S.N. Silim · R.D. Guy · T.B. Patterson
N.J. Livingston

# Plasticity in water-use efficiency of *Picea sitchensis*, *P. glauca* and their natural hybrids

**Abstract** Plasticity in water-use efficiency (WUE) was examined in populations of *Picea glauca* (Moench) Voss and *P. sitchensis* (Bong.) Carr. and their natural hybrids from an ecocline along the Skeena Valley, British Columbia, which runs from the dry continental interior (*P. glauca*) to the wet maritime Pacific coast (*P. sitchensis*). Seedlings were grown in a growth chamber and kept well-watered or repeatedly droughted for 3 months. Mean population photosynthetic WUE and total tissue $\delta^{13}C$ values were strongly correlated within and across treatments ($r=0.95$). There were also strong correlations ($r=0.60$–$0.80$) between individual seedling total tissue $\delta^{13}C$ and dry mass, and $\delta^{13}C$ and net photosynthesis ($A$), indicating that variation in $A$ was primarily responsible for differences in $\delta^{13}C$. When kept well-watered, *P. sitchensis* and the hybrids had higher $\delta^{13}C$ (–27.8‰ and –27.5‰, respectively) and higher dry mass (2.17 g and 1.99 g, respectively) than *P. glauca* (–28.2‰ and 1.68 g). Species ranking was reversed by repeated drought, with *P. glauca* and the hybrids having higher $\delta^{13}C$ (–25.6‰ and –25.5‰, respectively) and dry mass (1.10 g and 1.08 g, respectively) than *P. sitchensis* (–26.4‰ and 0.98 g). *P. glauca* had a smaller decrease in dry mass (35%) and a bigger increase in $\delta^{13}C$ (by 2.7‰) than *P. sitchensis* (55% and 1.4‰, respectively), with the hybrids in between (45% and 2.0‰, respectively). Drought also had a greater effect on $A$ in *P. sitchensis* (36% reduction) than in *P. glauca* (14% reduction) or the hybrids (24% reduction). Thus *P. glauca* and, to a lesser extent, hybrid populations, performed better and were more plastic than *P. sitchensis* in response to water defi-cit. Under the well-watered treatment, the hybrids behaved more like *P. sitchensis* in growth and WUE. These patterns are consistent with the seasonal variation in moisture availability that occurs along the introgression zone.

**Keywords** White spruce · Sitka spruce · Introgression zone · Carbon isotope discrimination · Drought response

S.N. Silim · R.D. Guy (✉) · T.B. Patterson
Department of Forest Sciences, University of British Columbia, Vancouver, Canada
e-mail: guy@interchange.ubc.ca
Fax: +1-604-8229102

N.J. Livingston
Biology Department, University of Victoria, Victoria, Canada

*Present address*:
S.N. Silim, Department of Biological Sciences,
University of Illinois at Chicago, Chicago, Illinois, USA

## Introduction

Phenotypic plasticity, the ability of an individual organism to alter its form or function in response to changes in environmental conditions, is believed to be an advantageous evolutionary response to environmental heterogeneity (Bradshaw 1965, 1973). Plants that grow in environments that are variable or unpredictable are expected to have a high degree of plasticity and high potential to acclimate (Bazzaz 1991).

*Picea glauca* (Moench) Voss (white spruce), a dominant species of northern conifer forests in North America, has an extremely wide geographical and ecological range extending from Newfoundland in the east to northern British Columbia and Alaska in the west (Nienstaedt and Zasada 1990). It is abundant across environments encompassing a wide range of moisture availability, soil fertility and temperature and can grow to about 30 m in height with a diameter of about 0.9 m at maturity, and live up to 200–300 years. *Picea sitchensis* (Bong.) Carr. (Sitka spruce) occurs along a narrow belt of the Pacific coast from northern California to southern Alaska, its eastward range restricted by steep mountain ranges close to the Pacific Ocean (Harris 1990). It can commonly live for 700–800 years and may exceed 60 m in height with a diameter of about 3 m at maturity. In British Columbia and southern Alaska, both species occur along a similar latitudinal range but within distinct climatic and ecological regions, physically separated from each other. The fog belt of the Pacific Coast, where *P. sitchensis* occurs, is characterised by high summer and winter precipita-

tion, low evaporative demands and moderate temperatures. In contrast, strong seasonal variations in temperature (cold winters and hot summers), limited precipitation and high evaporative demands characterise the interior where *P. glauca* is found (Daubenmire 1968).

