

Induction of photosynthesis and importance of limitations during the induction phase in sun and shade leaves of five ecologically contrasting tree species from the temperate zone

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Summary We examined the principal differences in photosynthetic characteristics between sun and shade foliage and determined the relative importance of biochemical and stomatal limitations during photosynthetic induction. Temperate-zone broadleaf and conifer tree species, ranging widely in shade tolerance, were investigated from one locality in the Czech Republic. The study species included strongly shade-tolerant *Abies alba* Mill. and *Tilia cordata* Mill., less shade-tolerant *Fagus sylvatica* L. and *Acer pseudoplatanus* L. and sun-demanding *Picea abies* (L.) Karst.

In the fully activated photosynthetic state, sun foliage of all species had significantly higher maximum CO₂ assimilation rates, maximum stomatal conductance and maximum rates of carboxylation than shade foliage. Compared with shade leaves, sun leaves had significantly higher nocturnal stomatal conductances. In all species, shade foliage tended to have higher induction states 60 s after leaf illumination than sun foliage. Sun and shade foliage did not differ in the rate of disappearance of the transient biochemical limitation during the induction phase. Longer time periods were required to reach 90% photosynthetic induction and 90% stomatal induction in sun foliage than in shade foliage of the less shade-tolerant *F. sylvatica* and *A. pseudoplatanus* and in sun-demanding *P. abies*; however, in sun foliage of the strongly shade-tolerant species *T. cordata* and *A. alba*, the time needed for photosynthetic induction was similar to, or less than, that for shade foliage. Shade but not sun needles of *P. abies* and *A. alba* had significantly slower induction kinetics than the broadleaf tree species. Among species, the sun-demanding *P. abies* exhibited the shortest stomatal induction times in both sun and shade leaves. Independently of shade tolerance ranking, the transient stomatal and total limitations that characterize photosynthetic induction were relieved significantly earlier in shade foliage than in sun foliage. Sun foliage generally exhibited a hyperbolic photosynthetic induction response, whereas a sigmoidal induction response was more frequent in shade foliage. The different relative proportions of transient biochemical and

stomatal limitations during photosynthetic induction in sun and shade foliage indicate an essential role of stomata in photosynthetic limitation during induction, mainly in shade foliage, with a consequent influence on the shape of the photosynthetic induction curve.

Keywords: dynamic light environment, gas exchange, photosynthetic limitations, sun/shade acclimation.

Introduction

Under natural conditions, most leaves are exposed to continually changing solar irradiances caused by variable cloud cover or self-shading of leaves in the crown periphery and leaf movement due to turbulence. Light undergoing short-term fluctuations in irradiance accounts for over 70% of the daily illumination in forest understories (Chazdon 1988), and represents the major energy source for understory vegetation and leaves in the inner tree crown (Valladares et al. 1997, Schulte et al. 2003). Effective utilization of fluctuating irradiance requires fast photosynthetic induction after leaf illumination (Pearcy 1990, Pearcy et al. 1994).

In trees, sun leaves, i.e., leaves that have developed in high incident irradiances, possess much higher photosynthetic rates than shade leaves (Boardman 1977, Lichtenthaler 1981, Lichtenthaler and Babani 2004), yet most of the leaves in forest canopies, in particular canopies with a high leaf area index, are in deep shade (Urban et al. 2007). Therefore, effective utilization of a dynamic light environment, especially in the lower canopy, may account for the largest proportion of daily carbon gain in forest ecosystems (Pearcy 1990, Urban et al. 2007).

Three phases of photosynthetic induction can be distinguished. (1) During the first 1–2 minutes of leaf exposure to high irradiance, activities of enzymes involved in the regeneration of the primary CO₂ acceptor ribulose-1,5-bisphosphate (RuBP) increase (Kirschbaum and Pearcy 1988a, Pearcy et al.

1994). The limitation is thought to be caused by the rapid down-regulation of fructose-1,6-bisphosphate (FBPase) and possibly other enzymes involved in RuBP regeneration at low irradiances (Sassenrath-Cole and Pearcy 1992, Martin et al. 2000). (2) Incomplete activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), catalyzing the primary carboxylation reaction, is considered the key biochemical limitation during most of the induction period (Woodrow and Mott 1989, Mott and Woodrow 2000). Rubisco activates exponentially over about 10 minutes (Woodrow and Mott 1989). (3) Stomatal opening is the slowest step in the photosynthetic induction process, and reaching full induction can take over an hour (Kirschbaum et al. 1998, Tinoco-Ojanguren and Pearcy 1993, Allen and Pearcy 2000a). Stomata may also impose a secondary limitation on induction by slowing the rate of Rubisco activation via a low intercellular CO_2 concentration (Valladares et al. 1997, Allen and Pearcy 2000b).

Because the carbon gain of shade leaves and shade-acclimated plants is more dependent on continually changing solar irradiance, they are expected to have faster photosynthetic induction kinetics than sun leaves and sun-acclimated plants (Percy et al. 1994, Valladares et al. 1997). However, evidence for this hypothesis in the literature is contradictory. More rapid photosynthetic induction was described in shade plants of *Piper auritum* Kunth. (Tinoco-Ojanguren and Pearcy 1993) and shade leaves of *Fagus sylvatica* L. (Küppers and Schneider 1993) compared with sun plants and sun leaves, respectively. However, other studies found no consistent differences between species grown in forest gaps and understories (Poorter and Oberbauer 1993, Naumburg and Ellsworth 2000, Rijkers et al. 2000). Entirely contrary to this hypothesis, Tausz et al. (2005) reported significantly faster photosynthetic induction in the upper canopy compared with the low-level copice of *Nothofagus cunninghamii* (Hook.) Oerst.

