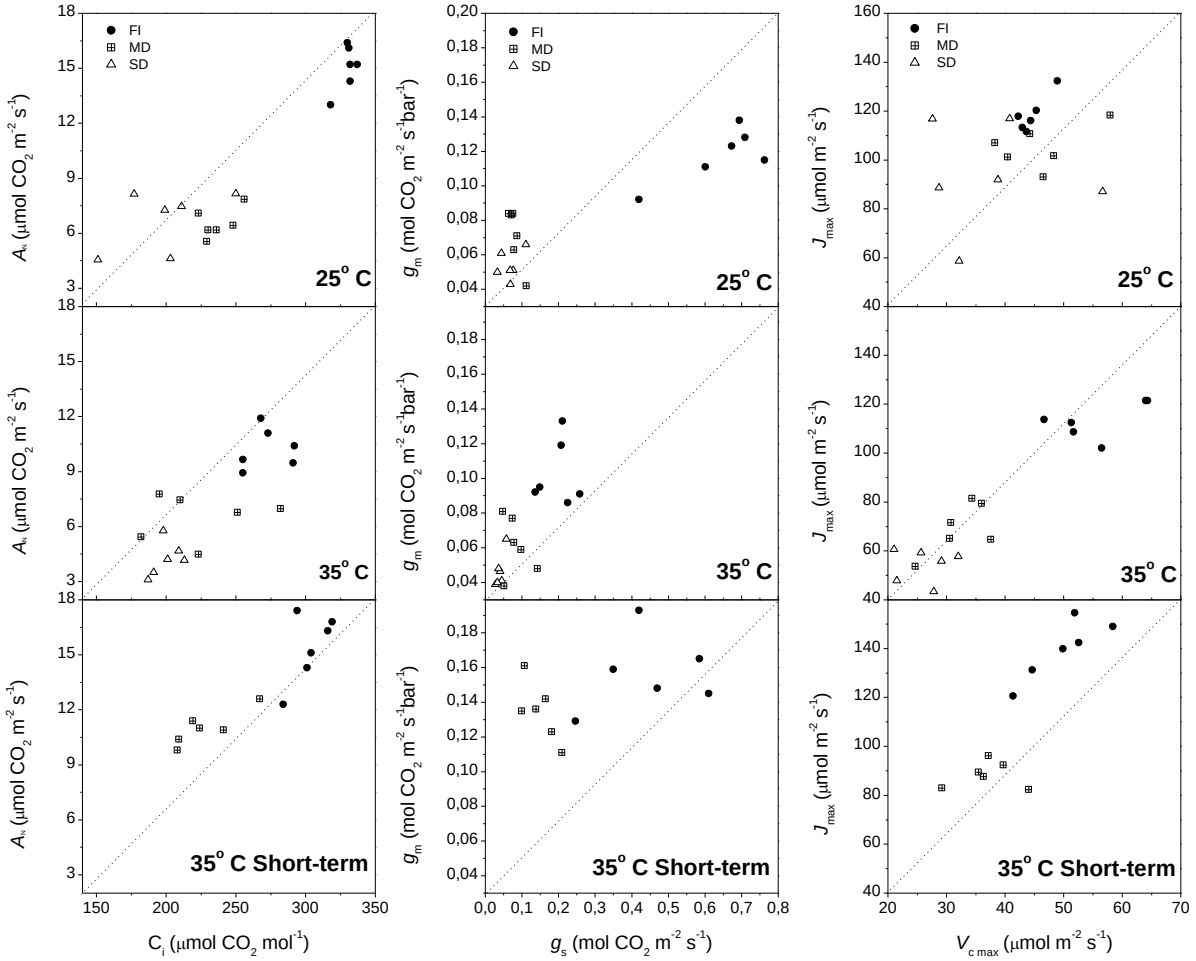

Figure 4. Relationship between net CO_2 assimilation (A_N) and intercellular CO_2 concentration (C_i), mesophyll (g_m) and stomatal conductance (g_s), maximum electron transport rate (J_{max}) and maximum rate of Rubisco carboxylation (V_{cmax}) extracted from CO_2 response curves at 380 μmol mol^{-1}, performed in leaves of *Eucalyptus globulus* grown at 25° C, 35° C and 35° C Short-term under different irrigation treatments: full irrigation (FI), moderate drought (MD) and severe drought (SD). Data represent individual measurements (n=6).