In areas where large river drainages connect the coast with the interior, such as the Skeena, Nass and Bulkley valleys, narrow unbroken transitions between the flora of the oceanic climate along the coast and the continental climate of the interior occur (Daubenmire 1968). In these areas, *P. sitchensis* and *P. glauca* hybridise introgressively. From the coast to interior the environment becomes progressively drier, more continental, and variable, within and between growing seasons. Coastal and interior ends of this ecocline are occupied by pure *P. sitchensis* or *P. glauca*, respectively, while the hybrids thrive in between (Daubenmire 1968). Because of this extreme environmental gradient and the variable climate of the interior, populations of the two species and their hybrids would be subjected to different selection pressures that operate at each point of the gradient. This should give rise to phenotypes adapted to the environment of their locality along the gradient. Indeed, introgressive hybridisation is considered to be an evolutionary mechanism allowing plant populations to exploit intermediate environments (Rieseberg and Carney 1998).

Introgression between *P. glauca* and *P. sitchensis* provides a unique opportunity to examine hybridisation and plasticity as alternative or complementary devices to accommodate spatial and temporal variation in the environment. Other than temperature, the major abiotic condition that differs between the habitats of the two species is moisture availability. Plants exposed to frequent moisture deficits in their natural habitats are believed to have evolved mechanisms that conserve water, whereas those from wet habitats are expected to utilise water less sparingly (Anderson et al. 1996). Water-use efficiency (WUE) is the dry matter produced over the life of a crop divided by the total volume of water used; or the amount of carbon gained per unit leaf water lost. In unpredictable environments, a flexible WUE could allow full exploitation of transiently favourable conditions, without compromising production. Where conditions are more uniform, either wet or dry, plasticity in WUE may not be so critical and, thus, have no adaptive advantage.

The stable carbon isotope composition ($\delta^{13}C$) of plant tissue has been widely used as an integrative measure of WUE in ecological and agronomic studies. Plants discriminate against $^{13}CO_2$ over the lighter and more abundant $^{12}CO_2$ during photosynthesis, resulting in $\delta^{13}C$ values that are lower than the $CO_2$ source. In C3 plants this discrimination is determined largely by the ratio of the concentration of intercellular to atmospheric $CO_2$, $c_i/c_a$ (Farquhar et al. 1982, 1989), which is, in turn, directly affected by the balance between net assimilation rate ($A$) and stomatal conductance ($g_s$). $c_i/c_a$ is inversely related to the ratio of $A$ and transpiration ($E$), if environmental vapour pressure deficit is held constant (Farquhar et al. 1989). There exists, therefore, an inverse linear relationship between plant isotope discrimination and *A/E*, or instantaneous water-use efficiency.

Differences in $c_i/c_a$ and thus $\delta^{13}C$, occur as a result of variations in either stomatal conductance or photosynthetic capacity (Hubick et al. 1986; Condon et al. 1987; Ehleringer 1990). The effects of environmental or physiological factors on *A* or $g_s$ in C3 plants will, therefore, be reflected in tissue $\delta^{13}C$ values. A high $\delta^{13}C$ caused by a large photosynthetic capacity, in contrast to one caused by decreased $g_s$, should be positively correlated with $CO_2$ assimilation rate and dry matter production (Hubick et al. 1986; O'Leary 1988).

This study investigates physiological adaptations and plasticity in WUE in seedling populations of *P. glauca* and *P. sitchensis*, and their hybrids originating from the Skeena River valley of British Columbia. In recent years, considerable interest in elucidating the nature and degree of differences in WUE among populations of native tree species has been shown, much of it devoted to examining carbon isotope discrimination in relation to the environment (e.g. Read and Farquhar 1991; Meinzer et al. 1992; Zhang and Marshall 1994; Flanagan and Johnsen 1995; Sun et al. 1996; Lauteri et al. 1997; Fan et al. 1999; Olivas-Garcia et al. 2000). Less attention has been given to differences in plasticity in this character. Most studies, however, have reported little genotype by environment interaction at the population or family level (e.g. Zhang and Marshall 1994; Sun et al. 1996; Guy and Holowachuk 2001). We hypothesised that this may not be the case at greater genetic distances (i.e. between closely related species) and, furthermore, that plasticity in WUE should reflect the degree of temporal variation at the habitat of origin.

## Materials and methods

### Plant material and growth conditions

Seeds used in this study were obtained from bulk seed collections of the British Columbia Ministry of Forests. They were collected from natural stands of *P. sitchensis*, *P. sitchensis* × *P. glauca* putative hybrids, and *P. glauca* along the Skeena River valley (Fig. 1). A total of 14 seedlots were used; 4 were from *P. sitchensis* stands, 5 were from *P. glauca* stands, and 5 were of *P. sitchensis* × *P. glauca* hybrids from stands within the introgression zone.

