Interaction of drought and elevated CO2 concentration on photosynthetic down-regulation and susceptibility to photoinhibition in Japanese white birch seedlings grown with limited N availability

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Summary The interaction of drought and elevated carbon dioxide concentration ([CO2]) on carboxylation capacity of Rubisco (Vcmax) and susceptibility to photoinhibition may be an important determinant of plant responses to seasonal fluctuations in precipitation in an anticipated elevated [CO2] environment. Japanese white birch (Betula platyphylla var. japonica) leaves that developed wholly during a period of drought showed an increase in leaf nitrogen and a decrease in leaf carbohydrates that could ameliorate photosynthetic down-regulation, defined as a decrease in Vcmax in response to elevated [CO2]. Photochemical quenching (qP) was decreased by elevated [CO2] but increased by drought when compared at a given intercellular [CO2] (Ci), indicating that elevated [CO2] could increase the risk of photoinhibition, whereas long-term drought could alleviate the risk of photoinhibition. However, only a small variation in qP was measured among seedlings in the various water availability × [CO2] treatment combinations, consistent with the small treatment differences in chronic photoinhibition among the seedlings, as indicated by the ratio of variable to maximum chlorophyll fluorescence after overnight dark-adaptation. Our results suggest that the offsetting responses—reduced Vcmax plus increased Ci at elevated [CO2] and increased Vcmax plus reduced Ci under drought conditions—resulted in a narrow range of susceptibility to photoinhibition at the growth [CO2] in Japanese white birch seedlings grown in various water availability × [CO2] treatment combinations.

Keywords: Betula platyphylla, electron transport rate, leaf N concentration, leaf sugar and starch concentration, photochemical quenching, shoot/root ratio.

Introduction Long-term exposure to elevated atmospheric carbon dioxide concentration ([CO2]) can cause photosynthetic down-regulation, typically a decrease in the carboxylation capacity of Rubisco (Vcmax), accompanied by a reduction in area-based leaf nitrogen concentration (Narea) and an accumulation of carbohydrates, especially when nitrogen supply is limited or rooting volumes are restricted (Sage 1994, Drake et al. 1997, Sims et al. 1998, Stitt and Krapp 1999). The failure of leaves to maintain their initial stimulation of net CO2 uptake rate in response to elevated [CO2] could be attributed almost entirely to the decrease in Vcmax (Rogers and Humphries 2000). Photosynthetic down-regulation induced by elevated [CO2] can occur even in the absence of rooting restriction in free-air carbon dioxide enrichment (FACE) experiments, as has been observed in wheat (Nie et al. 1995), loblolly pine (Rogers and Ellsworth 2002), poplars (Bernacchi et al. 2003) and even in the nitrogen-fixing species, soybean (Bernacchi et al. 2005).

Japanese white birch (Betula platyphylla var. japonica), which is broadly distributed across the Eurasian continent, has a high sink strength that is related to its indeterminate growth pattern (Kikuzawa 1983) and high growth rate (Koike 1988). Therefore, a sustained photosynthetic stimulation in response to elevated [CO2] might be expected under fertile growing conditions, as reported for the ecologically similar Populus (Gielgen and Ceulemans 2001). As a pioneer species, however, Japanese white birch is often found in infertile habitats such as those with volcanogenous regosols, which are widely distributed in northern Japan. Under such conditions, photosynthetic down-regulation in response to elevated [CO2] has been observed in Japanese white birch (Kitao et al. 2005).

