Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought

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Summary
Container-grown black spruce (Picea mariana (Mill.) B.S.P.) seedlings were planted in trays containing a sand and peat mixture, and placed in a climate-controlled greenhouse. One group of seedlings was kept well-watered, and another group was subjected to three cycles of drought. Gas exchange analysis showed that mesophyll photosynthetic function was largely unimpaired by drought. In contrast, stomatal conductance was sensitive to drought, although it became less sensitive with each drought cycle. Both stomatal and mesophyll conductances increased with time in control and drought-stressed seedlings, but mesophyll conductance increased with time more rapidly than did stomatal conductance. Limitation of photosynthetic rate was dominated by the mesophyll. In control seedlings, relative stomatal limitation increased from 6 to 16% by the end of the experiment. In drought-stressed seedlings, relative stomatal limitation of photosynthesis reached 40% during the first drought, but decreased to near control values immediately after rewatering. Because the third, most severe drought had only a minor effect on stomatal conductance, relative stomatal limitation of photosynthesis was similar to that in control seedlings by the end of the experiment. Inhibition of ontogenetic change during drought stress may be responsible for the apparent acclimation of mesophyll photosynthetic processes. We conclude that it would be more effective to select for high photosynthetic capacity than for reduced stomatal sensitivity when breeding for increased drought resistance in black spruce seedlings.

Keywords: acclimation, container stock, drought resistance, mesophyll conductance, Picea mariana, stomatal limitation, stomatal sensitivity.

Introduction
Young conifer seedlings are subject to a variety of environmental stresses when they are planted on clear-cut sites. Of these stresses, drought is perhaps the most prevalent (Burdett 1990). Even in the boreal forest, a decrease in water content of the surface horizons of the soil can subject shallow-rooted, newly planted seedlings to severe water stress. Seedlings that can acclimate to the first drought may be better able to survive subsequent droughts. Because water stress inhibits the growth of conifer seedlings, any physiological or morphological adjustment that ameliorates the growth reduction due to water stress will improve their rate of establishment.

The growth of a plant depends on a series of physical and biochemical processes that transfer CO₂ from the surrounding air into organic molecules, and, for this reason, much work on acclimation to water stress in trees has focused on the role of stomata in limiting gas exchange in response to stress (e.g., Unterscheutz et al. 1974, Seiler 1985, van den Driessche 1991). Because mesophyll processes involved in the transfer of CO₂ into the chloroplasts and CO₂ fixation may also be limiting during drought stress, there have been several studies of photosynthetic as well as stomatal acclimation to water stress in trees (e.g., Seiler and Johnson 1985, 1988, Walters and Reich 1989). However, few studies have examined the relative contributions of stomatal and nonstomatal acclimation to net photosynthetic acclimation to drought in trees (Epron and Dreyer 1990, Seiler and Cazell 1990) and nonwoody plants (Hutmacher and Krieg 1983, Matthews and Boyer 1984, Congiac et al. 1987).

To identify the traits that contribute most to drought acclimation in black spruce seedlings, we subjected seedlings to three cycles of drought and measured their photosynthetic responses to CO₂ during the drought and recovery periods. The objectives of this study were (a) to test for acclimation to drought stress in net photosynthesis of black spruce seedlings over three drought cycles, (b) to determine the relative stomatal and nonstomatal contributions to the drought response, and (c) to identify the mesophyll processes, if any, involved in acclimation.