These inconsistencies reflect, in part, differences in experimental design. Photosynthetic induction kinetics are influenced by: (1) the plant's acclimation to the growth environment, e.g., forest understory, forest gap or open area, which results in biochemical and anatomical changes in leaves (Cao and Booth 2001, Lichtenthaler and Babani 2004); (2) the previous light history of the plant (Han et al. 1999, Cai et al. 2005), which affects the rate of photosynthetic enzyme activation; and (3) ecological factors, such as temperature (Küppers and Schneider 1993) and leaf water status (Tinoco-Ojanguren and Pearcy 1993, Allen and Pearcy 2000a).

In the present study, we compared the photosynthetic induction in sun and shade leaves of five ecologically contrasting temperate-zone tree species: the deciduous *Fagus sylvatica*, *Acer pseudoplatanus* L. and *Tilia cordata* Mill. and the coniferous *Picea abies* (L.) Karst. and *Abies alba* Mill. These species differ in shade tolerance but grow in the same forest. All measurements were made under similar experimental conditions. Our objectives were to: (1) examine the effects of light acclimation on photosynthetic induction kinetics; (2) identify the transient dynamic biochemical and stomatal limitations during induction; (3) assess possible relationships between in-

duction kinetics and species' shade tolerance; and (4) determine if foliage of conifers responds in a similar way to that of broad-leaved trees.

Material and methods

Site description and plant material

Measurements were conducted in a natural stand of trees in a forest located in the Moravian-Silesian Beskydy Mountains (Bílý Kříž; 49°30' N, 18°32' E, 908 m a.s.l.) in the Czech Republic. The climate of the area is characterized by a mean annual temperature of 5.5 °C, a mean annual relative air humidity of 80% and total rainfall of 1400 mm (means of last 10 years). The geological bedrock is formed by Mesozoic Godula sandstone (flysch type) and is overlain by ferric podzols.

Physiological measurements were performed in mid-July, 2005 on fully developed sun and shade foliage of 20–40-year-old trees of European beech (*Fagus sylvatica*), maple (*Acer pseudoplatanus*), linden (*Tilia cordata*), white fir (*Abies alba*) and Norway spruce (*Picea abies*). The species differ in shade tolerance (Úradniček et al. 2001). *Abies alba* and *T. cordata* are strongly shade-tolerant, *F. sylvatica* and *A. pseudoplatanus* are intermediate in shade-tolerance and *P. abies* is sun-demanding.

Long-term measurements of photosynthetic photon flux (PPF) within the experimental stand, which has a hemi-surface leaf area index, i.e., half of the total needle surface area per unit ground surface area, of about 11, show a strong differentiation between sun and shade canopy spaces (Pokorný and Marek 2000). Shade foliage in the inner tree crown received up to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF on sunny days, whereas sun foliage was exposed to a maximum of 1200–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Branches with the desired sun or shade leaves were cut from the trees. The cut end of each branch was immediately recut under water to remove xylem embolisms and kept in the water during the measurements. The branches were taken from healthy trees that showed no signs of drought or photobleaching that may be experienced on hot dry summer days. Under the experimental conditions, there were no significant differences between attached and cut branches in CO_2 assimilation rate (A) or stomatal conductance (g_s).

Gas-exchange measurements

Fully dark-adapted (at least after 3 h) foliage was measured at night. The foliage tested was exposed to constant saturating irradiance (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) from the LED light source of a LI-6400-02B (Li-Cor, Lincoln, NE). The time courses of actual photosynthetic characteristics, i.e., A , g_s and intercellular CO_2 concentration (C_i), were automatically recorded (at 10-s intervals) with a Li-Cor LI-6400, open gas exchange system. The foliage was kept inside the assimilation chamber at a constant ambient CO_2 concentration ($375 \pm 5 \mu\text{mol mol}^{-1}$), air humidity ($60 \pm 5\%$) and leaf temperature ($20 \pm 1^\circ\text{C}$) throughout all measurements. Air flow rate through the assimilation chamber was maintained at 500 $\mu\text{mol s}^{-1}$.

From the photosynthetic induction curves, we calculated the time to reach 90% of full induction (T_{90}) and the photosynthetic CO_2 assimilation rate after a 60-s exposure to saturating irradiance as a percentage of the leaf's maximum A (IS_{60}) (Chazdon and Pearcy 1986, Valladares et al. 1997, Schulte et al. 2003).

After full photosynthetic induction (about 1 h), the initial part of the A/C_i response curve was produced starting at an ambient CO_2 concentration of $375 \mu\text{mol mol}^{-1}$ and decreasing stepwise to $50 \mu\text{mol mol}^{-1}$. The values of the A/C_i response curves were used to calculate the maximum rate of in vivo Rubisco carboxylation (V_{cmax}) and the CO_2 compensation concentration in the absence of photorespiration (Γ^*) using the equations of Farquhar et al. (1980).