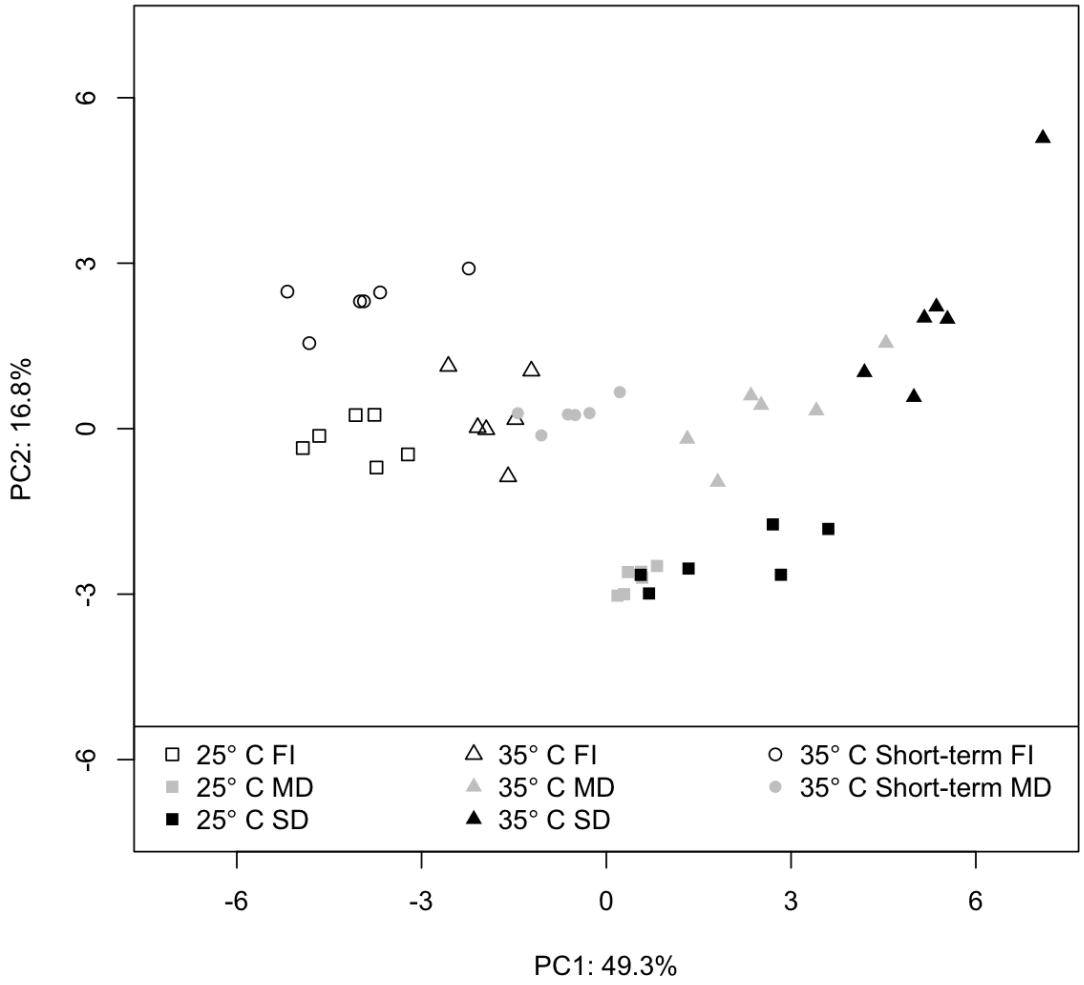

Figure 5. Distribution of the irrigation regimes in the thermal treatments for first and second principal components, PC1 and PC2 axes, respectively.

DISCUSSION

Effects on photochemical apparatus

It has been reported that the photochemical apparatus presents high robustness and resilience to drought (CORNIC et al., 1989; EPRON & DREYER, 1992, 1993). In fact, our results demonstrated that photochemical efficiency of PSII was not markedly sensitive to water deficit, mainly in plants at 25^{o} C and 35^{o} C Short–term (Table 1). Chlorophyll fluorescence parameters evaluated in dark–adapted leaves of *E. globulus* indicated that the photochemical apparatus was more affected by water deficit when plants were exposed to long–term 35^{o} C, since a substantial increase in F_0 and F_M and decrease in F_V/F_M and F_V/F_0 were observed. This is compatible with results shown by HAVAUX (1992) which reported that exposure of potato leaves to high temperatures caused an increase in PSII activity in water–stressed plants. A prolonged exposition to 35^{o} C caused a higher damage to the photochemical apparatus in *E. globulus*, since PSII is highly thermolabile and its activity is greatly reduced or even partially stopped under high temperatures (BUKHOV et al., 1999; CAMEJO et al., 2005), mainly due its localization in the thylakoid membranes which generally are damaged by heat. Moreover, the chlorophyll fluorescence yield in the dark–adapted state indicated that the photochemical apparatus was apparently more sensitive to temperature than to water deficit (compare values under FI between 25^{o} C and 35^{o} C with differences between 25^{o} C FI, MD and SD in Table 1).