Materials and methods

Seedling growing conditions
Black spruce seeds from a lowland Quebec population were planted in nursery containers (Rigipot, IPL, St-Damien, Quebec) with 50 ml cavities filled with a 3/1 (v/v) peat/vermiculite medium. Seedlings were raised under standard conditions in a commercial greenhouse for one growing season, hardened outside and then placed in black plastic bags in a coldroom at 2 °C for 3 months.
In January, the seedlings were moved to a greenhouse and transplanted from the nursery containers to 15 × 60 cm trays, each containing 10 seedlings. The trays were filled to a depth of 10 cm with a 3:1 (v/v) fine sand/peat mixture. Trays were watered daily by an automatic overhead watering boom equipped with fine spray nozzles. Seedlings were fertilized using the watering system twice per week, with each tray receiving approximately 47 mg N, P, K (20-20-20) and 1.5 mg using the watering system twice per week, with each tray watered daily by an automatic overhead watering boom of 10 cm with a 3/1 (v/v) fine sand/peat mixture. Trays were each containing 10 seedlings. The trays were filled to a depth with calibrated gypsum blocks (Soilmoisture Equipment Corporation, Santa Barbara, CA), and predawn xylem water potentials was measured at the end of each drought period before being placed in the 4-l cuvette. Once the seedling was sealed in the gas exchange cuvette, the relative humidity was raised to 85% by pumping ambient air through a gassing wash bottle, filled with water at room temperature, into the gas exchange system in open mode. The high humidity represented the upper calibration limit of the humidity sensor (personal communication, J.M. Welles, Li-Cor Inc., Lincoln, NB) and was used to avoid stomatal closure in response to high VPD in the water-stressed seedlings (Li-Cor 1991), which would have prevented the gas exchange analysis and is known to provoke heterogeneous stomatal closure in some species. After closure, the system was equilibrated under the measurement conditions for 20 to 30 min before measurements were taken.

Gas exchange measurements were taken continuously as the seedling depleted the CO₂ in the closed system. Observations of CO₂ and water fluxes were averaged for every 10 μmol mol⁻¹ change in CO₂ concentration. Under severe water stress, some seedlings had completely closed stomata, and the gas exchange was too slow to be measured. These seedlings were not included in the analysis.

Gas exchange parameters calculated were Aₚ (μmol CO₂ m⁻² s⁻¹), transpiration rate (E) and stomatal conductance to water vapor (gₛ). The program of the LI-6200 was placed in a Ray Leach “Contenedor” (Stuewe and Sons, Corvallis, OR) in a controlled environment chamber. On each measurement day, all four seedlings were either from the control group or from the treatment group.

The response of net photosynthesis (Aₚ) to intercellular CO₂ concentration (cᵢ), i.e., the A/cᵢ relationship, was measured by the nonsteady state method (Davis et al. 1987, McDermitt et al. 1989) in a closed-circuit gas analysis system (LI-6200, Li-Cor Inc., Lincoln, NB). To standardize the measurement process, the seedlings were placed in a controlled environment chamber and kept in the dark in a loosely closed box until needed. The concentration of CO₂ in the chamber was elevated as a result of respiration by the observer and was crudely regulated to between 600 and 700 μmol mol⁻¹. Water-cooled high pressure sodium and mercury lamps were suspended above the cuvette and provided 1000 μmol m⁻² s⁻¹ PAR at the level of the cuvette. For additional heat dissipation, a transparent acrylic tray filled with water was placed between the lamp and the cuvette. A fan circulated ambient air around the cuvette to increase convective cooling. Each seedling was allowed to adjust to the measurement light conditions for 30 min before being placed in the 4-l cuvette. Once the seedling was sealed in the gas exchange cuvette, the relative humidity was raised to 85% by pumping ambient air through a gassing wash bottle, filled with water at room temperature, into the gas exchange system in open mode. The high humidity represented the upper calibration limit of the humidity sensor (personal communication, J.M. Welles, Li-Cor Inc., Lincoln, NB) and was used to avoid stomatal closure in response to high VPD in the water-stressed seedlings (Li-Cor 1991), which would have prevented the gas exchange analysis and is known to provoke heterogeneous stomatal closure in some species. After closure, the system was equilibrated under the measurement conditions for 20 to 30 min before measurements were taken.

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Drought</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midday Ψₛₗₒᵣₗ (MPa) Treatment</td>
<td>-1.18 ± 0.520 (3)</td>
<td>-0.41 ± 0.139 (4)</td>
<td>&gt; -2.00 * (4)</td>
<td></td>
</tr>
<tr>
<td>Predawn Ψₛ (MPa) Control</td>
<td>-0.40 ± 0.020 (4)</td>
<td>-0.75 ± 0.052 (16)</td>
<td>-0.59 ± 0.034 (16)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>-1.17 ± 0.091 (15)</td>
<td>-0.98 ± 0.153 (16)</td>
<td>-2.62 ± 0.162 (16)</td>
<td></td>
</tr>
</tbody>
</table>
altered to account for the leak rate of the system as described in Li-Cor (1991). When the CO₂ depletion rate slowed in the lower part of the curve, CO₂ was removed by opening the CO₂ scrubbing circuit of the LI-6200 for 45 s, followed by a 5-min equilibration time before logging of observations resumed. The average change in CO₂ concentration per unit time during the scrub cycle was similar to that during the continuous upper portion of the curve. Ambient CO₂ concentration used for leak rate corrections was measured immediately after finishing a curve. The needles were dried for 48 h in a forced air oven at 70 °C, and dry weights of new and old needles were determined on cooled samples.