Forty seedlings from each seedlot were grown in a growth chamber for 3 months, in a peat-vermiculite mixture in 50-cm³ plastic containers arranged on plastic trays. During the experimental period, the trays were rotated once every week to reduce location effects within the growth chamber. Day-time and night-time temperatures were maintained at 23°C and 18°C, respectively, with an 18 h photoperiod at about 300 µmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) and about 65% relative humidity. All the containers were watered every other day and fertilised once a week for 3 weeks after germination. Following this, 20 seedlings from each population were subjected to a simulated drought condition while the rest were watered regularly. The watering regimes consisted of the following treatments: (1) watered every other day (well-watered treatment), and (2) watered only when the soil water potential reached about –2 MPa (drought treatment). This point was determined by weighing individual containers and re-watering when the average pot weight for the population reached a predetermined value corresponding to a soil water potential of about

**Fig. 1** Map of British Columbia showing the origins of the different populations of *Picea sitchensis* (*1–4*), *P. glauca* (*10–14*) and their hybrids (*5–9*) along the Skeena River Valley. British Columbia Ministry of Forests seedlot numbers and elevations of the seed sources are: 2794, 92 m; 4115, 20 m; 4116, 100 m; 4112, 30 m; 2775, 61 m; 2857, 61 m; 1004, 305 m; 1855, 579 m; 3988, 749 m; 1409, 762m; 4075, 790 m; 4925, 990m; 4416, 975m; and 4924, 914 m; respectively for populations 1–14
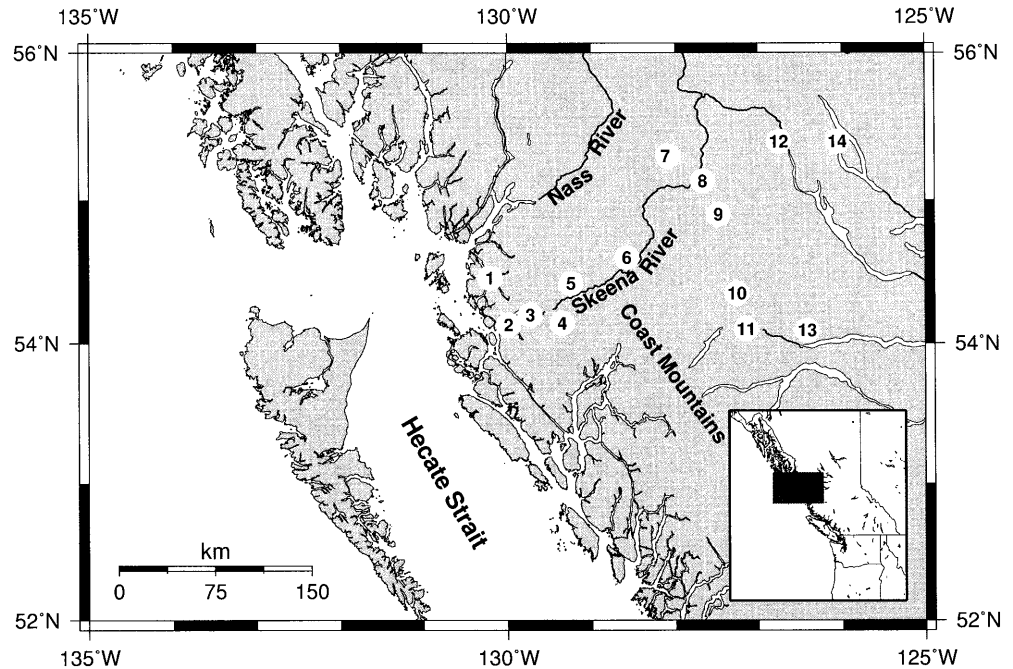


–2 MPa. This value was obtained by relating water potential (measured psychrometically) and peat weight as the peat was gradually dried down. Adjustments were made for the weight of the growing seedlings in the containers, based on an estimate of the relationship between seedling height and weight. All plants were fertilised with commercial 20:8:20 N-P-K fertiliser and micronutrients (Plant Products, Brampton, Ontario) at a rate of 5 mM N, on the same day that the droughted plants were watered.

Gas exchange measurements

After nine drought cycles of about 6 days each, $A$, $g_s$ and $E$ were measured on five randomly picked seedlings from each population in the drought and the well-watered treatments on the same day, 1 day after watering. Another measurement was done at the end of the drought cycle, in seedlings subjected to the drought treatment. Because the seedlings were actively growing measurements were done on the portion of the shoots where needle growth was complete. Measurements were performed using an LI-6200 portable photosynthesis system (Li-Cor, Lincoln, Neb.) at approximately 750 μmol m$^{-2}$ s$^{-1}$ PPFD, provided by a halogen lamp. This PPFD was found to be saturating for the plants. The ambient temperature in the measuring cuvette was about 24°C with a relative humidity of about 60% and a $CO_2$ concentration of about 360 μmol mol$^{-1}$. $A$, $E$, $g_s$, $c_i/c_a$ and instantaneous WUE were calculated according to von Caemmerer and Farquhar (1981). The projected area of the needles used in gas exchange analysis was determined using an LI-3100 leaf area meter (Li-Cor).