The decrease in F_V/F_M coupled to an increase in F_0 indicates that plants grown at 35^{o} C under MD and SD conditions suffered damage in the photochemical apparatus and, consequently, presented photoinhibition in response to water deficit. This suggests that photoinhibition only occurs after a long–term exposure to 35^{o} C in conjunction with water deficit, once plants of *E. globulus* grown at 35^{o} C Short–term under drought showed no signs of photochemical damage. Thus, the absence of a substantial reduction in the efficiency of photosystem II indicates a considerable stability of this machinery in *E. globulus*, mainly in short–term exposure to moderately

high temperatures. Moreover, the short–term exposure to 35^{o} C apparently induced a protective mechanism in plants under water deficit, since it was observed a decrease in F_0 while F_M was practically unchanged, and F_V/F_M and F_V/F_0 increased slightly under MD when compared do FI (Table 1).

In contrast to F_V/F_M, ΦPSII and qP indicated that *E. globulus* was more sensitive to water deficit than to heat stress since the courses of both parameters were lower in plants under water deficit, while remaining practically unchanged with the temperature increase in well–watered plants (Figure 2). Interestingly, we observed that water deficit had a more pronounced effect in chlorophyll fluorescence parameters measured in light rather than in dark–adapted leaves, which showed more sensitivity to temperature increase. Possibly, this difference reflects the fact that dark–adapted leaves have closed stomata implying in less means to dissipate energy and probably resulting in greater temperature sensitivity. This could superimpose the effect of water deficit, which apparently acts mainly through stomatal closure.

The suppression of NPQ due to temperature stress, observed between 25^{o} C FI and 35^{o} C FI (Figure 2), has been previously reported in barley by KALITUHO et al. (2003). NPQ increase under water deficit, observed in all thermal treatments but significantly less in both 35^{o} C treatments, has been associated with a photoprotective role by avoiding excessive energy at the photosystem (OSMOND, 1994). Thus, apparently the short–term exposure to 35^{o} C did not cause significant damage since there was no reduction in NPQ, whereas the response to water deficit could have been compromise by both short and long–term treatments. Furthermore, the indications of photoinhibition at 35^{o} C under MD and SD conditions may be related with limitations in the NPQ, since damage to PSII is attenuated by NPQ or locally by cyclic electron transport (RUMEAU et al., 2007). Variations in ETR in all irrigation and thermal treatments followed similar trends to A_N, probably due to the fact that Rubisco activity is highly correlated with ETR (KRALL et al., 1995). Previous studies showed that heat shock alters the photosynthetic activity via suppression of chloroplast electron transport (PASTENES & HORTON, 1996; FELLER et al., 1998), which is compatible with the ETR reduction observed at 35^{o} C, possibly also related with an increase in the Rubisco oxygenase activity leading to higher photorespiration. However, at 35^{o} C Short–term, there was no decrease in ETR under FI while there

were higher values and a lower reduction of ETR under MD when compared with other thermal treatments, suggesting an increase in tolerance to water deficit.

As proposed by hypothesis *i*, which states that *E. globulus* show different responses to short and long–term exposure to 35^{o} C, the chlorophyll fluorescence parameters indicated that the photochemical apparatus was differently affected by the time of exposure to 35^{o} C. Whereas the long–term exposition compromised more the photochemical functions, the short–term treatment even had a potential protective effect, ensuring the photochemical efficiency and increasing the tolerance to water deficit. Hypothesis *ii*, stating the effect of both stresses combined will not be simply the sum of each condition applied separately, was also supported since the combined effect of temperature and water deficit revealed to be synergistic in some cases, especially through chlorophyll fluorescence parameters measured in dark–adapted leaves at 35^{o} C, and even enhancing tolerance mechanisms, as indicated by the 35^{o} C Short–term treatment.