Curve-fitting and parameter calculations

Various nonlinear functions were fitted to the CO₂ response ($A/c_a$) data. Two three-parameter functions, the monomolecular (or Mitscherlich) function and the rectangular hyperbolic function, did not produce satisfactory fits. The four-parameter nonrectangular hyperbola suggested by Jones (1983) gave the best fit to the data:

$$A_{\text{net}} = \frac{A_{\text{max}} (c_i - \Gamma - A_{\text{net}} R_i)}{K_m + c_i - \Gamma - A_{\text{net}} R_i}.$$  \hspace{1cm} (1)

The fitted parameters ($K_m$, $A_{\text{max}}$, $\Gamma$ and $R_i$) of the hyperbolic function correspond to physiological properties of the photosynthetic system. The symbol $A_{\text{max}}$ is the CO₂ saturated rate of net photosynthesis and represents the maximum rate of RuBP regeneration by the Calvin cycle (Farquhar et al. 1980, von Caemmerer and Farquhar 1981). The symbol $\Gamma$ is the CO₂ compensation point, which is primarily determined by photorespiration rates. The symbol $K_m$ is a constant for Rubisco affinity for CO₂, but it may also be influenced by the light reactions (Jones 1983). The symbol $R_i$ is a measure of the resistance to liquid phase transport of CO₂ within the mesophyll, but may also be determined by the activity of the carboxylase which may be acting well below its maximum rate in the lower, near-linear portion of the response curve (Jones 1983). The carboxylation efficiency of Rubisco associated with changes in concentration or activity ($V_{\text{rubisco}}$) was calculated from the first derivative of the function, evaluated at $A = 0$, and is closely related to $1/R_i$.

Stomatal and mesophyll conductances to CO₂ for an atmospheric concentration of 350 μmol mol⁻¹ were obtained from the response curve data following the procedure described by Jones (1985). Stomatal conductance ($g_s$) defined the slope of the supply curve connecting $c_i$ with $c_1$ on the demand curve. Mesophyll conductance ($g_m$) was calculated as the slope of the demand ($A/c_a$) curve at its intersection with the supply curve. Stomatal limitation of photosynthesis ($L_s$) was calculated as in Jones (1985, Equation 17):

$$L_s = \frac{1/g_s}{1/g_s + 1/g_m}.$$  \hspace{1cm} (2)

Statistical analysis

For the control seedlings, the calculated variables were tested for linear and quadratic responses over the four control measurement dates. Analyses of variance and $a$ priori contrasts were performed to assess (a) the differences in response among the three drought cycles, and (b) the effect of the drought and recovery treatment versus control conditions. Before the analysis of variance, data variables were transformed as necessary to uphold the assumptions of normality and heteroscedasticity for the analysis of variance. No transformation was required for $A_{\text{max}}$ $V_{\text{rubisco}}$ and $g_s$. All analyses were performed using the General Linear Models procedure of SAS. Effects were considered statistically significant when the probability of a Type I error was 0.05 or less.

Results

Conductance to CO₂ and photosynthetic limitation

Stomatal conductance of control seedlings increased with seedling age, though only during Cycle 3 (Figure 1). Seedlings recovering from drought showed a similar trend, but stomatal conductance was generally lower in recovering seedlings than in control seedlings, especially in Cycles 1 and 3 (Cycle 2...
produced only a mild stress; Table 1). Drought caused a large decrease in stomatal conductance, but recovery following re-watering was rapid. The drought-induced reduction in stomatal conductance was least in Cycle 3, even though this was the most severe drought.

Mesophyll conductance increased linearly with age in control (Table 2) and drought-stressed seedlings (Figure 1), and there was no significant difference between control and drought-stressed seedlings (Table 3).