Acclimation to long-term drought is associated with high Narea in dry-site perennial species (Cunningham et al. 1999, Reich et al. 1999, Wright et al. 2001, Wright and Westoby 2002). Increased Narea in leaves developed during an imposed long-term water stress was found in cork oak (Quercus suber L.) (Aranda et al. 2005) and Japanese mountain birch (Betula ermanii Cham.) (Kitao et al. 2003a). Low precipitation during the period of leaf development also results in increased Narea and light-saturated photosynthetic rates in seedlings of several deciduous broad-leaved tree species (Kitaoa...
In this study, we hypothesized that susceptibility to photoinhibition is the result of modifications in the carboxylation capacity of Rubisco and the differences in $C_i$ resulting from the interacting effects of the growth $[CO_2]$ and water availability. Specifically, we postulate that when plants are subject to contrasting combinations of $[CO_2]$ and water availability, the offsetting responses (i.e., reduced $V_{max}$ + increased $C_i$ at elevated $[CO_2]$ and increased $V_{max}$ + reduced $C_i$ under drought) result in a similar narrow range of susceptibility to photoinhibition in plants in all treatments. To test this hypothesis, we evaluated $C_i$-dependent photosynthetic characteristics of leaves of N-limited Japanese white birch (Betula platyphylla var. japonica) seedlings that developed under conditions of low and high water availability in ambient or elevated $[CO_2]$. Accurate assessment of the risk of photoinhibition requires comparing plant responses at the same $C_i$. This was achieved by determining $V_{max}$ from the $C_i$-dependent net photosynthetic response in seedlings exposed to the factorial combination of $[CO_2]$ and water availability treatments.

**Materials and methods**

**Plant materials**

One-year-old seedlings of Japanese white birch (Betula platyphylla var. japonica) from a commercial nursery were transplanted to individual free-draining plastic pots (diameter: 15 cm, depth: 13 cm and volume: about 1.5 l) filled with a clay loam soil mixed with Kanuma pumice soil (1:1 v/v). Pots were placed individually in trays (diameter: 15 cm, depth 5 cm) to prevent nutrient drainage. A preliminary experiment with birch seedlings grown in various sized pots and subjected to limited N and water availability showed that the small pots were necessary to induce sufficient water stress. Seedling height at transplanting was 15 to 20 cm, and initial seedling dry mass (dw) was about 0.5 g. Seedlings were placed in a phytotron at Hokkaido Research Center, FFPRI, in Sapporo, Japan (43° N, 141° E; 180 m a.s.l.) in natural daylight (about 90% of full sunlight) at 26/16 °C (day/night) for 2 months (May 30 to July 31). Each $CO_2$ treatment—360 (ambient $[CO_2]$ treatment: A) and 720 µmol mol−1 (elevated $[CO_2]$ treatment: E)—was replicated in three chambers; details of the $CO_2$ treatments have been described previously (Koike 1995, Koike et al. 1996, Yazaki et al. 2001, 2004). Twelve seedlings were grown in each chamber. Six randomly selected seedlings in each chamber were irrigated daily with 100 ml of water or nutrient solution (well-watered treatment: W). The other six seedlings received 100 ml of nutrient solution once per week only (drought treatment: D). Each seedling received a total of 100 mg N during the experiment, a sufficiently limited supply to induce photosynthetic down-regulation in seedlings grown in elevated $[CO_2]$ based on an earlier study (Kitao et al. 2005). Predawn leaf water potential was measured with a pressure chamber (Model 3000, Soilmoisture Equipment Corp., Santa Barbara, CA) just before the scheduled watering.