Effects on carbon assimilation

The effects of water deficit on photosynthesis were widely described by several authors (CHAVES, 1991; CHAVES et al., 2003; CORNIC, 2000, FLEXAS et al., 2004; CHAVES et al., 2009; LAWLOR & TEZARA, 2009) and there is a general consensus that the decrease in the photosynthetic rate is caused primarily by stomatal closure, which limits the availability of CO_2 within the leaf. As expected, we verified a substantial decrease in g_s in plants under water deficit, in all thermal treatments studied, with a proportional decrease in C_i (Figure 3, 4). Interestingly, a short–term exposure to 35^{o} C did not cause a substantial stomatal closure under FI, and furthermore resulted in a lower reduction in g_s in response to water deficit, allowing the maintenance of high A_N rates compared to 25^{o} C and 35^{o} C. Possibly, the short–term exposure to 35^{o} C induces a protective mechanism which allows the maintenance of a high g_s, increasing the tolerance to water deficit.

Moderately high temperatures increased stomatal limitation (L_s) both under FI and water deficit (Table 2). Moreover, long term exposure to 35 ^{o} C induced a

substantial decrease in g_s in well–watered plants, probably causing the observed reduction in A_N and C_i (Figure 3). Such decrease in A_N under FI at 35^o C indicates a down–acclimation of well–watered plants when exposed to moderately high temperatures for a long time. Moderate heat stress reduces A_N and g_s in many plant species due to decreases in the activation state of Rubisco (CRAFTS–BRANDNER & SALVUCCI, 2002), once Rubisco activase is thermolabile (FELLER et al., 1998; SALVUCCI et al., 2001). The heat–induced deactivation of Rubisco is the primary constraint on photosynthesis at moderately high temperatures, showing that chlorophyll fluorescence signals from PSII are not affected by the same temperatures (CRAFTS–BRANDNER & SALVUCCI, 2000; HALDIMANN & FELLER, 2004) and injuries or death may occur only after long–term exposure. Accordingly, we verified that the thermal treatments caused more pronounced effects in the carbon assimilation than in the photochemical apparatus, and injuries occurred mainly after a long–term exposure to high temperature (Figure 2, 3).

The reduction in g_m is another component that may influence carbon availability to photosynthesis under stress conditions, being regulated almost simultaneously to g_s (FLEXAS et al., 2008). LAISK et al. (1998) proposed that the decrease in A_N in response to temperature might be partially related with g_m, while several authors reported g_m reduction under water stress (SCARTAZZA et al., 1998; FLEXAS et al., 2002, 2008; GALMÉS et al., 2007). Accordingly, we also observed a decrease in g_m in response to water deficit and temperature increase, possibly underlying the decrease in A_N (Figure 4). However, the short–term exposure to 35^o C induced the maintenance of a high g_m under water deficit, which probably enabled a high C_c even with the low g_s thus contributing to the high A_N under MD (Figure 4). Jointly, the maintenance of high V_{cmax} and J_{max} rates could also have contributed to the high A_N rates under MD at 35^o C Short–term, since under long–term exposure to 35^o C there was a more pronounced decrease in V_{cmax} and J_{max} under drought. Previous studies have found that the J_{max}:V_{cmax} ratio tends to decrease with increasing growth temperature (BUNCE, 2000; KATTGE & KNORR, 2007). However, the short-term exposure to 35^o C slightly increased this ratio, showing the highest J_{max}:V_{cmax} under MD, also contributing to the maintenance of a high A_N under water deficit (Table 1).

The increase in R_d is another factor that contributed to the decrease in net CO_2 assimilation rate (Table 2). WAHID et al. (2007) reported that elevated temperature causes a heat–induced imbalance in photosynthesis and respiration since, in general, photosynthetic rate decreases while dark and photo–respiration increase considerably under high temperatures. Correspondingly, we observed a substantial increase in R_d in short and long–term exposure to 35^o C under FI, while plants under water deficit showed a decrease in R_d in all thermal treatments. This response may save energy under stress conditions. However, higher R_d did not correlate directly with photosynthetic performance given that plants at 35^o C Short–term showed the highest R_d rate in both FI and MD, but maintained equal or higher A_N than the remaining thermal treatments under all irrigation conditions. Decreases in R_d with water deficit observed at 25^o C and 35^o C did not lower the LCP, probably because the apparent quantum efficiency (α) had a significant decreasing trend with drought severity. This was not the case at 35^o C Short–term which showed the lowest reduction in α and a decrease in LCP under water deficit, having significantly higher α than the remaining thermal treatments under MD. In fact, although $A_{max\ light}$ decreased with water deficit in all thermal treatments, the lowest reduction occurred at 35^o C Short–term which presented the highest maximum assimilation rate under MD, being a further indicative of the increase in tolerance to water deficit.