After gas exchange measurements were complete, the peat was carefully and thoroughly washed from the roots. Seedlings were frozen in liquid $N_2$ then freeze-dried and later used for dry mass and δ$^{13}$C determinations.

Carbon isotope composition and dry mass production

Six seedlings, the five from gas exchange measurements above, plus one additional, for each population and treatment were used for δ$^{13}$C and dry mass determinations. Whole seedlings were pulverised to fine powder using a stainless-steel planetary ball mill. One milligram subsamples were analysed for δ$^{13}$C using a model 1106 Elemental Analyser (Carlo Erba, Valencia, Calif., USA) interfaced to a Prism triple-collecting ratio mass spectrometer (VG Isotech, Middlewich, UK).

δ$^{13}$C is defined as the $^{13}$C/$^{12}$C of organic C in a plant sample relative to an arbitrary standard, Vienna Peedee belimnite (VPDB):

$$\delta^{13}C\ (‰)=(R_{sample}-R_{standard}/R_{standard})\times1{,}000$$

where $R_{sample}$ and $R_{standard}$ are the ratios of $^{13}$C/$^{12}$C in the sample and in the standard, respectively. One random duplicate measurement for every 20 samples was used to check the accuracy of the analysis.

Data analysis

The experiment was laid out in a completely randomised block design. All the statistical analyses on the data were performed using Systat version 4.0 (SYSTAT, Evanston, Ill.). Correlation analysis was performed to examine the relationships between δ$^{13}$C and seedling dry mass, δ$^{13}$C and $A$, and δ$^{13}$C and $g_s$. A nested factorial analysis of variance was performed to compare differences between watering treatments, among species (i.e. *P. glauca*, *P. sitchensis*, and the grouped hybrids) and among populations (within species) in δ$^{13}$C, $A$, $g_s$, WUE, dry mass and $c_i/c_a$.

# Results

## Effect of watering regime

The two watering regimes resulted in significant ($P<0.01$) differences in $A$, dry mass, $g_s$, WUE and δ$^{13}$C (data not shown). Compared to the well-watered seedlings, seedlings which had undergone the drought treatment during their growth had about 20% lower $A$, 50% lower $g_s$ (measured 1 day after watering) and 45% lower dry mass, while WUE was about 35% higher and δ$^{13}$C was about 2‰ higher. Significantly greater differences in gas exchange parameters occurred during periods when

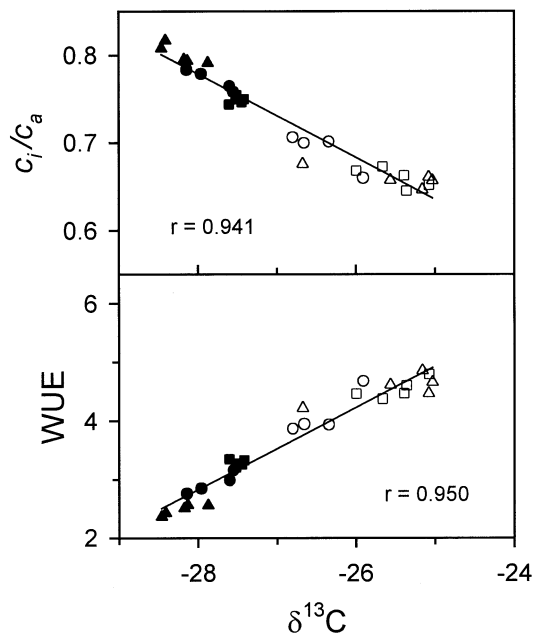**Fig. 2** The relationship between $\delta^{13}C$ (‰) and $c_i/c_a$, and $\delta^{13}C$ and WUE in populations of *P. sitchensis* (○), *P. glauca* (△) and their hybrids (□). The well-watered and droughted treatments are shown as *solid* and *open* symbols, respectively. Each point represents means of five measurements for $c_i/c_a$ and WUE, and six measurements for $\delta^{13}C$. Error bars omitted for clarity. All gas exchange measurements were taken at the beginning of the drought cycle

the droughted plants were experiencing moisture stress. At the end of the drought cycle, $A$ and $g_s$ averaged about 70% and 85% lower, respectively, in the droughted seedlings as compared to the well-watered ones, whilst WUE was about 55% higher.