**$CO_2$ assimilation rates and chlorophyll fluorescence measured in situ under treatment conditions**

Measurements began 50 days after the start of the treatments. Light-saturated $CO_2$ assimilation rate ($A_{sat}$) and chlorophyll fluorescence were measured simultaneously on intact fully expanded mature leaves (about 30 days old) with a leaf chamber fluorometer (Li 6400-40, Li-Cor, Lincoln, NE) combined with a portable photosynthesis system (LI-6400, Li-Cor). Seedlings were measured in the phytotron at their respective growing conditions the day before irrigation in the drought
treatment. We measured CO₂ assimilation rate and chlorophyll fluorescence at the growth [CO₂] under a saturating photosynthetic photon flux (PPF) of 1000 µmol m⁻² s⁻¹ provided by a red/blue LED array (Li-6400-40, Li-Cor) with blue light comprising 10% of total PPF. Leaf temperature was held constant at 25 °C, and relative humidity of inlet air ranged from 63 to 72%. Quantum yield of electron transport, Φₚₛₚₛ (=( Fₘ – F₀)/Fₘ ) was calculated from the fluorescence yield in actinic light (Fₐ) and maximum fluorescence (Fₘ) (determined after a 1-s pulse of saturating light of 7000 µmol m⁻² s⁻¹ PPF). We determined minimum fluorescence following light adaptation (Fₚ'), by turning off the actinic light and applying far-red light immediately after a saturating light pulse. From Fₚ', we derived photochemical quenching (qₑ) and Fₚ'/Fₘ according to Schreiber et al. (1994) (where Fₚ' = Fₘ – Fₚ'). Leaf absorptance was calculated based on a calibration curve between SPAD readings (SPADₘₐₓ, measured with a SPAD chlorophyll meter, SPAD 502, Minolta, Osaka, Japan) and leaf absorptance. Leaf absorptance (at 400–700 nm with a halogen light source) was measured with a spectroradiometer (LI-1800C, Li-Cor) and an external integrating sphere (LI-1800-12S, Li-Cor). The calibration curve is described as: leaf absorptance with the halogen light = 1.14SPADₘₐₓ/(12.3 + SPADₘₐₓ) – 0.01SPADₘₐₓ (n = 24, r² = 0.99, P < 0.0001). To account for the difference in spectral composition between the halogen light source and the red/blue LED, the actual absorptance with the LED (d₁₀) was d₁₀ = 0.974dₐ₁ + 0.053 (n = 16, r² = 0.99, p < 0.0001), where dₐ₁ is absorptance with the halogen lamp. Electron transport rate (ETR) was calculated as ETR = 0.5Φₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₛₚₜ Fₚ' = Fₘ – F₀). We measured CO₂ assimilation rate and chlorophyll fluorescence in saturating light (1000 µmol m⁻² s⁻¹) across a range of CO₂ concentrations. Leaf temperature was maintained at 25 °C and relative humidity at the inlet of the chamber was kept between 70 and 80%. The carboxylation capacity of Rubisco was derived from the Aₛₑ/Cᵣ response below a Cᵣ of 300 µmol mol⁻¹ according to the photosynthesis model (Farquhar et al. 1980, von Caemmerer et al. 1994, von Caemmerer 2000). We calculated NPQ as NPQ = Fₘ/Fₚ' – 1 (Björkman and Demmig-Adams 1994). **Leaf N concentration** After the gas exchange and chlorophyll fluorescence measurements, N concentration was determined in the same leaves by a combustion method (Bremner 1996) with an NC analysis system comprising a nitrogen/carbon determination unit (Sumigraph, NC-800, Sumika Chem. Anal. Service, Osaka, Japan), a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) and a data processor (Chromatopac, C-R6A, Shimadzu, Kyoto, Japan). **Sugar and starch concentrations** We measured leaf sugar and starch concentrations in leaves adjacent to those used for the gas exchange and chlorophyll fluorescence measurements. Leaf samples were collected early in the morning when carbohydrate concentrations best reflect the source–sink relationship following diurnal leaf CO₂ assimilation and nocturnal mobilization to other organs. Leaf sugar and starch concentrations were determined by a perchloric acid method. Leaf sugars were first extracted with boiling ethanol followed by extraction of leaf starch solubilized with perchloric acid (Method 1 in Rose et al. 1991). Solubilized starch was then hydrolyzed to glucose with concentrated sulfuric acid and analyzed colorimetrically after reaction with anthrone. **Statistical analysis** All statistics are based on the individual plot (CO₂ × water regime) in each chamber as the sample unit (n = 3). Three to six seedlings were measured in each plot. A mean value from these seedlings was used as the estimate for that sample unit. A two-factorial ANOVA (CO₂ × water regime) was used to test treatment differences in chlorophyll fluorescence and gas exchange parameters (Aₛₑ, Cᵣ, ETR, Fₚ'/Fₘ and qₑ) biomass and leaf N and carbohydrate concentrations (StatView V5.0, 1998; SAS Institute, Cary, NC). For the n-dependent responses of Aₛₑ, ETR, qₑ and NPQ, an ANOVA for a split-plot design was used with CO₂ and water regime as the main-plot factors and Cᵣ (five predetermined values) as the sub-plot factor (JMP, 2003; SAS Institute). The derived chlorophyll fluorescence parameters Fₚ'/Fₘ, Fₚ'/Fₘ' and qₑ, were arcsine-transformed before statistical analysis (Sokal and Rohlf 1981). **Results** **Seedling growth** The lowest predawn leaf water potential (measured just before the scheduled watering), which was in equilibrium with soil water potential at its lowest, was AW: –0.13 ± 0.02, AD: –0.52 ± 0.04, EW: –0.12 ± 0.02 and ED: –0.39 ± 0.03 MPa (means ± 1 SE, n = 3) (A: ambient [CO₂] treatment; E: elevated [CO₂] treatment; W: daily irrigation; D: weekly irrigation). Total seedling biomass was significantly increased by elevated [CO₂] and significantly decreased by drought (Figure 1; Table 1). Compared with well-watered seedlings, aboveground biomass (leaves and stem) of drought-treated seedlings showed a greater decrease relative to root biomass, resulting in
a significantly lower shoot:root (S:R) ratio. In contrast, elevated [CO₂] had little effect on the S:R ratio. The S:R ratios of the treated seedlings were: AW, 1.9 ± 0.01; AD, 1.5 ± 0.04; EW, 1.8 ± 0.14; and ED, 1.5 ± 0.07 (means ± SE, n = 3).