Carbon assimilation evaluated through gas–exchange parameters showed that plants respond differently to water deficit according to the thermal treatment. A general response to drought is the reduction in g_s and g_m, which implies in a reduction of CO_2 diffusion in the mesophyll consequently decreasing the photosynthetic activity, as evidenced by decreases in α and A_{max} (Table 2). Moreover, V_{cmax} and J_{max} also decreased substantially with water deficit, mainly under long–term exposure to 35^o C. The combination of water deficit and a long–term exposure to 35^o C caused a down–acclimation and had the most negative effect in photosynthesis, while a short–term exposure to 35^o C implied in a less compromised photosynthetic apparatus having, a higher performance under drought even when compared to the control 25^o C condition, supporting hypothesis *i* and *ii*. Possibly, the main factor accounting for the higher photosynthetic efficiency of plants exposed to 35^o C Short–term under water deficit is the maintenance of a relatively high stomatal and mesophyll conductance, showing an especially lower reduction in g_m in response

to drought (Figure 4). Since the response to a combination of heat and drought differed from the superposition of individual responses, as observed mainly at 35^{o} C, the hypothesis *ii* was also confirmed by our results.

The combination of PC1, capturing 49.3% of the variance contained in the data, with PC2, 16.8%, yielded a considerably coherent clusters of the replicates according to irrigation and thermal treatments (Figure 5). There was a clear gradient of irrigation treatments along the main axis of variation, indicating a consistent overall effect of water deficit in the global change of physiological variables. Conversely, PC2 mainly accounted for the separation among thermal treatments, which are less discernible along PC1. This is in general agreement with the statistical results obtained with the Tukey's test, since the main parameters composing PC1 (α, A_N, $A_{max\ light}$, L_s and J_{max}) differed more with respect to irrigation, whereas the parameters composing PC2 (LSP, LCP, F_V/F_0, F_V/F_M and R_d) differed more according to thermal treatment.

CONCLUSIONS

The co–occurrence of water deficit and moderately high temperature in *E. globulus* amplified the reduction of photosynthetic rates, compared to the single effects, mainly after a long–term exposition to 35^{o} C. This effect was less pronounced in the exposure to 35^{o} C Short–term, which apparently increased the tolerance to water deficit given the observed highest overall photosynthetic efficiency under drought. When compared to other thermal treatments under water deficit, the short–term exposure to 35^{o} C induced higher A_N, $A_{max\ light}$, α, ETR, V_{cmax}, J_{max}, g_m, g_s and C_i, lower F_0 and LCP, lower reduction in ΦPSII and qP and no reduction in F_M, F_V/F_M and F_V/F_0, suggesting an enhancement of photosynthetic capacity under water deficit, possibly through a cross–tolerance mechanism.

Hypothesis *i* was supported by the results presented herein since the effects of short and long–term exposure to 35^{o} C differed, being a longer exposure generally more injurious to the photosynthetic efficiency. Furthermore, the short–term exposure to 35^{o} C showed a fortunate protective effect under water deficit, apparently as a

result of maintaining a high stomatal and mesophyll conductance. Hypothesis *ii* was also supported by the results since water deficit and moderately high temperature had a singular effect when applied in combination, distinct from the mere sum of their individual effects. This is in accordance to MITTLER (2006) who proposes that a particular stress combination should be regarded as a singular state of abiotic stress requiring a new defense or acclimation response.

Our results allowed to evaluate the individual and combined effects of water deficit and different times of exposure to a moderately high temperature, which is of crucial importance given that both stresses often co–occur in the field. This is particularly relevant given the lack of knowledge regarding physiological responses to combined stress types, especially in *Eucalyptus*, and the imminent global climate change which will likely increase the incidence of drought and moderately high temperature. Thus, the present study showed that the acclimation to a long–term exposure to 35^{o} C impose some restrictions to photosynthetic activity mainly under water deficit, while a short–term exposure enhances the photosynthetic efficiency under water deficit, conferring some degree of tolerance. Beyond contributing to the understanding of *E. globulus* physiological responses to realistic stress conditions, we hope to assert the importance of evaluating the effects of stress combinations which can reveal non expected responses, while potentially providing insights into underlying physiological mechanisms involved in acclimation.