Model of induction limitations

The model proposed by Woodrow and Mott (1989) was used to separate the transient dynamic biochemical and stomatal limitations that disappeared during photosynthetic induction. In this model, stomatal limitations to photosynthesis are removed by recalculating the photosynthetic rate to a constant C_i . The rate of CO_2 assimilation without stomatal limitation (A^*) was calculated as:

$$A^* = \frac{(A + R_d)(C_{\text{if}} - \Gamma^*)}{C_i - \Gamma^*} - R_d \quad (1)$$

where C_{if} is the final C_i (C_i at the end of the induction period) and R_d is the dark respiration rate. Subsequently, we calculated the transient dynamic stomatal (LS) and biochemical (LB) limitations that disappeared during the photosynthetic induction phase as:

$$\text{LS} = \frac{A^* - A}{A_{\text{max}} + R_d} \quad (2)$$

$$\text{LB} = \frac{A_{\text{max}} - A^*}{A_{\text{max}} + R_d} \quad (3)$$

where A_{max} is the maximum CO_2 assimilation rate at the end of the induction period. Total transient dynamic limitation (LT) during photosynthetic induction was calculated as $\text{LS} + \text{LB}$.

Statistical analysis

An LSD test, following a one-way analysis of variance, was performed to evaluate the significant differences between sun and shade leaves. Differences were tested at probability levels 0.05 and 0.01.

Results

Photosynthetic and respiration parameters of sun and shade foliage

Photosynthetic parameters of sun and shade foliage revealed typical photosynthetic adaptations to the light environment, with no apparent differences between foliage of broadleaf trees and conifers (Table 1). Across species, A_{max} was significantly ($P < 0.01$) higher in sun foliage (1.6-fold in *T. cordata* and up to 5-fold in *P. abies*) than in shade foliage (Table 1). Independently of absolute A_{max} values, we found a gradient of difference in A_{max} between sun and shade foliage that followed the gradient in shade tolerance of the species. Thus, relative to that of shade foliage, A_{max} of sun foliage was lower in the strongly shade-tolerant species *T. cordata* (1.6-fold) and *A. alba* (2.2-fold) than in the less shade-tolerant *F. sylvatica* (2.9-fold) and *A. pseudoplatanus* (4.3-fold), and highest in the sun-demanding *P. abies* (5.0-fold).

The higher photosynthetic rates in sun foliage were associated with significantly ($P < 0.01$) higher g_{smax} compared with shade foliage (0.0–0.28 versus 0.03–0.09 $\text{mol m}^{-2} \text{s}^{-1}$). Also,

Table 1. Summary of photosynthetic parameters (mean \pm standard deviation; $n = 12$) of sun and shade foliage estimated under steady-state conditions. Abbreviations: A_{max} , maximum CO_2 assimilation rate estimated at the end of photosynthetic induction; R_d , dark respiration rate (nocturnal); C_{ii} , initial (nocturnal) intercellular CO_2 concentration estimated before photosynthetic induction; C_{if} , final intercellular CO_2 concentration estimated after photosynthetic induction; g_{smax} , maximum stomatal conductance after photosynthetic induction; and V_{cmax} , maximum rate of in vivo Rubisco carboxylation.

Species		A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	C_{ii} ($\mu\text{mol mol}^{-1}$)	C_{if} ($\mu\text{mol mol}^{-1}$)	g_{smax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
<i>Acer pseudoplatanus</i>	Sun	9.5 ± 0.3	1.7 ± 0.2	505 ± 27	209 ± 6	0.10 ± 0.02	77 ± 5.4
	Shade	2.2 ± 0.2	0.6 ± 0.1	536 ± 17	219 ± 15	0.03 ± 0.01	15 ± 4.3
<i>Fagus sylvatica</i>	Sun	12.6 ± 0.2	1.3 ± 0.2	408 ± 8	260 ± 2	0.23 ± 0.02	60 ± 2.1
	Shade	4.3 ± 0.3	0.4 ± 0.1	511 ± 19	269 ± 5	0.07 ± 0.01	35 ± 1.1
<i>Tilia cordata</i>	Sun	12.2 ± 0.3	1.3 ± 0.2	404 ± 3	251 ± 2	0.18 ± 0.03	63 ± 6.1
	Shade	4.7 ± 0.3	0.5 ± 0.2	601 ± 15	264 ± 3	0.09 ± 0.01	31 ± 7.3
<i>Abies alba</i>	Sun	7.5 ± 0.3	0.6 ± 0.2	411 ± 5	119 ± 4	0.28 ± 0.04	40 ± 4.5
	Shade	3.7 ± 0.3	0.2 ± 0.03	470 ± 18	180 ± 29	0.04 ± 0.01	18 ± 2.1
<i>Picea abies</i>	Sun	10.4 ± 0.3	1.1 ± 0.1	408 ± 12	175 ± 3	0.09 ± 0.02	37 ± 2.7
	Shade	2.1 ± 0.2	0.7 ± 0.2	614 ± 24	194 ± 16	0.03 ± 0.01	13 ± 1.0

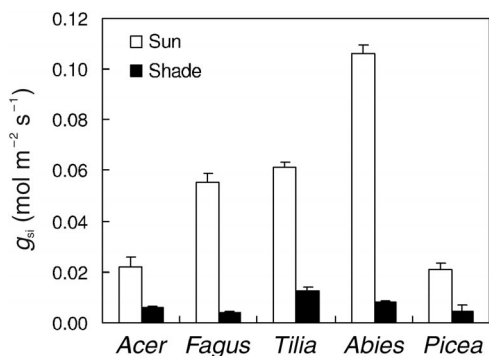


Figure 1. Initial nocturnal stomatal conductance before exposure to saturating irradiance (g_{si}). Values are means \pm standard deviations ($n = 7$) for sun (open columns) and shade (filled columns) foliage of *Acer pseudoplatanus*, *Fagus sylvatica*, *Tilia cordata*, *Abies alba* and *Picea abies*. The differences between sun and shade leaves are highly significant ($P < 0.01$).