Gas exchange and chlorophyll fluorescence measured in situ under growing conditions

The Fv/Fm ratio measured after overnight dark-adaptation was unaffected by the CO₂ treatments, water regimes and their interaction (Table 1; for daily and weekly irrigation respectively, ratios of 0.775 ± 0.005 and 0.788 ± 0.002 were found for ambient [CO₂], and 0.777 ± 0.006 and 0.777 ± 0.002 were found for elevated [CO₂]). Simultaneous measurements of gas exchange and chlorophyll fluorescence were made the day before a scheduled watering when soil water availability in the drought treatment was at its lowest. Because there was a significant (P < 0.05) effect of the interaction of CO₂ × water regimes on intercellular CO₂ concentration (Cᵢ) (Table 1), significant differences in Cᵢ among the combinations were retested by a one-way ANOVA and the Tukey-Kramer post-hoc test. The ranking of Cᵢ was EW: 553 ± 13 > ED: 374 ± 18 > AW: 300 ± 13 µmol mol⁻¹ (means ± SE, different superscript letters indicate statistically significant difference at P < 0.05) (Figure 2). Light-saturated CO₂ assimilation rates were unaffected by drought in either CO₂ treatment, although Cᵢ differed significantly between the treatments (Figure 2A; Table 1). Elevated [CO₂] marginally enhanced A_sat (P = 0.056) compared with seedlings grown in ambient [CO₂]. No significant effect of CO₂ treatment or water regime was observed on electron transport rate (ETR, Figure 2B) or photochemical efficiency of open photosystem II (Fv'/Fm′, Figure 2C; Table 1; Björkman and Demmig-Adams 1994). Photochemical quenching (qP) significantly decreased in response to elevated [CO₂] but increased in response to drought (Figure 5; Table 2). Drought-treated seedlings, however, had significantly higher values of A_sat compared with well-watered seedlings. Both ETR and qP showed similar patterns as described for A_sat (Figure 3B and 3C; Table 2). There was a significant increase in NPQ in response to elevated [CO₂] and a significant decrease in response to drought (Figure 3D; Table 2). The effects of the interactions of Cᵢ × water and Cᵢ × CO₂ on A_sat, ETR, qP and NPQ were all significant: the only nonsignificant interaction being that of Cᵢ × water on qP (Table 2).

Sugar and starch concentrations

Leaf sugar concentration was higher in well-watered seedlings than in drought-treated seedlings, but elevated [CO₂] had no effect on sugar concentration (Figure 4A; Table 1). Starch concentration increased significantly in response to both elevated [CO₂] and daily watering (Figure 4B; Table 1).

Relationship between area-based leaf N and the carboxylation capacity of Rubisco

Both N_area and V_cmax significantly decreased in response to elevated [CO₂] but increased in response to drought (Figure 5; Table 1). Therefore, higher N_area values were associated with higher V_cmax values.