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CONSIDERAÇÕES FINAIS

Além de aspectos ecofisiológicos e da aclimatação do aparato fotossintético, o fator comum entre os estudos aqui apresentados é o papel da plasticidade fenotípica em diversas condições ambientais. Plasticidade fenotípica pode ser definida como a capacidade de um determinado genótipo expressar fenótipos variados em resposta às variações do ambiente. Adjacente a esse conceito, está o fato de que a habilidade de um organismo responder às condições do seu entorno é crítica para o seu estabelecimento e manutenção, especialmente em ambientes heterogêneos. Dessa forma, a ocorrência de uma espécie vegetal em nichos ecológicos distintos pode representar a sua capacidade de expressar plasticidade fenotípica, a qual pode permitir o sucesso reprodutivo sob condições que seriam consideradas adversas em outra circunstância. Sendo assim, a plasticidade fenotípica é especialmente importante em ambientes com alta heterogeneidade, pois possibilita a ocorrência de indivíduos da mesma espécie em ambientes distintos.

A aclimatação é considerada um caso especial de plasticidade, uma vez que envolve a adaptação morfo-fisiológica de um fenótipo durante seu ciclo de vida, diferentemente da adaptação evolutiva. A aclimatação possibilita a ampliação dos limites fisiológicos da planta, através da mudança de um estado estável para um novo estado estável em resposta a uma determinada variação ambiental. Assim, tal modulação metabólica permite que o organismo atinja um novo nível de estabilidade fisiológica sob uma determinada condição, restabelecendo a homeostase. Contudo, foi verificado no presente estudo que esse novo nível de estabilidade não faz com que a planta apresente, necessariamente, um rendimento fotossintético maior, no entanto ele possibilita a manutenção do desenvolvimento do indivíduo sob condições limitantes. Um exemplo é a diminuição da taxa de assimilação de CO_2 em plantas de *Eucalyptus globulus* aclimatadas a 35º C, comparado a plantas nas mesmas condições a 25º C, o que foi caracterizada como *down-acclimation*. Sendo assim, uma planta pode estar aclimatada a uma determinada condição, mas apresentar taxas fotossintéticas comparativamente menores nessa nova condição, indicando o envolvimento de outros parâmetros nesse processo.

Como suposto inicialmente, o conjunto dos resultados obtidos nos quatro experimentos permitiu verificar que as respostas fotossintéticas foram

consideravelmente afetadas pelo ambiente de crescimento das plantas. As análises realizadas nos experimentos com espécies nativas (capítulos 1, 2 e 3) mostraram que as espécies estudadas responderam de forma diferenciada à disponibilidade de recursos no ambiente, possuindo capacidades distintas de aclimatação a ambientes considerados contrastantes àqueles onde geralmente ocorrem. Porém, essa diferença entre espécies não está essencialmente relacionada ao seu grupo ecológico, indicando que a classificação de espécies tropicais em grupos ecológicos, feita segundo sua tolerância ao sombreamento, pode ser considerada uma medida muito subjetiva uma vez que a tolerância à sombra não é um critério indicativo da capacidade de aclimatação de uma espécie. A adoção de critérios quantitativos, obtidos através da conjugação de estudos ecofisiológicos e fitossociológicos, pode ser mais efetiva na classificação das espécies, principalmente em ambientes tropicais com alta heterogeneidade luminosa. A integração de ferramentas ecofisiológicas e fitossociológicas pode ser uma interessante proposta interdisciplinar, além de consistir em uma ferramenta potencialmente robusta para a caracterização de ambientes naturais e para a investigação do significado ecológico–evolutivo da interação planta–ambiente.

Uma proposta de classificação, que emergiu após a realização da presente tese, é a criação de um índice multivariado de aclimatação que auxiliaria na determinação da capacidade de aclimatação relativa de uma dada espécie, baseado no nível de variação dos parâmetros avaliados entre ambientes, conforme realizado no capítulo 1. Esse índice poderia ser utilizado como uma ferramenta complementar à classificação ecológica das espécies, auxiliando na medida do potencial de aclimatação a diferentes condições ambientais. Outros índices que expressam quantitativamente o potencial de aclimatação de uma espécie foram propostos anteriormente, como o coeficiente de adaptação à sombra (SAC) criado por Laisk et al. (2005) e o índice de plasticidade fenotípica proposto por Valladares et al. (2000a, b), cuja aplicação é mais indicada para estudos comparativos de espécies e ambientes. A utilização de índices que expressam quantitativamente a capacidade de aclimatação de diferentes espécies requer uma interpretação cautelosa de seus resultados, sendo de fundamental importância a consideração do significado biológico dos parâmetros avaliados e inseridos na análise, bem como seu padrão de variação nas condições analisadas. Além disso, outro fator a ser considerado é a