R_d values were significantly ($P < 0.01$) higher in sun foliage than in shade foliage (from 1.6-fold in *P. abies* to 3.3-fold in *F. sylvatica*). In all species, nocturnal values of stomatal conductance (g_{si}) were considerably lower ($P < 0.01$) in shade foliage than in sun foliage (Figure 1). The low values for g_{si} resulted in significantly ($P < 0.05$) higher C_{ii} values in shade foliage (from only 6% in *A. pseudoplatanus* to 27% in *P. abies*) than in sun foliage (Table 1). In addition, the C_{if} values were higher in shade foliage compared with sun foliage (Table 1), although the difference was not statistically significant. There were no major differences in C_{if} values between deciduous

leaves and conifer needles.

Maximum rates of in vivo Rubisco carboxylation, as determined in the fully light-adapted leaves from the A/C_i response curves, were significantly higher in sun foliage (from 1.7-fold in *F. sylvatica* to 5.1-fold in *A. pseudoplatanus*) than in shade foliage (V_{cmax} in Table 1). Thus, all of the photosynthetic parameters we studied differed between sun and shade foliage.

Time course of photosynthetic induction

Figures 2A and 2C show the development of A during photosynthetic induction in sun and shade foliage of the strongly shade-tolerant *T. cordata* and the sun-demanding *P. abies*. In both species, A was higher in sun foliage than in shade foliage. Similar induction curves for A were obtained for the other three tree species (data not shown). In *P. abies*, the difference in A between sun and shade needles was larger throughout the induction period than the corresponding difference between sun and shade leaves of *T. cordata* (Figures 2A and 2C). In the strongly shade-tolerant *A. alba*, the difference in the induction of A between sun and shade needles was as small as in *T. cordata*, whereas the differences in *A. pseudoplatanus* and *F. sylvatica* was greater, although less than that in the sun-demanding *P. abies*.

The time course of induction of CO_2 assimilation varied among species from hyperbolic to sigmoidal (Figures 2A and 2C). Hyperbolic responses were detected in sun foliage, whereas sigmoidal responses were typical for shade foliage. The only exception was found in the leaves of the less shade-tolerant *A. pseudoplatanus*, where a sigmoidal rise in CO_2 assimilation was observed in both sun and shade leaves.

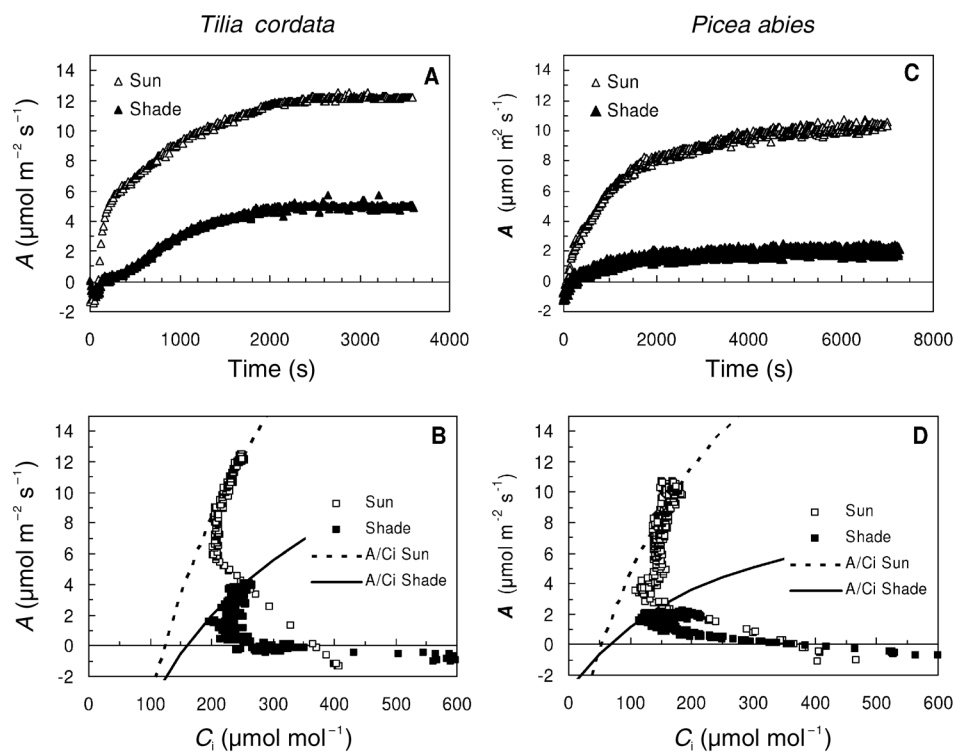


Figure 2. (A, C) Time course of CO_2 assimilation rate (A) during photosynthetic induction. (B, D) Relationship between A and intercellular CO_2 concentration (C_i) during photosynthetic induction. Typical examples of sun and shade foliage of the strongly shade-tolerant *Tilia cordata* (A, B) and the sun-demanding *Picea abies* (C, D) are presented. Lines in B and D represent the A/C_i response curves estimated under steady-state conditions in fully light-induced leaves; broken lines denote sun leaves, and solid lines denote shade leaves.

