Characterization of the photosynthetic induction response in a *Populus* species with stomata barely responding to light changes

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Summary  The photosynthetic induction response is constrained by stomatal and biochemical limitations. However, leaves in some plants like *Populus koreana* × *trichocarpa* cv. Peace (a hybrid clone) may have little stomatal limitation because their stomata barely respond to changes in photon flux density (PFD). We examined the induction responses of leaves of well-watered and dehydrated *P. koreana* × *trichocarpa* plants grown in a high-light or a low-light regime. With an increase in PFD from 50 to 500 μmol m⁻² s⁻¹, steady-state stomatal conductance (gₛ) increased by only 0.25–8.2%, regardless of the initial gₛ, but steady-state assimilation rate (A) increased by 550–1810%. Photosynthetic induction times required to reach 50% (IT₅₀) and 90% (IT₉₀) of A at high PFD were 60–90 s and 210–360 s, respectively. Examination of the dynamic relationships between A and gₛ, and between A and intercellular CO₂ concentration, indicated that the induction limitation was imposed completely by the biochemical components within 30–40 s after the PFD increase. Values of IT₅₀ and IT₉₀ were significantly higher in low-light leaves than in high-light leaves, whereas the induction state at 60 s and the induction efficiency at 60 and 120 s after the increase in PFD were lower in low-light leaves than in high-light leaves. Dehydration reduced leaf water potential (Ψ) significantly, resulting in a significantly decreased initial gₛ. Leaf water potential had no significant effects on induction time in high-light leaves, but a low Ψ significantly reduced the induction time in low-light leaves. We conclude that the photosynthetic induction response was limited almost completely by biochemical components because the stomata barely responded to light changes. The biochemical limitation appeared to be higher in low-light leaves than in high-light leaves. Mild water stress may have reduced steady-state A and gₛ, but it had little effect on the photosynthetic induction response in high-light leaves.

Keywords: light fluctuation, sunflecks, water stress.

Introduction


Under conditions of fluctuating PFD, photosynthetic carbon gain depends on the extent of induction limitation following an increase in PFD, and on post-illumination CO₂ fixation after a decrease in PFD. A typical time course of photosynthetic induction involves a fast and a slow induction phase (Pearcy et al. 1996). The fast phase is limited mainly by ribulose 1,5-bisphosphate (RuBP) regeneration, and the slow phase is constrained by the combined effects of stomatal conductance (gₛ) and light activation of Rubisco. Recently, a high degree of coordination was observed between gₛ and biochemical capacity (Allen and Pearcy 2000a), and the whole time course of induction may be determined by a combination of stomatal opening time and rates of activation of light-regulated enzymes (Allen and Pearcy 2000b). In most plants, stomatal limitation plays a major role in the induction response. Some species, however, are able to maintain a constant high gₛ and exhibit little or no stomatal response to PFD variations (Furukawa et al. 1990, Roden and Pearcy 1993). Although leaves of such species may be efficient in utilizing rapidly fluctuating light, they are susceptible to water stress because a constant high gₛ facilitates water loss. However, a constant high gₛ may be advantageous for species growing in wet or riparian environments or for deeply rooted species (Knapp and Smith 1990, Allen and Pearcy 2000a, 2000b). This may explain why a lack of stomatal responsiveness to light has been reported in some wetland plant species (Jones 1988, Schulz et al. 1991, Koch and Rawlik 1993, Torn and Chapin 1993). Knowledge of induction characteristics in leaves of plants with stomata insensitive to light changes may provide an insight into understanding both the relative role of biochemical components in the induction response and the ecological significance of the photosynthetic induction response.