Discussion

Elevated [CO₂] stimulated both A_sat (Figure 2A) and seedling dry mass (Figure 1) irrespective of water regime, as has been reported by others (Oechel et al. 1995, Scarascia-Mugnozza et al. 1996, Huxman et al. 1998, Norby et al. 1999, Belote et al. 2003). The A_sat/Cᵢ response of our seedlings is also consistent with other studies (Sage 1994, Stitt and Krapp 1999) in that the

Table 1. Summary of the two-factorial ANOVA to test for the effects of growth [CO₂] (F₁,₈), water (F₁,₈) and their interaction (F₁,₈) on total dry mass, photochemical efficiency (Fv/Fm), intercellular CO₂ concentration (Cᵢ), light-saturated net photosynthetic rate (A_sat), electron transport rate (ETR), photochemical efficiency of open photosystem II (Fv'/Fm′), photochemical quenching (qP) measured in situ, and leaf total sugar concentration, leaf starch concentration, area-based leaf nitrogen concentration (N_area) and carboxylation capacity of Rubisco (V_cmax). Significance level: *, P < 0.05; **, P < 0.01; and ***, P < 0.001.

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<tr>
<td></td>
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<tr>
<td>Total dry mass</td>
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<tr>
<td>Fv/Fm</td>
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<td>Cᵢ</td>
<td>237***</td>
<td>98.9***</td>
<td>10.3*</td>
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<tr>
<td>A_sat</td>
<td>4.98</td>
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<td>0.11</td>
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<tr>
<td>qP</td>
<td>8.36*</td>
<td>0.31</td>
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<td>Leaf total sugar</td>
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<tr>
<td>Leaf starch</td>
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<td>0.02</td>
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seedlings grown with limited N in elevated [CO2] underwent photosynthetic down-regulation with decreased \( V_{\text{cmax}} \) (Figure 3A and 5). Therefore, when compared with seedlings grown in ambient [CO2], the higher in situ \( A_{\text{sat}} \) in seedlings grown at elevated [CO2] appears to be attributable to the higher \( C_i \) in elevated [CO2] (Figure 2A) even though stomatal conductance decreased in response to both drought and elevated [CO2] (data not shown). In both CO2 treatments, leaves developed during drought were able to achieve the same \( A_{\text{sat}} \) as leaves formed when plants were well watered, but at a much lower \( C_i \) (Figure 2A); moreover, they showed a higher \( A_{\text{sat}} \) at the same \( C_i \) than leaves of the well-irrigated seedlings (Figure 3A). These results suggest that an extended drought occurring during leaf development can counteract the down-regulation of photosynthesis in elevated [CO2]. Similar results were reported for drought-treated chaparral plants (Oechel et al. 1995) and in a Mojave Desert shrub, *Larrea tridentata* (Huxman et al. 1998) grown in elevated [CO2]. A drought-in-

**Figure 2.** Light-saturated net photosynthetic rate (\( A_{\text{sat}} \)), electron transport rate (ETR), photochemical efficiency of open PSII (\( F'_v/F'_m' \)) and photochemical quenching (\( q_P \)) of intact leaves of Japanese white birch seedlings measured under the growing conditions. Abbreviations: A, ambient CO2 concentration ([CO2]) treatment; E, elevated [CO2] treatment; W, daily irrigation; and D, weekly irrigation. Measurements were made when soil water content was at its lowest, at a photosynthetic photon flux of 1000 µmol m\(^{-2}\) s\(^{-1}\) and at a leaf temperature of 25 °C. Values are means ± SE (\( n = 3 \)).

**Figure 3.** Light-saturated net photosynthetic rate (\( A_{\text{sat}} \)), electron transport rate (ETR), photochemical quenching (\( q_P \)) and non-photochemical quenching (NPQ) in Japanese white birch seedlings as a function of intercellular CO2 concentration ([CO2]) at full hydration. Abbreviations: A, ambient [CO2] treatment; E, elevated [CO2] treatment; W, daily irrigation; and D, weekly irrigation. Measurements were made at a photosynthetic photon flux of 1000 µmol m\(^{-2}\) s\(^{-1}\) and a leaf temperature of 25 °C at various ambient CO2 concentrations. Values are means ± SE (\( n = 3 \)).
duced increase in $A_{sat}$ associated with higher $N_{area}$ has also been reported in other deciduous broad-leaved tree species native to northern Japan (Kitaoka and Koike 2005).