## Relationship between $\delta^{13}C$ and WUE

A strong positive correlation was observed between mean total tissue $\delta^{13}C$ ($r=0.950$) and WUE, and a strong negative correlation between $\delta^{13}C$ and $c_i/c_a$ ($r=0.941$) for all the populations and treatments (Fig. 2). Generally, populations with the lowest WUE and $\delta^{13}C$ values under the well-watered treatment belonged to *P. glauca*, while hybrid populations and *P. sitchensis* had higher WUE and $\delta^{13}C$ values. Under the drought treatment, however,

species ranking was changed; populations of *P. sitchensis* generally had lower WUE and $\delta^{13}C$ values, whereas the *P. glauca* and hybrid populations had higher $\delta^{13}C$ values and WUE. A similar trend was found between $\delta^{13}C$ and $c_i/c_a$ among populations of the two species and their hybrids.

### Species-level variation in drought response

In the well-watered treatment, *P. sitchensis* had the highest total dry mass and $A$ followed by the hybrids, while *P. glauca* had the lowest dry mass and $A$ (Table 1). Under the drought treatment, however, *P. sitchensis* had the lowest dry mass and $A$ while both *P. glauca* and the hybrids had slightly, but significantly ($P<0.01$), higher values. Overall, the drought treatment reduced $A$ and dry mass in *P. sitchensis* much more (36% and 55%, respectively) than in either *P. glauca* (14% and 35%, respectively) or the hybrids (24% and 45%, respectively).

Mean total tissue $\delta^{13}C$ value, under the well-watered treatment, was lowest in *P. glauca* seedlings followed by *P. sitchensis*, while the hybrids collectively had the highest value (Table 1). In seedlings raised under the drought condition, on the other hand, *P. sitchensis* had a significantly ($P<0.01$) lower mean $\delta^{13}C$ value than either *P. glauca* or the hybrids. Thus, *P. sitchensis*, again, had the smallest change in $\delta^{13}C$ value (an increase of 1.4‰) in response to the drought treatment. *P. glauca* had the greatest increase in $\delta^{13}C$ (2.7‰) while the hybrids averaged an increase of 2‰, intermediate to the two species.

$g_s$ in the well-watered seedlings was similar in *P. sitchensis* and *P. glauca* but slightly lower in the hybrids (Table 1). In seedlings raised under the drought condition, *P. sitchensis* seedlings had a slightly higher $g_s$ (significant at $P<0.05$) than either *P. glauca* or the hybrids although these differences were small. In contrast to the magnitude of reductions in $A$, above, as a result of the drought treatment, $g_s$ was reduced by about the same magnitude in *P. sitchensis*, *P. glauca* and the hybrids (54%, 55% and 52% reductions, respectively).

Figure 3 shows the relationship between the measured mean population $A$ and the corresponding calculated $c_i$ for seedlings raised under the well-watered conditions and those raised under the drought cycle (including measurements at the end of the drought cycle) in the two species and their hybrids. When grown under the well-

**Table 1** Dry mass accumulation, net photosynthesis, $\delta^{13}C$ and stomatal conductance in *Picea sitchensis*, *P. glauca* and their hybrids under the well-watered and drought treatments. Values are means ± SE of 20–30 observations. All gas exchange measurements were taken at the beginning of the drought cycle

| Species | Total plant dry mass (g dry wt) | | Net photosynthesis (µmol $CO_2$ m$^{-2}$ s$^{-1}$) | | $\delta^{13}C$ (‰) | | Stomatal conductance (mol $H_2O$ m$^{-2}$ s$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | Watered | Drought | Watered | Drought | Watered | Drought | Watered | Drought |
| *P. sitchensis* | 2.17±0.07 | 0.98±0.04 | 6.92±0.15 | 4.60±0.10 | −27.81±0.10 | −26.43±0.16 | 0.148±0.004 | 0.071±0.002 |
| *P. glauca* | 1.68±0.09 | 1.10±0.05 | 5.78±0.12 | 4.97±0.11 | −28.22±0.11 | −25.55±0.15 | 0.147±0.004 | 0.066±0.002 |
| Hybrids | 1.99±0.06 | 1.08±0.03 | 6.26±0.13 | 4.84±0.12 | −27.50±0.11 | −25.49±0.14 | 0.138±0.003 | 0.065±0.002 |