The dynamic properties of the induction response can be modified by environmental factors. For example, induction limitation is greater in sun-acclimatized plants than in shade-acclimatized plants (Tang et al. 1993, 1994, Tinoco-Ojanguren and Pearcy 1993). Stomatal response is also faster in shade-tolerant species than in shade-intolerant species (Woods and Turner 1971). However, little evidence is available to determine whether biochemical limitation is greater in sun-acclimatized leaves compared with shade-acclimatized leaves.
Moreover, most of these studies focused only on the effect of modification of light regimes on induction response, but soil water content may also play an important role in the photosynthetic induction response. Although many studies have focused on the effects of water- or drought-stress on leaf photosynthesis in steady-state light (see review by Cornic and Massacci 1996), there have been few attempts to clarify the effects of drought on photosynthesis in fluctuating light environments (Allen and Pearcy 2000a, 2000b). A detailed knowledge of the effects of water availability on the induction response is needed to elucidate the ecological roles of fluctuating light regimes and to predict photosynthetic performance in natural environments.

We have characterized the steady-state and transient photosynthesis of a hybrid poplar species (Populus koreana × trichocarpa cv. Peace) grown in contrasting light regimes. Use of this poplar, which has stomata that stay open and barely respond to light changes, enabled us to focus on the photosynthetic induction response limited mainly by biochemical capacity. We assessed the extent to which photosynthetic induction depends on stomatal limitation and biochemical limitation. We also determined how growth light regime modifies photosynthetic induction response, and to what extent soil water-stress affects the photosynthetic induction response.

Materials and methods

Plant materials and growth conditions

All plants of Populus koreana × trichocarpa were grown in 5.2-dm³ pots (16-cm diameter, 26 cm high) in a 1:1 mixture of gravel and vermiculite for 2 months before measurement of gas exchange. The pots were placed in a phytotron (ACP-1L, Koito Corporation, Tokyo, Japan) at the National Institute for Environmental Studies, Japan. Light was provided by 400-W metal halide lamps (Toshiba Corp., Tokyo, Japan) for a 14-h photoperiod to two light regimes: a high-light regime of about 550 ± 12 µmol m⁻² s⁻¹ (H) and a low-light regime of 190 ± 10 µmol m⁻² s⁻¹ (L). Plants were watered daily and fertilized once a week with Magamp-K nutrient solution (Hyponex, Tokyo, Japan) diluted 1:1000. During gas exchange measurement, watering of dehydrated plants was withheld for 3 days for H plants and 5 days for L plants. Leaf water potentials of dehydrated (D) plants in the two light regimes were similar: –1.27 MPa for H plants and –1.19 MPa for L plants (Table 1). The mean water potential for well-watered (W) plants before gas exchange measurement was –0.85 MPa for H plants and –0.74 MPa for L plants. Day/night air temperatures were 25/20 °C, and air humidity was maintained around 75%. The four combinations of growth conditions, high-light + well-watered regime, low-light + well-watered regime, high-light + dehydrated regime, and low-light + dehydrated regime are referred to as HW, LW, HD and LD, respectively.

Gas exchange measurements

Photosynthesis was measured with a portable gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE). The air stream entering the chamber was first scrubbed of CO₂ by passage through soda lime, and the CO₂ concentration was then adjusted to 360 ± 1.5 µmol CO₂ mol⁻¹ air by injection of 10% CO₂ with a mass-flow controller (Model 5877, Ueshima Brooks Co. Ltd., Tokyo, Japan). To maintain a constant flow rate, the humidity in the chamber was not controlled by the LI-6400 itself, but rather by humidifying the air and then removing excess water vapor with a temperature-controlled condenser (RTE-111, Neslab Instrument Inc., Newington, NH). Because both CO₂ concentration and humidity were controlled by other systems, we were able to keep the flow rate constant at 500 µmol s⁻¹ for all of the measurements, which is essential for measurements of dynamic photosynthesis.

Light in the phytotron was supplied with 400-W metal-halide solar lamps (MLRBOC400F-U, Mitsubishi/Osram, Yokohama, Japan). A wire-screen filter covering the chamber was used to change incident PFD in the chamber. To measure the photosynthetic induction response, leaves were maintained in a low PFD of 50 ± 3 µmol m⁻² s⁻¹ for longer than 45 min, and then the PFD was increased in one step to 500 ± 5 µmol m⁻² s⁻¹. An auto-logging program was run to send data at 1–3-s intervals from the LI-6400 system to a computer (HP 200LX, Hewlett-Packard Co.). The data-logging intervals were set to 1 s, but the actual logging interval varied depending on how busy the instrument’s computer processor was. The mean interval was 2.08 s during measurement, but the actual intervals were used for the data analysis. Gas exchange parameters were calculated based on the equations described by von Caemmerer and Farquhar (1981).