In control seedlings, the relative stomatal limitation of photosynthesis increased linearly with age (Table 2), rising from 6% at the beginning of the experiment to about 16% at the end (Figure 1). Stomatal limitation increased to about 40% during the period of maximum drought stress in Cycle 1, but the effect was much less during the drought periods in Cycles 2 and 3 (Figure 1). Relative stomatal limitation quickly declined to near control values on re-watering, and there were no significant differences between recovering and control seedlings in Cycles 2 and 3 (Table 3). Overall, the rate of photosynthesis was less affected by stomatal conductance than by mesophyll conductance.

Table 2. Regression analysis of the linear response of the measured variables in control seedlings with respect to time. The parameter estimates for intercept and slope, and the t-test probability that slope is not equal to zero are presented.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>Slope</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>g_s</td>
<td>1.64</td>
<td>0.0114</td>
<td>0.070</td>
</tr>
<tr>
<td>g_m</td>
<td>0.035</td>
<td>0.0049</td>
<td>0.001</td>
</tr>
<tr>
<td>L_s</td>
<td>0.048</td>
<td>0.0025</td>
<td>0.002</td>
</tr>
<tr>
<td>A_max</td>
<td>59.5</td>
<td>1.47</td>
<td>0.002</td>
</tr>
<tr>
<td>Γ</td>
<td>−0.089</td>
<td>0.934</td>
<td>0.001</td>
</tr>
<tr>
<td>K_m</td>
<td>4.45</td>
<td>0.248</td>
<td>0.002</td>
</tr>
<tr>
<td>V_rubisco</td>
<td>0.261</td>
<td>0.0046</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3. Statistical summary for gas exchange and photosynthetic parameters for control seedlings and treatment seedlings at the end of the recovery periods following each drought. Probability values from a two-way ANOVA with contrasts are presented.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>g_s</th>
<th>g_m</th>
<th>L_s</th>
<th>A_max</th>
<th>G</th>
<th>K_m</th>
<th>V_rubisco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle</td>
<td>2</td>
<td>0.002</td>
<td>0.001</td>
<td>0.018</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.004</td>
<td>0.498</td>
<td>0.080</td>
<td>0.690</td>
<td>0.015</td>
<td>0.010</td>
<td>0.042</td>
</tr>
<tr>
<td>Cycle × treatment</td>
<td>2</td>
<td>0.441</td>
<td>0.673</td>
<td>0.383</td>
<td>0.934</td>
<td>0.003</td>
<td>0.294</td>
<td>0.508</td>
</tr>
</tbody>
</table>

**Gas exchange parameters**

Most of the gas exchange parameters measured in control seedlings increased with time (Table 2 and Figure 2), whereas the effect of drought varied among the gas exchange parameters and drought cycles.

Gas exchange parameters of both control and recovering seedlings showed increases mostly in Cycle 3 (Table 3); however, Γ also increased from Cycle 1 to Cycle 2. By Cycle 3, Γ was lower in recovering seedlings than in control seedlings. Both control and recovering seedlings exhibited similar A_max. Drought only affected A_max during Cycles 1 and 2 (Figure 2 and Table 3). The value of K_m decreased with drought, but tended to recover to values greater than those of control seedlings on re-watering, but this effect was only statistically significant in Cycle 2 (Table 2 and Figure 2). There was a significant difference in V_rubisco between control and recovering seedlings late in Cycle 2 (Table 2 and Figure 2). Drought only affected V_rubisco during Cycle 2.

**Discussion**

**Ontogenetic changes**

As seedlings go through a series of drought cycles, they respond not only to water stress, but also to increasing age and ontogenetic state. The A/c_i curves of control seedlings showed changes in photosynthetic properties over time. The values of A_max, V_rubisco, and K_m increased with age (Figure 2), resulting in an increase in mesophyll conductance with age (Figure 1). All of these processes changed in concert, suggesting that this ontogenetic response is a reflection of the increasing photosynthetic competence of the needles as they mature (Ludlow and Wilson 1971, Field 1987).

The apparent trend in Γ may not be real, because values
below 10 µmol mol$^{-1}$ are not likely to occur in a C$_3$ plant (normal range is 30 to 70 µmol mol$^{-1}$). The data points at the bottom of the curve, which have the greatest influence on the value of $\Gamma$, have a lower precision than those at the top of the curve, due to the very low rates of gas exchange at low $e_c$. Unlike the other gas exchange parameters, the calculated values of $\Gamma$ were sensitive to the form of equation used to fit the data. The monomolecular function gave more realistic and consistent values of $\Gamma$ than the nonrectangular hyperbolic function, but the overall fit was not as good, especially for the upper portion of the curve.