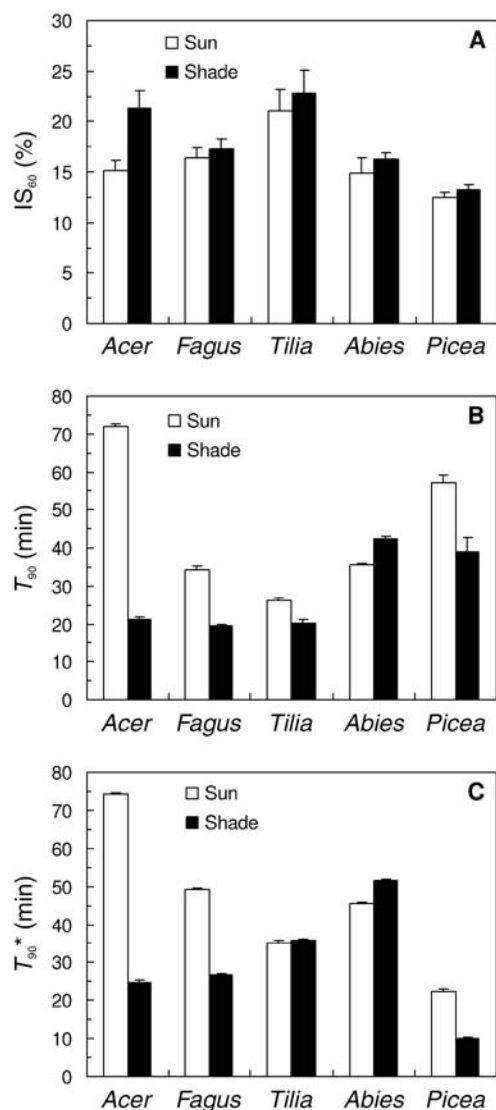


Figure 3. (A) The photosynthetic CO₂ assimilation rate (A) after a 60-s exposure to saturating irradiance (IS_{60}) as a percentage of the leaf's maximal A (A_{max}). (B) Time required to reach 90% of A_{max} (T_{90}). (C) Time required to reach 90% of maximum stomatal conductance (T_{90}^*). Values are means \pm standard deviations ($n = 12$) for sun- (open columns) and shade-acclimated (filled columns) foliage of *Acer pseudoplatanus*, *Fagus sylvatica*, *Tilia cordata*, *Abies alba* and *Picea abies*.

In both cases the rise in g_s lagged the increase in A . Exponential photosynthetic induction responses were usually associated with high g_{si} values, whereas sigmoidal induction responses were more frequently found in shade leaves exhibiting low g_{si} values (Figure 1).

After a 60-s exposure to saturating PPF, there was a tendency for somewhat higher IS_{60} values in shade foliage than in sun foliage (Figure 3A), but the difference was not significant ($P > 0.05$), except for *A. pseudoplatanus* where the IS_{60} value of shade leaves was significantly higher ($P < 0.01$) than that of sun leaves. The IS_{60} values ranged from 12% in *P. abies* to 23% in *T. cordata* in both sun and shade foliage (Figure 3A). In

contrast, T_{90} of shade foliage was significantly ($P < 0.01$) shorter than in sun foliage (Figure 3B), except in the highly shade-tolerant *A. alba* and *T. cordata*. However, shade leaves of the less shade-tolerant *F. sylvatica* and *A. pseudoplatanus* and the sun-demanding *P. abies* had 55, 30 and 66% lower T_{90} values, respectively, than sun leaves. In addition, significantly higher T_{90} values were observed in shade foliage of conifers (about 40 min in *P. abies* and *A. alba*) than of the broad-leaved species (about 20 min in *A. pseudoplatanus*, *F. sylvatica* and *T. cordata*). In sun-exposed leaves and needles, however, we detected no major difference in T_{90} values between the broadleaf and the conifer species.

Sun and shade foliage differed in the induction kinetics of g_s . After a 60-s exposure to saturating PPF, no significant increase in g_s was observed (unpublished data). In the less shade-tolerant *F. sylvatica* and *A. pseudoplatanus* and in the sun-demanding *P. abies*, T_{90}^* values were lower by 45–67% in shade foliage than in sun foliage (Figure 3C). In contrast, there were no differences in T_{90}^* values between sun and shade foliage in the strongly shade-tolerant *A. alba* and *T. cordata*. It is notable that the shortest T_{90} values in both sun and shade foliage were in the sun-demanding *P. abies*, with 21 min for sun needles and 9 min for shade needles (Figure 3C).

Biochemical versus stomatal limitations during induction

The relationship between A and C_i during photosynthetic induction at constant saturating PPF (Figures 2B and 2D) provides a detailed insight into LS and LB that disappears during photosynthetic induction. The lines in Figures 2B and 2D show A/C_i curves, determined under steady-state conditions and light saturation in fully induced leaves, and represent the demand function for CO₂ (Schulte et al. 2003) in sun (bold lines) and shade leaves (dashed lines). The initial slope of the demand function is proportional to V_{cmax} presented in Table 1. If the limitation during photosynthetic induction was caused only by insufficient g_s , A should rise along the demand curve. Lower A/C_i values thus indicate the biochemical co-limitation of CO₂ uptake.