Photosynthetic induction state (IS) was calculated based on the equation of Chazdon and Pearcy (1986):

\[
IS = \frac{A_h - A_w}{A_h - A_w},
\]

where \(A_h\) and \(A_w\) are steady-state assimilation rates before and after the PFD increase, respectively, and \(A_i\) is the instantaneous assimilation rate at time \(t\) after the increase in PFD at time 0. Because IS emphasizes the instantaneous induction state, to evaluate carbon gain during the induction response we integrated \(A\) and calculated photosynthetic induction efficiencies for various induction times (IE\(_T\)), based on an equation of Tang et al. (1994):

\[
IE_T = \int_0^T A_i dt - T A_w
\]

where \(T\) is induction time.

It is critical to consider the problem of time lags in the gas analysis system when examining rapid photosynthetic response. The variation of time lags depends mainly on flow rate, chamber volume and tubing volume. In the LI-6400 photosynthetic measuring system, there is no tubing that runs from the leaf chamber to the analyzer because the gas analyzers are located in the sensor head. This allows leaf dynamics to be measured in real time. However, there are volume-related

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Table 1. Steady-state assimilation rate (A), stomatal conductance (gs), intercellular CO2 concentration (ci), transpiration rate (E), dark respiration (R), apparent quantum yield (Q) and potential water-use efficiency (A/ gs) in Populus koreana × trichocarpa grown under contrasting light regimes. Gas exchange was measured in plants from a well-watered condition (W) and a water deficit condition (D). Means ± SD (n = 3–5) within the same row followed by different letters are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Well-watered (W)</th>
<th>Dehydrated (D)</th>
</tr>
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<tbody>
<tr>
<td><strong>PFD 50 µmol m−2 s−1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (µmol CO2 m−2 s−1)</td>
<td>1.20 ± 0.147 a</td>
<td>0.710 ± 0.187 b</td>
</tr>
<tr>
<td>g0 (mol H2O m−2 s−1)</td>
<td>0.499 ± 0.280 a</td>
<td>0.315 ± 0.050 b</td>
</tr>
<tr>
<td>c1 (µmol CO2 mol−1 air)</td>
<td>363 ± 2 a</td>
<td>364 ± 4 a</td>
</tr>
<tr>
<td>E (mol H2O m−2 s−1)</td>
<td>4.19 ± 0.10 a</td>
<td>3.23 ± 0.26 b</td>
</tr>
<tr>
<td>A/g0 (µmol CO2 mol−1 H2O)</td>
<td>2.46 ± 0.37 a</td>
<td>2.88 ± 1.37 a</td>
</tr>
<tr>
<td><strong>PFD 500 µmol m−2 s−1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (µmol CO2 m−2 s−1)</td>
<td>15.4 ± 0.20 a</td>
<td>13.1 ± 0.76 b</td>
</tr>
<tr>
<td>g0 (mol H2O m−2 s−1)</td>
<td>0.509 ± 0.030 a</td>
<td>0.324 ± 0.045 b</td>
</tr>
<tr>
<td>c1 (µmol CO2 mol−1 air)</td>
<td>295 ± 2.87 a</td>
<td>263 ± 13 b</td>
</tr>
<tr>
<td>E (mol H2O m−2 s−1)</td>
<td>4.72 ± 0.16 a</td>
<td>3.67 ± 0.30 b</td>
</tr>
<tr>
<td>A/g0 (µmol CO2 mol−1 H2O)</td>
<td>30.6 ± 1.5 a</td>
<td>43.7 ± 7.8 a</td>
</tr>
<tr>
<td>R (µmol CO2 m−2 s−1)</td>
<td>1.53 ± 0.25 a</td>
<td>1.39 ± 0.18 ab</td>
</tr>
<tr>
<td>Q (µmol CO2 mol−1 photons)</td>
<td>0.057 ± 0.008 a</td>
<td>0.039 ± 0.001 b</td>
</tr>
</tbody>
</table>