Although the decrease in growth in response to drought led to a reduction in the strength of the carbohydrate sink, a higher root to shoot ratio (Figure 1) could improve the source:sink imbalance and prevent leaves from accumulating carbohydrate even at elevated [$CO_2$] (Figure 4). However, in this 2-month study we were unable to determine whether the shift in root to shoot ratio was driven directly by water availability or by delayed growth and development (i.e., ontogenetic drift) caused by drought (McConnaughay and Coleman 1999). Further studies on biomass allocation and ontogenetic drift in well-watered and drought-stressed plants grown for longer periods in elevated [$CO_2$] are needed to clarify if drought alleviates leaf carbohydrate accumulation in elevated [$CO_2$] consistently throughout the growing season. The high $N_{area}$ observed in leaves that developed during drought (Figure 5) appears to be a common phenomenon in plants that inhabit dry sites (Cunningham et al. 1999, Reich et al. 1999, Wright et al. 2001, Wright and Westoby 2002) and in leaves developed during prolonged drought (Aranda et al. 2005, Kitaou et al. 2003a, Kitaoka and Koike 2005, Pancovi et al. 1999). These responses all tend to alleviate photosynthetic down-regulation at elevated [$CO_2$].

Similar to the patterns observed in $A_{sat}$, ETR at a given $C_i$ increased in response to drought and decreased in response to elevated [$CO_2$] (Figure 3B), suggesting a higher capacity of ETR in drought-stressed seedlings than in well-watered seedlings. In another study, we found high ETR and $N_{area}$ in leaves of Japanese mountain birch that developed during long-term drought (Kitao et al. 2003a). Likewise, $q_P$ was decreased by elevated [$CO_2$] but increased by drought when treatments were compared at a given $C_i$ (Figure 3C). This suggests that elevated [$CO_2$] increases the risk of photoinhibition, because a decrease in $q_P$ is closely associated with increased susceptibility to photoinhibition (Ögren and Rosenqvist 1992, Öquist and Huner 1993). In contrast, the high $q_P$ under long-term drought conditions could alleviate the risk of photoinhibition.

Thermal energy dissipation and electron transport are important factors in preventing photoinhibition by keeping PSII...

Table 2. Summary of $F$ statistics of the split-plot ANOVA to test for the effects of growth [$CO_2$] ($F_{1,8}$), water ($F_{1,8}$), $CO_2$ × water ($F_{1,8}$), $CO_2$ × intercellular [$CO_2$] [$C_i$] ($F_{4,32}$), water × $C_i$ ($F_{4,32}$) and $CO_2$ × water × $C_i$ ($F_{4,32}$) on $C_i$-dependent light saturated net photosynthetic rate ($A_{sat}$), electron transport rate (ETR), photochemical quenching ($q_P$) and non-photochemical quenching (NPQ). Significance level: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

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<th>$C_i$</th>
<th>CO$_2$ × $C_i$</th>
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<td>$A_{sat}$</td>
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<td>1208***</td>
<td>3.71*</td>
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<td>ETR</td>
<td>16.5**</td>
<td>13.2**</td>
<td>0.23</td>
<td>273***</td>
<td>9.23***</td>
<td>4.54**</td>
<td>0.05</td>
</tr>
<tr>
<td>$q_P$</td>
<td>16.3**</td>
<td>9.91*</td>
<td>0.05</td>
<td>300***</td>
<td>9.09***</td>
<td>1.85</td>
<td>0.36</td>
</tr>
<tr>
<td>NPQ</td>
<td>14.6**</td>
<td>15.4**</td>
<td>0.05</td>
<td>166***</td>
<td>5.23**</td>
<td>6.52***</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Figure 4. Leaf total soluble sugar (A) and starch (B) concentrations of Japanese white birch seedlings grown in ambient or elevated [$CO_2$] with either daily irrigation (open bar) or weekly irrigation (closed bar). Values are means ± SE ($n = 3$).