Stomatal conductance increased with age. Relative stomatal limitation of photosynthesis also increased with age, because mesophyll conductance increased faster than stomatal conductance (Table 2 and Figure 2). Although both $g_a$ and $g_m$ increase with age in most plants, the relative rates of increase vary among species (e.g., Ludlow and Wilson 1971, Field 1987, Mebrahtu and Hanover 1991). Consequently, the change in relative stomatal limitation of photosynthesis can be positive, negative or constant, depending on the species.

Acclimation

Acclimation in drought-stressed seedlings is manifested as either a change in the degree of response to drought, or a persistent difference between recovering seedlings and the controls. Although the values of photosynthetic variables in treated and control seedlings followed similar trends with time, acclimation responses to the drought and recovery cycles were seen. The finding that $g_a$, $A_{\text{max}}$, and $\Gamma$ reacted to drought and rewatering in the first two cycles, but not in the third and most severe drought cycle, indicates that these variables exhibited decreasing sensitivity to drought (Figures 1 and 2). It is possible that stomatal sensitivity to drought decreased with both seedling age and drought history. Although a reduction in stomatal sensitivity with age has been reported for orange trees (Syvertsen et al. 1981), stomatal sensitivity to drought usually increases with age, especially in agricultural species (Field 1987).

A persistent difference between the control and recovering seedlings 7 days after drought relief was observed for $g_a$, $K_m$ and $V_{\text{rubisco}}$ (Figures 1 and 2, and Table 3). However, only $g_a$ showed a consistent difference over the three cycles. The responses of the other mesophyll photosynthesis variables were either significant only in the second cycle ($K_m$ and $V_{\text{rubisco}}$, or inconsistent in trend ($\Gamma$), possibly due to measurement error. In general, mesophyll photosynthetic function, as reflected by mesophyll conductance, was largely independent of water stress. In a broad range of species, stomatal closure occurs before mesophyll photosynthetic function is greatly affected by water stress (Gollan et al. 1985, Teskey et al. 1986, Kirschbaum 1987, Grieu et al. 1988, Grossnickle and Russell 1991). In several studies, mesophyll photosynthetic capacity under saturating CO$_2$ showed little response to dehydration at relative water contents as low as 50–70%, which is beyond the usual turgor loss point (Kaiser 1987, Cornic et al. 1989, Epron and Dreyer 1990).

It is possible that we observed only an apparent acclimation. Drought stress treatments can suspend or slow down the normal process of photosynthetic development (Ludlow and Ng 1974). During the recovery periods between droughts, the ontogenetic trends in $g_a$, $\Gamma$, $A_{\text{max}}$ and $V_{\text{rubisco}}$ continued at roughly the same rates in both control and drought-stressed seedlings (cf. slopes in Figures 1 and 2). The apparent acclimation response could, therefore, be due to an offset in the development curves of control and drought-stressed seedlings caused by the slowing or cessation of development during the drought periods.
Photosynthetic limitation and drought resistance

The finding that stomatal limitation was less than mesophyll limitation in black spruce has also been observed in other conifers (Teskey et al. 1986, Grieu et al. 1988, Seiler and Cazell 1990, Grossnickle and Russell 1991) and in C3 plants in general (Bunce 1977, Briggs et al. 1986, Ni and Pallardy 1992). The relative stomatal limitation at the beginning of the experiment was lower than most reported values; however, stomatal limitation increased with seedling age and was similar to values found in other black spruce trees (calculated from data in Macdonald and Lieffers 1990, Greenway et al. 1992).

The functional consequences of the drought-insensitivity of mesophyll photosynthetic processes combined with the small relative stomatal limitation of photosynthesis are illustrated by plotting $A_{\text{net}}$ against $c_i$, as determined at the operating point ($c_a = 350 \mu$mol mol$^{-1}$, Figure 3). For all seedlings pooled, there was a poor correlation between $A_{\text{net}}$ and $c_i$ ($R^2 = 0.019$). However, when the data were separated into well-watered (including recovering seedlings) and drought-stressed subsets, the regressions explained 45 and 31% of the variation, respectively. In drought-stressed seedlings, photosynthesis decreased with decreasing $c_i$ as the stomata closed, whereas in well-watered seedlings the relationship was negative. The photosynthetic rate largely determined $c_i$ and stomatal conductance had little influence. Thus, contrary to observations on several species by Wong et al. (1979), photosynthetic rate in our seedlings was largely uncoupled from stomatal behavior, except during periods of maximum drought when stomatal conductance was very low.