Results

Photosynthetic induction response

After PFD was increased from 50 to 500 µmol m−2 s−1, stomatal conductance (gs) exhibited little or no increase despite variation in the initial gs, which differed in leaves from different light regimes (Figure 1, Table 1). The steady-state gs of leaves in high PFD (500 µmol m−2 s−1) was only 0.25–8.2% higher than that of leaves in low PFD (Figure 2, Table 1). Moreover, in response to the increase in PFD, the increase in gs, if any, was rapid, reaching a steady-state value for the high PFD within 30–40 s.

In response to an increase in PFD, assimilation rate (A) required about 60–90 s to reach 50% (IT90) and 210–360 s to reach 90% (IT90) of the steady-state A of leaves in high PFD (Figures 1 and 3). Steady-state A of leaves in high PFD was 550–1810% higher than that of leaves in low PFD. Photosynthetic induction periods differed among leaves from different light regimes (Figure 2). Both IT90 and IT90 were significantly higher in low-light + well-watered plants (HW) than in high-light + well-watered (HW) plants (Figure 3). Both induction state (IS) and induction efficiency (IE) tended to be lower in LW leaves than in HW leaves (Table 2).

To understand further the stomatal limitation on photosynthetic induction response, we examined the effects of gs and intercellular CO2 concentration (ci) on A (Figures 4 and 5). The increase in A was independent of gs, after 15–30 s (Figure 4). In low-light + dehydrated leaves (LD), A increased from 2 to 8 µmol m−2 s−1 with almost no change in gs. Moreover, in all cases, A increased almost linearly with a decrease in ci (Figure 5). At high PFD, steady-state ci remained at about 300–320 µmol mol−1, except in LD leaves where it dropped to 170 µmol mol−1.

Steady-state gas exchange

There was an acclimation in steady-state A to light regime after a period of 2 months (Table 1). Assimilation rate measured at a PFD of 500 µmol m−2 s−1 (A500) was significantly higher in H leaves than in L leaves. The A500 decreased significantly in response to dehydration and was about 33% lower in LD leaves than in LW leaves.

Steady-state gs was significantly lower in LD leaves than in HW leaves (Table 1). In LD leaves, gs was only 20% of that in LW leaves. Steady-state ci exhibited little variation among leaves from different growth conditions when measured at a PFD of 50 µmol m−2 s−1, but was significantly lower in LD

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leaves than in LW leaves. Among treatments, LD leaves showed the lowest $c_i$ (< 200 µmol mol$^{-1}$) when measured at a PFD of 500 µmol m$^{-2}$ s$^{-1}$.

The LD leaves showed a significantly lower transpiration rate ($E$) than the LW leaves at both measurement PFDs. Potential water-use efficiency—the ratio of assimilation rate to stomatal conductance ($A/g_s$)—was much higher at high PFD than at low PFD. The $A/g_s$ ratio was 10 times higher in HW leaves at a PFD of 500 than at 50 µmol m$^{-2}$ s$^{-1}$. The HW leaves also showed significantly higher apparent quantum yield than the leaves from other treatments (Table 1).

**Discussion**

**Biochemical and stomatal limitations in induction response**

The photosynthetic induction response is constrained by both stomatal and biochemical limitations, although the relative role of the limitations may differ during different periods of induction (Pearcy 1990, Pearcy et al. 1996, Allen and Pearcy 1997).
2000a). It has also been shown that stomatal conductance and biochemical activity co-vary in tropical rainforest shrub species (Allen and Pearcy 2000b). However, it is often difficult to characterize the independent roles of the two components because of their interaction during the induction. The stomata of *P. koreana × trichocarpa* leaves barely respond to changes in PFD, which makes these leaves suitable for characterization of biochemical limitation during the induction response. We identified two important features in the induction response of stomatal conductance in these poplar leaves. First, *g*$_{s}$ changed less than 9% with a change in PFD from 50 to 500 µmol m$^{-2}$ s$^{-1}$, whereas *A* increased 550–1810% during the same period. Second, in addition to the extremely small change in *g*$_{s}$, the increase in *g*$_{s}$ was very rapid and was complete within less than 1 min (Figures 1 and 2). Similarly, *g*$_{s}$ of *P. koreana × trichocarpa* plants grown under artificial conditions showed no change in *g*$_{s}$ between darkness and PFDs of 25 and 50 µmol m$^{-2}$ s$^{-1}$, but there was a slight change in *g*$_{s}$ of leaves of the same species under natural conditions (Y. Tang, unpublished data).