Figure 5. Relationship between leaf nitrogen (N) concentration and carboxylation capacity of Rubisco ($V_{\text{max}}$) in Japanese white birch seedlings grown in ambient or elevated carbon dioxide concentration ([CO$_2$]) with either daily irrigation or weekly irrigation. Abbreviations: A, ambient [CO$_2$] treatment; E, elevated [CO$_2$] treatment; W, daily irrigation; and D, weekly irrigation. Values are means ± SE ($n = 3$).
reaction centers open, i.e., by maintaining high $q_P$ (Ögren 1991, Park et al. 1996, Baroli and Melis 1998, Niyogi 1999, Kitao et al. 2003a, 2003b). Because NPQ is proportional to thermal energy dissipation (Björkman and Demmig-Adams 1994), the increases in thermal energy dissipation observed with decreasing $C_i$ in seedlings in all of the water availability and CO$_2$ treatment combinations (Figure 3D) might contribute to preventing photoinduction as stomata began to close (Kitao et al. 2003a). The lower NPQ and higher ETR (compared at a given $C_i$; Figure 3B and 3D) in drought-treated seedlings than in well-watered seedlings suggest a greater dependence on energy dissipation through ETR than on thermal energy dissipation in maintaining higher $q_P$ (Figure 3C).

By comparing treated seedlings with a $C_i$ corresponding to the growth [CO$_2$] (Figure 2B), we were able to demonstrate that the higher capacity of ETR in drought-treated seedlings compensated for their lower $C_i$ even when seedlings were in their driest state. However, the reduced capacity of ETR in seedlings grown in elevated [CO$_2$] (Figure 3B) counteracted their higher $C_i$. These offsetting results are akin to homeostatic adjustments because seedlings in all combinations of the [CO$_2$] and water availability treatments had a similar apparent ETR in situ and only a small variation in $q_P$ (Figure 2B and 2D; Rosenqvist 2001, Einhorn et al. 2004). Furthermore, thermal energy dissipation, indicated by a decrease in $F_v/F_m$ (Demmig-Adams et al. 1996, Verhoeven et al. 1998), did not differ among seedlings in the various treatment combinations (Figure 2C). This is consistent with the responses of NPQ to $C_i$ measured at the respective operating [CO$_2$] among treatments (Figure 3D). Consequently, we predict little difference among the treatment seedlings in their susceptibility to photoinduction in situ. This prediction is supported by the lack of a significant difference in $F_v/F_m$ among the treatments after an overnight dark-adaptation (Krause et al. 1995). In contrast, short-term drought stress caused by the dehydration of detached leaves or by withholding water to expanded mature leaves can lead to a reduction in ETR that is accompanied by an increase in NPQ and a decrease in $q_P$ primarily associated with CO$_2$ depletion because of stomatal closure (Cornic and Briantais 1991, Epron and Dreyer 1992). This phenomenon would correspond to a decrease in ETR with decreasing $C_i$ (below 600 µmol mol$^{-1}$) accompanied by a decrease in $q_P$ and an increase in NPQ in our seedlings (Figure 3). Contrary to the short-term drought response of expanded mature leaves, leaves developed during long-term drought increased their capacity for electron flow (Figure 3B) and maintained a $q_P$ comparable with that of leaves of well-watered seedlings at their respective growth [CO$_2$] (Figure 2D). Thus, it is likely that long-term drought triggers a different set of photosynthetic adjustments from short-term drought.

In conclusion, drought during leaf development can induce functional adjustments including an increase in $N_{dea}$ and a decrease in leaf carbohydrate concentrations, which could alleviate photosynthetic down-regulation, i.e., the decrease in $V_{max}$ in response to elevated [CO$_2$]. As a result, leaves developed under conditions of frequent irrigation at elevated [CO$_2$] had a lower carboxylation capacity than leaves developed under conditions of drought at ambient [CO$_2$], and also showed a higher risk of photoinhibition, as indicated by their lower $q_P$ when compared at a given $C_i$ and when compared at their respective growth $C_i$. A similar situation can occur under natural conditions where ample precipitation during leaf development and expansion in spring is followed by sparse rainfall in summer. Leaves that develop fully in elevated [CO$_2$] when soil water content is high are likely to have a relatively low capacity to tolerate photoinhibition. If such leaves were then subjected to drought stress, they would likely suffer greater photoinhibition through a reduction in $C_i$ because of stomatal closure than leaves that developed during drought. Therefore, knowing the susceptibility to photoinhibition associated with variations in $V_{max}$ (such as those caused by CO$_2$ and water availability) at the prevailing $C_i$ may be useful in predicting plant responses to seasonal fluctuations in precipitation in an anticipated higher [CO$_2$] environment.

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References