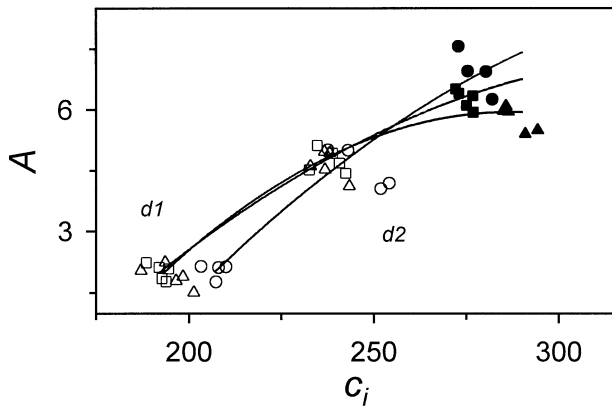**Fig. 3** The relationship between net photosynthesis, $A$ (µmol $CO_2$ m$^{-2}$ s$^{-1}$) and the observed corresponding intercellular concentration of $CO_2$, $c_i$, in populations of *P. sitchensis*, *P. glauca* and their hybrids. Symbols as in Fig. 2. *d1* and *d2* represent the end and beginning of the drought cycle, respectively
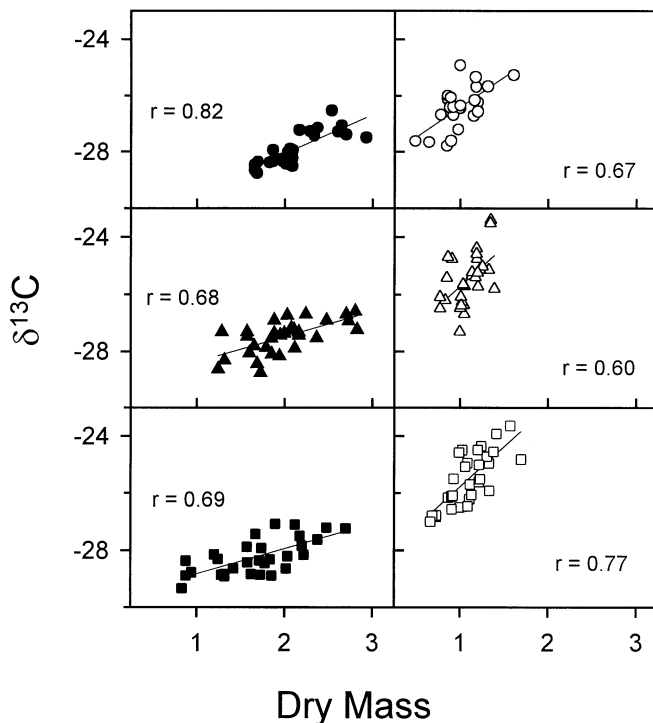


**Fig. 4** Relationship between $\delta^{13}C$ (‰) and dry mass (g dry wt) in *P. sitchensis*, the hybrids and *P. glauca* under the well-watered and drought treatments. Symbols as in Fig. 2. Each data point represents a single seedling



**Fig. 5** Net photosynthesis, $A$ (µmol $CO_2$ m$^{-2}$ s$^{-1}$) and stomatal conductance, $g_s$ (mol $H_2O$ m$^{-2}$ s$^{-1}$) in relation to $\delta^{13}C$ (‰) in seedlings of *P. sitchensis*, the hybrids and *P. glauca*. Symbols as in Fig. 2. Each point represents a single seedling. All gas exchange measurements were taken at the beginning of the drought cycle

accumulated the most dry mass also had higher total tissue $\delta^{13}C$. This correlation was found under both the well-watered and drought treatments, although the slope of the relationship was steeper under the drought treatment.

Corresponding to the correlation between $\delta^{13}C$ and dry mass, above, there was also a strong positive correlation between $A$ and total tissue $\delta^{13}C$ ($r=0.71$–$0.79$, $P<0.05$) within species and treatments (Fig. 5); individuals with the highest $\delta^{13}C$ also had the highest $A$. However, there was only a weak or no correlation between $\delta^{13}C$ and $g_s$. Between the watering treatments, on the other hand, both $A$ and $g_s$ were negatively correlated with $\delta^{13}C$ (data not shown), as is commonly the case. The slope of this relationship was different among species and their hybrids, however; the biggest change in $\delta^{13}C$ with a change in $A$ was in *P. glauca* followed by the hybrids, while *P. sitchensis* had the smallest change (see also Table 1). A similar but weaker pattern was found in the relationship between $\delta^{13}C$ and $g_s$.

Population-level variation in drought response

Within each species and the hybrids, significant ($P<0.01$) population differences were observed in $A$, $\delta^{13}C$, and dry mass production under both watering regimes (Fig. 6). Generally, however, population differences in $A$ and dry mass, but not in $\delta^{13}C$, were smaller under the drought

watered condition, *P. sitchensis* populations had higher $A$ than *P. glauca* populations for a given $c_i$ level, while the hybrids were intermediate. When grown under the drought conditions, however, the pattern was reversed; both *P. glauca* and the hybrid populations maintained higher levels of $A$ but at a lower $c_i$, than *P. sitchensis* populations.