In cases of heterogeneous stomatal closure, the calculated values of $c_i$ are higher than the real values, which can give the appearance of mesophyll photosynthesis decreasing along with stomatal conductance. The lack of such a coordinated response to drought stress in our experiment supports our assumption that heterogeneous stomatal closure did not occur. Although there have been no reports of heterogeneous stomatal closure in conifers, we minimized the probability of patchy stomatal closure by taking measurements soon after full light exposure in high humidity on homobaric leaves that had been exposed to a slowly increasing drought stress.

Gollan et al. (1985) postulated that the ability to maintain constant mesophyll photosynthetic processes during water stress, where photosynthesis is reduced only by a reduction in stomatal conductance, is characteristic of a tolerance to soil drought. In black spruce, mesophyll response was negligible under drought conditions that produced soil water potentials of $-1.5 \text{ MPa}$. Limited mesophyll response to drought has been noted in several species including Abies bornmuelleri Matt. ($-1.0 \text{ MPa}$, Guehl et al. 1991), Eucalyptus pauciflora Sieb. ex Spreng. (Kirschbaum 1987), Pseudotsuga macrocarpa (Tor.) Mayr ($-1.5 \text{ MPa}$) and Pseudotsuga menziesii (Mirb.) Franco ($-1.9 \text{ MPa}$, Grieu et al. 1988). In Cedrus atlantica Manetti, the entire drought response was due to changes in mesophyll processes (Grieu et al. 1988, Guehl et al. 1991).

The drought resistance strategy of black spruce seedlings appears to be one of maximizing, rather than optimizing, photosynthetic rate (Tan et al. 1992). Under drought conditions, when stomatal closure limits CO$_2$ uptake as well as water loss, photosynthetic efficiency remains relatively unaffected. Maximizing growth, particularly of the roots, is important in establishment of young seedlings (van den Driessche 1987, Burdett 1990). Although high mesophyll photosynthetic capacity may be of little value during severe droughts when stomata close completely, during moderate droughts the maintenance of photosynthetic capability may improve the overall net carbon gain and growth of black spruce seedlings.

We were unable to demonstrate unequivocally that the drought treatments induced true active acclimation rather than delaying the ontogenetic processes in black spruce seedlings. However, our results show that photosynthetic capacity remained relatively unaffected by drought treatments causing predawn water potentials of $-1.5 \text{ MPa}$, although stomatal conductance was greatly reduced under such conditions. Stomatal conductance accounted for less than 20% of photosynthetic limitation under most conditions. Based on the variation in photosynthetic capacity ($A_{\text{max}}$) and efficiency ($V_{\text{rubisco}}$) found among black spruce seedlings, and the limited degree of control of photosynthetic rate by the stomata, we conclude that it would be more effective to select for high photosynthetic capacity than for reduced stomatal sensitivity when breeding for increased drought resistance in black spruce (cf. Lauer and Boyer 1992).

Acknowledgments

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Figure 3. The relationship between net photosynthetic rate ($A_{\text{net}}$) and intercellular CO$_2$ concentration ($c_i$) in black spruce seedlings, calculated for an ambient CO$_2$ concentration of 350 $\mu$mol mol$^{-1}$. Circles are control seedlings, triangles are seedlings under drought stress, and squares are seedlings recovering from drought. Open squares and triangles are seedlings in the middle of their respective recovery and drought phases; filled symbols are seedlings at the ends of their respective recovery and drought phases. Regression lines shown are for control and recovering seedlings: $A_{\text{net}} = 336.9 - 0.941c_i$ ($R^2 = 0.311$), and for drought-treated seedlings: $A_{\text{net}} = -39.39 + 0.407c_i$ ($R^2 = 0.447$).
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Li-Cor, Inc. 1991. A protocol for measuring assimilation rate versus internal CO2 concentration using the LI-6200. Application Note #103. LI-COR Inc., Lincoln, NB.