An analysis of the relative importance of these induction limitations was performed according to the model proposed by Woodrow and Mott (1989). Time courses of the extinction of these transient limitations that appear during photosynthetic induction (LT, LB and LS) are presented in Figures 4A–D. The differences between sun and shade leaves and species were evaluated on the basis of parameters derived from these time courses and are presented in Table 2.

In all species, the time to reach zero LT (T_{tot}) was significantly ($P < 0.05$) lower in shade foliage than in sun foliage (Table 2). In the strongly shade-tolerant species *A. alba* and *T. cordata*, T_{tot} of shade foliage was only 6–19% lower than in sun foliage, whereas it was 45–226% lower in the less shade-tolerant *A. pseudoplatanus* and *F. sylvatica* as well as in the sun-demanding *P. abies*. Because the measurements were carried out at night on fully dark-adapted leaves, the transient LB was highest (100%) immediately after switching on the light, and rapidly declined to 0%. In contrast, LS was negligi-

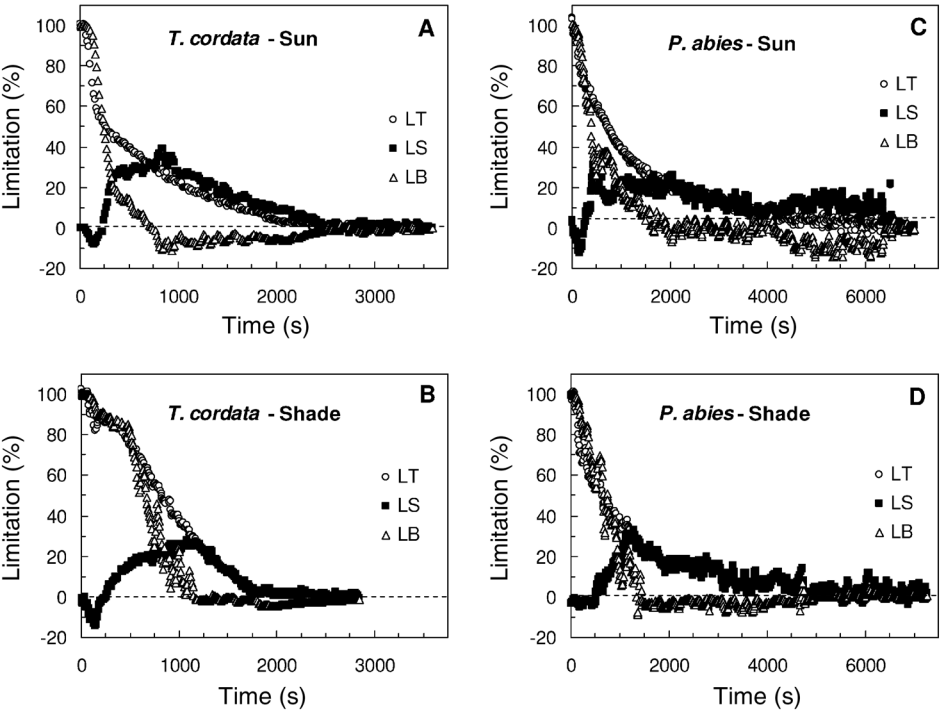


Figure 4. Estimates of relative limitations during photosynthetic induction in (A) sun leaves and (B) shade leaves of *Tilia cordata* and (C) sun needles and (D) shade needles of *Picea abies*. Symbols: ○, total transient dynamic limitation (LT); △, transient dynamic biochemical limitation (LB); and ■, transient dynamic stomatal limitation (LS) during photosynthetic induction. These limitations were calculated according to the model proposed by Woodrow and Mott (1989).

ble initially, but rose to 24–58% (see LS_{max} in Table 2). We observed a faster decline in LB to zero (T_{bio}) in shade foliage than in sun foliage of *A. pseudoplatanus* (2.8-fold) and *A. alba* (1.6-fold), whereas the rate of decline was similar for shade and sun foliage of *F. sylvatica* and *P. abies*, and in shade leaves of *T. cordata* it was 1.6-fold higher than in sun leaves. In all species, LS declined to zero earlier in shade foliage than in sun foliage. A correlation between the time required to remove these transient limitations and the tolerance of trees to shade was not observed. However, shade needles required significantly longer times to overcome T_{tot} and the stomatal limitation (T_{st}) compared with shade leaves (Table 2). Transient

stomatal limitations were lower at the time they exceeded the biochemical limitations (Cross point) in sun than in shade foliage in all species, and the difference was significant ($P < 0.01$), except for the less shade-tolerant *A. pseudoplatanus* and the sun-demanding *P. abies* (Table 2).

Discussion

Possible impacts of the experimental design

A wide range of times for full photosynthetic induction has been reported in the literature: from 3 min in *Hybanthus pruni-*

Table 2. Mean values (\pm standard deviation; $n = 12$) of the times required to remove transient dynamic limitations during photosynthetic induction in sun and shade leaves; i.e., total limitation (T_{tot}), biochemical limitation (T_{bio}) and stomatal limitation (T_{st}). All limitations were estimated according to a model proposed by Woodrow and Mott (1989). In addition, LS_{max} represents the maximum stomatal limitation during the induction phase as a percentage of the total limitation. At the beginning of the induction phase, the biochemical limitations always amounted to 100% of the total limitation. Cross point (Cross) represents the time when the stomatal limitation became larger than the biochemical limitation.