importância de variáveis que não foram contempladas na análise. Aspectos como a variabilidade funcional entre folhas e a estratégia de alocação de recursos, podem ser críticos para o estabelecimento de uma espécie num determinado ambiente, não sendo contemplados ao abordar apenas no metabolismo fotossintético.

A utilização do índice multivariado de aclimatação é indicada para estudos comparativos, uma vez que depende dos dados em análise, contudo sua utilização em estudos de larga escala pode ser uma ferramenta complementar na caracterização do perfil ecofisiológico de diferentes espécies florestais. De forma geral, o emprego de índices na caracterização fisiológica de diferentes espécies pode resultar em uma interpretação mais objetiva a cerca da sua ocorrência em diferentes nichos ecológicos. Entretanto, é necessário conjugar várias dimensões da ecofisiologia e da fitossociologia, uma vez que muitos fatores ecológicos influenciam na ocorrência de uma determinada espécie em um dado ambiente, por exemplo, a presença de agentes dispersores, o padrão de alocação de recursos que a espécie apresenta, entre outros fatores. Assim, a utilização dos referidos índices *per se* não leva ao entendimento do padrão de ocorrência de uma espécie, mas contribui para uma visão mais objetiva e integrada do seu perfil ecofisiológico.

Outro aspecto abordado no presente estudo, que por sua vez está relacionado com a proposta descrita acima, é o indício de que muitas espécies tropicais podem apresentar requerimento de luz intermediário, uma vez que as espécies não se mostraram ser estritamente de sol ou de sombra. Deste modo, apesar de o presente trabalho ter contemplado o estudo de um número reduzido de espécies, foram verificados indícios, principalmente no capítulo 1, de que as espécies tropicais podem apresentar demanda luminosa intermediária, como descrito anteriormente por WRIGHT et al. (2003).

Tanto em experimentos com espécies nativas como com cultivadas, foi possível verificar que a aclimatação ocorre em diferentes níveis, os quais operam em escalas de tempo diferentes, como descrito por WALTERS (2005). A aclimatação do aparato fotossintético pode ser considerada rápida, supostamente pelo efeito direto do ambiente sobre o seu funcionamento, enquanto alterações estruturais certamente demandam mais tempo para sua detecção. Foi observado também que as espécies diferem claramente em seu potencial de aclimatação, refletindo quão conservativo ou flexível é o metabolismo fotossintético da espécie. Enquanto algumas espécies

são capazes de se aclimatar mais facilmente a uma determinada condição, outras espécies não apresentam a mesma habilidade que, apesar de estar relacionado com a estratégia ecológica das espécies, parece ser independente do seu grupo sucessional. Dessa forma, foi observado que a autonomia das espécies, em relação ao seu ambiente de crescimento, varia significativamente, enquanto algumas espécies ajustam o seu rendimento energético de acordo com a oferta de recursos, outras mantêm o metabolismo mais estável independente das alterações na disponibilidade de recursos do ambiente, como também reportado por SOUZA et al. (2009). Um exemplo são as diferenças na taxa de assimilação máxima de CO_2 observadas entre as espécies crescidas em ambientes com disponibilidade luminosa distintas, conforme apresentado no capítulo 1. Se uma espécie não altera sua taxa fotossintética em um ambiente, cuja disponibilidade de recursos é substancialmente maior, isso pode indicar uma limitação fisiológica em utilizar recursos adicionais, ao passo que o ajuste metabólico visando explorar ao máximo os recursos pode refletir a elevada flexibilidade fisiológica da espécie. Tais observações podem estar relacionadas com estratégias mais conservativas ou mais flexíveis, o que por sua vez indica a estratégia ecológica da espécie, conforme descrito acima.