The lack of stomatal responsiveness to light variation may play an important role in some plants. In some wetland species, *g*$_{s}$ remains relatively constant throughout the day (Koch and Rawlik 1993), and in other species, stomata are reported to remain open continuously, even in darkness or when assimilation rate is zero (Jones 1988, Schulz et al. 1991, Torn and Chapin 1993). Recently, Thomas et al. (1999) found that stomata of several tropical tree species from a riparian closed canopy did not optimize the unit marginal water cost of plant carbon gain when photon flux density varied experimentally, suggesting that stomata of these riparian species may be unresponsive to light changes. We found that there was no significant stomatal response to light variation in the poplar species *P. koreana* Rehd. (authors’ unpublished observations).

The small change in *g*$_{s}$ compared with the large change in *A* indicates that photosynthetic induction in poplar leaves was almost entirely a response of biochemical limitation. Moreover, the extremely short period required for *g*$_{s}$ to reach the steady-state for the high PFD implies that *g*$_{s}$ was not a limiting factor from about 1 min after the increase in PFD. In addition, the small change in *g*$_{s}$ in response to light change was independent of the initial stomatal conductance (Figure 1, Table 1). Support for this conclusion was obtained from analyses of the relationships between *g*$_{s}$ and *A*, and between *A* and *c*$_{i}$ (Figures 4 and 5). The relationship between *A* and *c*$_{i}$ during the induction response provides insight into the limitations of stomatal conductance and biochemical capacity on photosynthetic induction (Chazdon and Pearcy 1986, Pearcy et al. 1996). If the increase in *A* during induction is caused solely by an increase in *g*$_{s}$, then *A* and *c*$_{i}$ should increase along the steady-state *A/c*$_{i}$ curve. If the increase in assimilation rate is also limited by biochemical capacity, then *A* should fall below the steady-state *A/c*$_{i}$ curve. A linear dependency of *A* on *c*$_{i}$ indicates that the increase in assimilation rate was limited mainly by chemical capacity and not by *g*$_{s}$ (Figure 5).

### Biochemical limitation in leaves from contrasting light regimes

Tinoco-Ojjanguren and Pearcy (1992) reported that growth of the shade-intolerant species *Piper auritum* Kunth in low-light regimes resulted in a slow response of stomata to light flecks. Woods and Turner (1971) showed that stomatal response is faster in shade-tolerant species than in shade-intolerant spe-

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**Table 2. Photosynthetic induction state at 60 (IS$_{60}$) and 120 seconds (IS$_{120}$) and photosynthetic induction efficiency at 60 (IE$_{60}$) and 120 seconds (IE$_{120}$) after an increase in PFD. Means ± SE (n = 3–6) within the same row followed by different letters are significantly different (P < 0.05).**

<table>
<thead>
<tr>
<th>Induction state (IS)</th>
<th>Well-watered (W)</th>
<th>Dehydrated (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High-light regime (H)</td>
<td>Low-light regime (L)</td>
</tr>
<tr>
<td>IS$_{60}$</td>
<td>0.558 ± 0.009 a</td>
<td>0.438 ± 0.015 b</td>
</tr>
<tr>
<td>IS$_{120}$</td>
<td>0.793 ± 0.007 a</td>
<td>0.642 ± 0.066 ab</td>
</tr>
<tr>
<td>Induction efficiency (IE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE$_{60}$</td>
<td>0.258 ± 0.0010 a</td>
<td>0.207 ± 0.009 b</td>
</tr>
<tr>
<td>IE$_{120}$</td>
<td>0.431 ± 0.013 a</td>
<td>0.318 ± 0.006 b</td>
</tr>
</tbody>
</table>