Species		T_{tot} (min)	T_{bio} (min)	T_{st} (min)	LS_{max} (%)	Cross (min)
<i>Acer pseudoplatanus</i>	Sun	88 \pm 4.2	69 \pm 6.4	76 \pm 5.9	24 \pm 2	16 \pm 2.5
	Shade	27 \pm 3.3	25 \pm 1.0	34 \pm 1.0	25 \pm 2	19 \pm 1.7
<i>Fagus sylvatica</i>	Sun	45 \pm 3.5	12 \pm 2.8	38 \pm 3.1	58 \pm 3	5 \pm 0.5
	Shade	31 \pm 2.7	13 \pm 1.9	28 \pm 3.3	52 \pm 6	11 \pm 1.7
<i>Tilia cordata</i>	Sun	37 \pm 3.2	12 \pm 0.8	43 \pm 1.3	36 \pm 3	6 \pm 0.8
	Shade	31 \pm 4.1	19 \pm 1.5	29 \pm 1.9	25 \pm 4	14 \pm 1.7
<i>Abies alba</i>	Sun	52 \pm 5.0	34 \pm 4.8	52 \pm 5.0	40 \pm 4	6 \pm 1.5
	Shade	49 \pm 1.8	21 \pm 1.7	45 \pm 2.2	55 \pm 4	18 \pm 1.2
<i>Picea abies</i>	Sun	89 \pm 4.3	28 \pm 3.3	82 \pm 9.2	27 \pm 3	16 \pm 1.3
	Shade	60 \pm 5.8	25 \pm 2.1	69 \pm 7.3	31 \pm 5	17 \pm 2.3

folius (Violaceae) (a shade-tolerant angiosperm) to 115 min in *Pinus taeda* L. (a shade-intolerant gymnosperm) (see review by Naumburg and Ellsworth 2000). Our results with five temperate-zone tree species showed T_{90} values in the range of 25 to 73 min for sun leaves and 19 to 41 min for shade leaves (Figure 3B), which are well within the overall range reported for trees. Fairly low T_{90} values have been reported for trees grown in the shade or at low irradiances: 8.8 min for *Acer rubrum* grown in the forest understory (Naumburg and Ellsworth 2000), 21.5 min for *Picea sitchensis* grown in growth chambers at an irradiance of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Ögren and Sundin 1996) and 12 min for *Fagus sylvatica* grown in the forest understory (Schulte et al. 2003). The *F. sylvatica* we investigated had a T_{90} of 19 min for shade leaves and 35 min for sun leaves.

When comparing data in the literature for different species, one has to consider that the rate of photosynthetic induction is influenced by: (1) the plant's acclimation to the prevailing growth environment, i.e., forest understory, forest gap, open areas and sun or shade leaves, as shown here, resulting in particular biochemical and anatomical adaptations in leaves (Cao and Booth 2001, Lichtenthaler and Babani 2004); (2) the previous light history of the plants (Han et al. 1999, Cai et al. 2005), which affects the extent of photosynthetic enzyme activation; and (3) ecological factors, such as temperature (Küppers and Schneider 1993) and leaf water status (Tinoco-Ojanguren and Pearcy 1993, Allen and Pearcy 2000a).

To obtain comparable data from sun and shade foliage of our study species, we observed induction kinetics of fully dark-adapted foliage during the night. In addition, all samples investigated were maintained at a constant leaf temperature ($T_L = 20 \pm 1^\circ\text{C}$) and vapor pressure deficit ($\text{VPD} = 0.9 \pm 0.1 \text{ kPa}$). Nocturnal dark adaptation of leaves leads to full inactivation of the primary limiting enzymes, i.e., FBPase and Rubisco, and formation of nocturnal inhibitors, e.g., carboxyarabitol 1-phosphate (Martin et al. 2000). Therefore, the initial phase of the photosynthetic induction (Figures 2A and 2D) rose more slowly than has been observed for plants that had been pre-exposed to low irradiances (Han et al. 1999) or were kept in the dark for several minutes only (Schulte et al. 2003).

The induction kinetics of sun versus shade leaves

Our temperate-zone study species exhibited typical differences between sun and shade leaves that have developed under natural conditions in either full sun or in deep shade (Table 1). These acclimation adjustments are in accordance with previous results obtained at the level of chloroplasts (Lichtenthaler 1981, Lichtenthaler et al. 1981), leaves (Špunda et al. 1998, Lichtenthaler and Babani 2004) and whole plants (Chen and Klinka 1997, Han et al. 1999). In addition, significantly higher g_{si} values were found in sun foliage compared with shade foliage (Figure 1). In contrast to our observation, FitzJohn (2002) detected no relationship between g_{si} and photosynthetic capacity across a range of light environments in four shade-tolerant tree species (*Alectryon excelsum* Gaert., *Dysoxylum spectabile* (G. Forst.) Hook.f., *Melicocarpus ramiflorus*

Forst. & Forst.f. and *Piper excelsum* Forst.).