A utilização de recursos pelas plantas é severamente afetada por condições ambientais desfavoráveis ao desenvolvimento vegetal, contudo foi observado no presente trabalho que as diferentes espécies estudadas apresentam estratégias e mecanismos distintos de atenuação desses efeitos, as quais variam de acordo com a sua habilidade de aclimatação e de re-estabelecimento da homeostase. A aclimatação do aparato fotossintético é uma das etapas iniciais desse processo e atua conforme a estratégia ecológica e fisiológica das espécies, além de eventualmente alterar a tolerância das plantas a condições adversas. Conforme descrito, as restrições impostas pelo ambiente obrigam as plantas a transitarem entre diferentes estados fisiológicos, sendo que essa transição é representada pelo processo de aclimatação da planta. Assim, o modo no qual essa transição ocorre reflete o potencial de aclimatação da espécie. Os diversos ajustes no aparato fotossintético, descritos no presente trabalho em diferentes espécies e condições ambientais, demonstraram a importância da aclimatação fotossintética no ajuste fisiológico da planta a uma nova condição do ambiente. Assim sendo, a aclimatação

fotossintética é um processo metabólico crucial para o restabelecimento da homeostase da planta, após uma determinada variação do ambiente.

• Perspectivas Futuras

A interpretação dos resultados obtidos no presente trabalho, bem como a extrapolação dos efeitos observados para outros níveis e contextos, possibilitaram o surgimento de novas idéias e possibilidades de investigação. O amplo campo de estudo com espécies cultivadas e nativas, aliada à necessidade de uma melhor compreensão ecofisiológica dos ecossistemas brasileiros, deve ser considerada como uma fonte potencial de novas abordagens ecofisiológicas.

Dentre as novas possibilidades de estudo que o presente trabalho revelou, destacam-se:

- A realização de estudos ecofisiológicos com um número elevado de espécies e em diversos ambientes florestais pode contribuir para o melhor entendimento da demanda luminosa das espécies e do papel ecológico da luz nos ecossistemas brasileiros. A medida e análise de numerosas variáveis ecofisiológicas podem permitir a realização de estudos mais integrados sobre a influência do ambiente luminoso nas espécies vegetais, constituindo uma importante ferramenta para a adoção de estratégias de regeneração e conservação, já que pouco é conhecido a cerca da dinâmica luminosa de ambientes florestais. Além disso, essa abordagem pode permitir a avaliação da influência da plasticidade fenotípica na interação entre as espécies vegetais, o que permitiria inferências ecológicas e evolutivas ainda muito carentes para os ambientais florestais brasileiros;

- A realização de estudos em espécies filogeneticamente relacionadas, possibilitando avaliar a distribuição de diferentes perfis ecofisiológicos entre táxons;

- A execução de experimentos que envolvam medidas de variáveis representativas das diferentes escalas espaço-temporais onde a aclimatação a diferentes ambientes ocorre;

- A avaliação da contribuição efetiva dos *sunflecks* para o ganho de carbono e manutenção de espécies em ambientes de sub-bosque com diferentes densidades de dossel, a fim de verificar a dinâmica luminosa gerada pela cobertura vegetal e o papel ecológico dos *sunflecks* nos ambientes florestais brasileiros, a qual já é muito bem caracterizada em outros ecossistemas;

- A investigação de mecanismos de facilitação e competição entre espécies tropicais que ocorrem no mesmo ambiente, ou que tenham algum outro tipo de interação, além da influência de condições ambientais adversas na intensidade dessas relações entre espécies vegetais;

- A estimativa da contribuição do tempo de vida da folha na capacidade de aclimatação da planta, com o objetivo de avaliar se espécies com tempo de vida foliar mais curto podem se aclimatar mais rapidamente pela substituição das suas folhas;

- A avaliação da influência da temperatura e deficiência hídrica por um período prolongado nos parâmetros de produtividade em E. globulus, que podem informar de uma maneira mais aplicada sobre os efeitos dessas condições na produtividade dessa espécie. Apesar das especulações a cerca dos efeitos das mudanças climáticas na produtividade de espécies florestais de interesse econômico, são necessários estudos adicionais que priorizem a relação dos efeitos fisiológicos ao crescimento e produção de tais espécies em longo prazo;

- A procura de fatores ambientais que ativem mecanismos de tolerância cruzada em espécies cultivadas de interesse econômico, a fim de investigar o aumento da tolerância das plantas a um determinado fator ambiental em decorrência de outro;

- A investigação de mecanismos que podem levar à tolerância cruzada entre temperatura elevada e deficiência hídrica em *E. globulus*, como o aumento da condutância do mesófilo, expressão de proteínas de choque de calor e alterações na cinética enzimática de proteínas de membrana.

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