Within each treatment and species, there was a strong positive correlation ($r=0.60$–$0.82$, $P<0.05$) between total tissue $\delta^{13}C$ and total dry mass (Fig. 4); individuals which
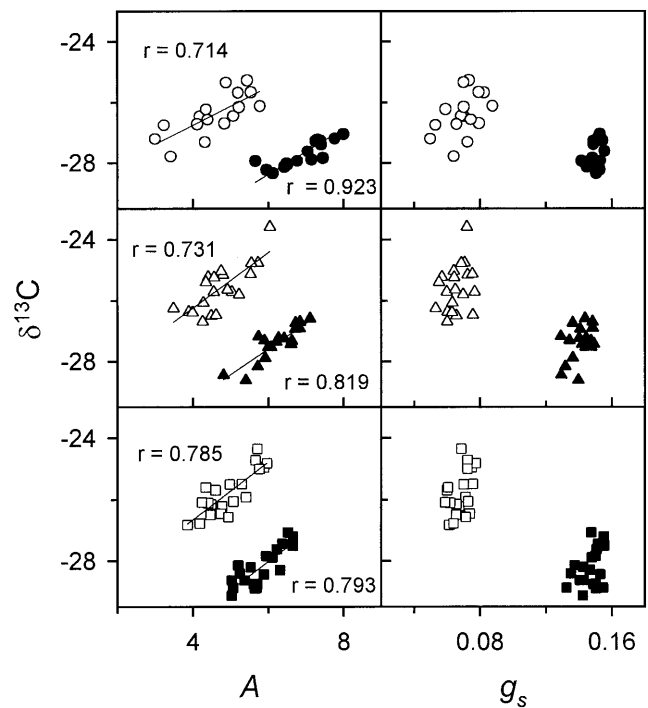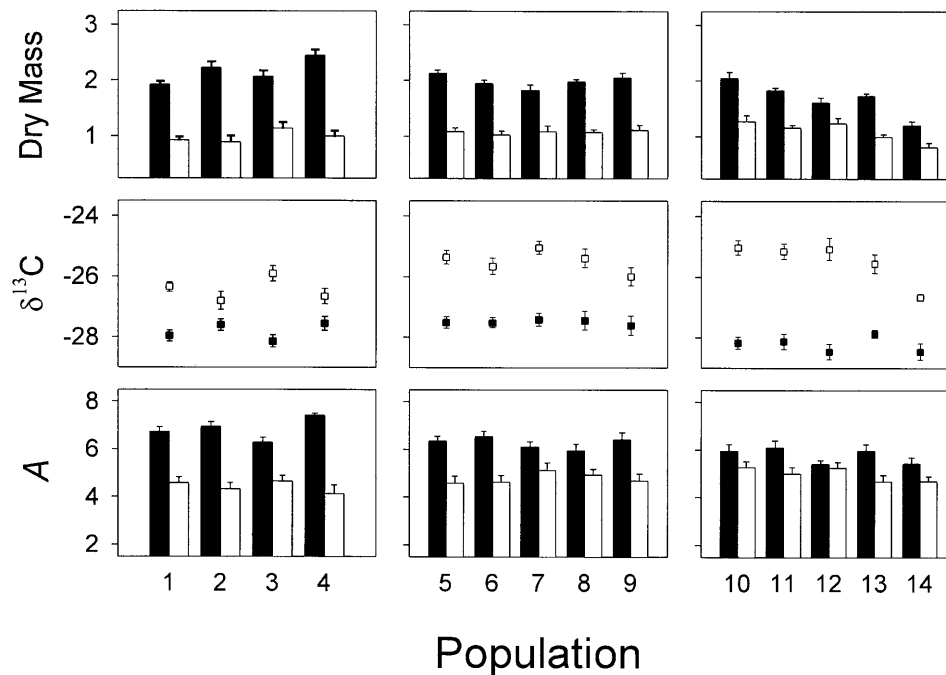
**Fig. 6** Dry mass (g dry wt), $\delta^{13}C$ (‰) and net photosynthesis, $A$ (µmol $CO_2$ $m^{-2}$ $s^{-1}$), in response to the watering treatment in seedlings of populations of *P. sitchensis* (1–4), hybrids (5–9) and *P. glauca* (10–14). *Solid* and *open* symbols are the well-watered and droughted seedlings, respectively. All gas exchange measurements were taken at the beginning of the drought cycle



treatment than under the well-watered treatment. The hybrid populations were also more uniform in $A$ and dry mass production than populations of either *P. glauca* or *P. sitchensis*. Differences between $\delta^{13}C$ values of hybrid populations were observed only under the drought treatment. In *P. sitchensis* and *P. glauca*, population differences were observed in all the variables, except $A$ under the drought treatment. Thus, observed population differences in $A$ under the well-watered treatment generally disappeared under the drought treatment when measured 1 day after watering. In contrast to the changes in ranking among species and their hybrid, the ranking and thus degree of response of the different populations within each species for each of the examined variables, did not change significantly as a result of the watering regime.