We observed more rapid photosynthetic induction kinetics (T_{90}) in shade foliage than in sun foliage, except in the strongly shade-tolerant *A. alba* (Figure 3B), apparently reflecting mainly a difference in the rate of stomatal opening (see Kirschbaum and Pearcy 1988a, 1988b). Similarly, understory-grown branches of *Pseudotsuga menziesii* (Mirb.) Franco (Chen and Klinka 1997) and shade leaves of *Fagus sylvatica* (Küppers and Schneider 1993) manifested significantly shorter times to full photosynthetic induction compared with leaves on sun-grown branches. Shade leaves tended to have higher IS_{60} values (Figures 3A) than sun leaves, apparently reflecting mainly the incomplete activation of enzymes (Kirschbaum and Pearcy 1988a, 1988b). However, the differences in IS_{60} values were not statistically significant. This corresponds to findings of Tinoco-Ojanguren and Pearcy (1993) who reported no differences in the time course of Rubisco activity between sun and shade plants of *Piper auritum*. The observed differences in induction kinetics were attributed to differences in stomatal behavior.

Ögren and Sundin (1996) hypothesized that shade plants and slow-growing sun plants have higher efficiencies in photosynthetic induction than fast-growing sun plants. This could be related to the presence of a higher electron transport capacity relative to carboxylation capacity, which seems to be associated with lower photosynthetic capacities. Marek et al. (1999) came to the same conclusion studying the differences between sun and shade needles of *Picea abies*. However, we observed no relationship ($r < 0.1$) between the induction parameters (IS_{60} , T_{90}) and carbon assimilation parameters (A_{max} , V_{cmax}) in the five species we studied (data not shown).

Transient dynamic limitations during induction

The model proposed by Woodrow and Mott (1989) has been used to separate LB and LS that disappear during photosynthetic induction. However, there are several problems with this method. First, the recalculation of photosynthetic rates (A^* in Equation 1) is linear, whereas a linear approximation to the A/C_i curve is only accurate at low C_i . Second, the recalculation is made to a constant C_{if} . At times when C_i is higher than C_{if} (e.g., at the start of the induction), A^* will be lower than A , resulting in negative stomatal limitations. Finally, it is assumed in the model that LT is the sum of LB and LS; however, limitations are nonlinearly additive (Chazdon and Pearcy 1986).

Because the leaves we investigated had been dark-adapted for at least 3 hours, the initial LB was 100% and declined gradually over time (Figure 4). However, no differences between sun and shade foliage were found for either broadleaf or conifer trees (Table 2). In contrast, LS and LT were eliminated significantly earlier in shade leaves than in sun leaves (T_{tot} and T_{st} in Table 2).

We found that sun leaves required longer induction times for stomatal conductance than shade leaves, as indicated by higher T_{90}^* values, in the sun-demanding *P. abies* and less shade-tolerant *A. pseudoplatanus* and *F. sylvatica*, whereas these times were similar (*T. cordata*) and shorter (*A. alba*) in strongly shade-tolerant species (Figure 3C). The observation that the

sun-demanding *P. abies* had the shortest T_{90}^* values for both sun and shade leaves, whereas the strongly shade-tolerant or less shade-tolerant tree species exhibited greater T_{90}^* values, points to an essential role of genetic adaptation and the particular light requirements of the species on the length of the stomatal induction period. In accordance with earlier results (e.g., Valladares et al. 1997), we observed that leaf photosynthesis rapidly increased in response to the onset of irradiance, whereas stomatal conductance responded more slowly (Figure 2). Therefore, the transient stomatal limitations were relatively more important during 60–90% of the time required for full photosynthetic induction (see Cross point values in Table 2). These results provide further support for the idea of a large potential role of stomata in limiting photosynthetic induction, particularly in shade leaves.

Consequences of ecologically contrasting species

Previous studies have suggested that shade-tolerant species have induction characteristics that allow more efficient utilization of continually changing irradiances than shade-intolerant species (Küppers et al. 1996). However, Naumburg and Ellsworth (2000) concluded, on the basis of a review of a wide range of woody species, that photosynthetic induction characteristics are generally not closely related to a species' shade tolerance ranking.

To evaluate the possible relationship between the shade-tolerance of trees and the rate of photosynthetic induction, ecologically contrasting trees from the same location in the Beskydy Mountains were investigated under the same microclimatic conditions. Our comparison of three broad-leaved species and two conifers of differing shade tolerances showed that the photosynthetic induction of CO_2 assimilation (e.g., IS_{60} and T_{90} values) is not closely related to species shade tolerance. Sun and shade leaves of strongly shade-tolerant species (*T. cordata*, *A. alba*) did not differ significantly in photosynthetic (T_{90} ; Figure 3B) or stomatal (T_{90}^* ; Figure 3C) induction times; however, both T_{90} and T_{90}^* were significantly shorter in shade leaves than in sun leaves of the less shade-tolerant *F. sylvatica* and *A. pseudoplatanus* and the sun-demanding *P. abies* (Figures 3B and 3C). Also, no clear correlation between shade-tolerance and the times required to overcome the transient limitations was observed. However, T_{tot} was lower in shade leaves than in sun leaves, and the difference was only 6–19% in the strongly shade-tolerant species, but 45–226% in the less shade-tolerant and sun-demanding species.

We found significantly slower photosynthetic induction kinetics in shade needles of the conifers than in shade leaves of the broadleaf species (Figure 3B), but this difference was not evident between sun needles and sun leaves. Our observations support the conclusion of Naumburg and Ellsworth (2000) that photosynthetic induction takes longer in gymnosperm species than in angiosperm species (42 min versus 13 min, respectively).

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