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**Figure 4. Dependence of assimilation (A) on stomatal conductance (g$_{s}$) during the photosynthetic induction response after an increase of PFD from 50 to 500 µmol m$^{-2}$ s$^{-1}$ in leaves of *Populus koreana × trichocarpa* plants grown under different light and water conditions. Treatments were: HW = high-light + well-watered; LW = low-light + well-watered; HD = high-light + dehydrated; and LD = low-light + dehydrated.**
cies. Other studies suggest that induction limitation may be greater in sun-acclimatized plants than in shade-acclimatized plants (Tang et al. 1993, 1994, Tinoco-Ojanguren and Pearcy 1993). However, there seems to be no unequivocal evidence to indicate whether photosynthetic enzyme regulation in response to light increase is more efficient in shade leaves than in sun leaves. The significantly longer induction time in LW leaves than in HW leaves appears to be the result of a higher biochemical limitation in LW leaves. The reasons may be as follows. First, the linear increases in A and c\textsubscript{i} during induction suggests that the change in A was not a result of a change in g\textsubscript{s}; any increase of A was thus caused by an increase in biochemical activity. Second, in well-watered plants, both induction state and induction efficiency were significantly higher in high-light leaves than in low-light leaves (Table 2). This indicates that HW leaves had a relatively larger biochemical capacity than LW leaves, because 40 s after the increase in PFD, g\textsubscript{s} no longer varied with the increase in A. The difference in induction response between H and L leaves was indistinct in dehydrated leaves partly because the low steady-state g\textsubscript{s} masked the effect of changes in biochemical components. Further studies are needed to clarify acclimation of the photosynthetic induction response to sun and shade environments under drought conditions.

**Effect of drought on constant and transient photosynthesis**

In most plants, stomatal closure is responsible for the decline in photosynthesis in leaves subjected to mild desiccation (see review by Cornic and Massacci 1996). Our results suggest that dehydration resulted in a decrease in stomatal conductance, and that the decrease was much greater in L leaves than in H leaves (Table 1), indicating that shade leaves of *P. koreana × trichocarpa* may have a lower resistance to dehydration than sun leaves. There may be a tradeoff between drought tolerance and shade tolerance in temperate tree species. Valladares and Pearcy (1997) suggested that *Heteromeles arbutifolia* (Ait.) M.J. Roem., a sclerophyll shrub, can sacrifice carbon gain for water conservation under severe water stress. *Populus koreana × trichocarpa* plants were grown under different light and water conditions. Treatments were: HW = high-light + well-watered; LW = low-light + well-watered; HD = high-light + dehydrated; and LD = low-light + dehydrated. See detailed explanations for a trajectory of A/c\textsubscript{i} during induction response by Pearcy 1994.
Populus koreana × trichocarpa is not considered a shade-tolerant species, but the low assimilation rate under low-light and well-watered conditions suggests that the species may undergo some acclimation to low light (Table 1, Figure 4). However, such an acclimation was not evident in plants in the dehydration regime. Furthermore, leaves of P. koreana × trichocarpa did not show acclimation to light regimes under natural conditions (data not shown). Roden and Pearcy (1993) have discussed the possibility that light acclimation in other poplar species is expressed under experimental conditions of uniform shade, but not under natural conditions. Previously, it has been reported that stomata of mature leaves of P. koreana × trichocarpa respond poorly to changes in leaf water potential (Furukawa et al. 1990) or soil water content (Ridolfi et al. 1996). However, we found that although stomatal conductance did not respond effectively to PFD changes, P. koreana × trichocarpa leaves did respond to soil water variation. Thus, L leaves were much more susceptible to dehydration than H leaves, suggesting that a high-light environment may reduce the susceptibility of stomata to decreased soil water content. In contrast, Cochard et al. (1996) reported that P. koreana × trichocarpa plants did not show any specific hydraulic property that could compensate for the lack of response to water deficit.

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