## Discussion

*Picea sitchensis* occurs in relatively rich habitats with minimal environmental stresses while *P. glauca* occupies habitats characterised by high environmental stresses and variable, often limited, resource supply. Generally, species or populations adapted to low-stress, high-resource environments develop mechanisms that utilise abundant resources, resulting in rapid growth and low stress resistance. Conversely, species or populations adapted to resource-limited environments and/or stressful environments, develop mechanisms that allow them to balance survival with growth more effectively (Grime 1979; Tilman 1988).

In this study, we characterised some mechanisms that permit the unique ecological distribution of spruce populations along the moisture gradient of the Skeena River valley. Distinct adaptive responses in gas exchange pa-

rameters, WUE and growth of the two species and their hybrids, as means of coping with the soil moisture deficit, were displayed. Through greater plasticity in photosynthetic characteristics, populations of *P. glauca* were able to ameliorate the costs of carbon gain much better than populations of *P. sitchensis* when soil moisture was limited, a behaviour consistent with the environments of their native habitats.

### Effect of watering regime

The reductions in $A$, $g_s$, growth and carbon isotope discrimination, and increase in WUE observed here, as a result of the drought treatment, are consistent with expected trends and patterns of responses commonly observed in other studies (e.g. DeLucia et al. 1989).

Soil moisture availability affects both leaf conductance and photosynthesis, but $g_s$ is believed to be more sensitive than $A$ (Schulze et al. 1989). Consistent with this, we observed that even when soil moisture was not limiting, seedlings which had been subjected to repeated drought during their growth had about 50% lower $g_s$ but only about 20% lower $A$ than the seedlings that were never water stressed. As a result of these adjustments in $g_s$ and $A$, WUE was enhanced (by about 35%) in the drought-hardened plants. Paralleling this increase in WUE was a decrease in carbon isotope discrimination, as indicated by higher tissue $\delta^{13}C$ values. Other studies have also found greater decreases in $g_s$ than in $A$ under water-limited conditions in forest trees (Monson and Grant 1989; Green and Mitchell 1992; Zhang and Marshall 1994) and crop plants (Ismail et al. 1994).

Growth of seedlings was also strongly affected by the drought treatment, as expected. Dry mass was reduced

by about 45% when the seedlings were grown under the water-limited conditions compared to the seedlings raised with ample soil moisture. The drought-induced reduction in growth can be partly explained by the reduction in $A$.

## Relationship between $\delta^{13}C$ and WUE

A positive correlation between population mean total tissue $\delta^{13}C$ and WUE, and a negative correlation between total tissue $\delta^{13}C$ and $c_i/c_a$ was observed under both watering treatments (Fig. 2), as theory would predict (Farquhar et al. 1982, 1989; O'Leary 1988). Repeated drought resulted in lower $g_s$ and lower $c_i/c_a$, even after watering. This, in turn, resulted in reduced carbon isotope discrimination and increased WUE, as noted above.

## Species-level variation in drought response

Significant differences in ecophysiological behaviour were observed between *P. glauca* and *P. sitchensis* in response to the watering regimes (Table 1), reflecting adaptations to their native habitats. The two species and their hybrids displayed different magnitudes of change in $\delta^{13}C$, $A$ and dry mass production in response to the drought treatment. Repeated drought resulted in a reversal of species ranking. For example, under the well-watered treatment, *P. sitchensis* and the hybrids had much higher total dry mass and $A$ than *P. glauca*. In the drought treatment, however, the pattern was reversed; both *P. glauca* and the hybrids had higher dry mass and $A$ than *P. sitchensis*. Mean total tissue $\delta^{13}C$ value, which was lowest in *P. glauca* and highest in the hybrids and *P. sitchensis* (see also Fig. 2) in the well-watered treatment, was lowest in *P. sitchensis* when soil moisture was limited. Thus, the drought treatment reduced dry mass and $A$ in *P. sitchensis* much more than in either *P. glauca* or the hybrids.

*P. glauca* appears to be better adapted to drought at a minimal cost to growth, a trait that was also displayed by the hybrids. *P. sitchensis*, on the other hand, is less well adapted to growth under conditions of poor or variable moisture supply, as evidenced by its greater reductions in dry mass and $A$, and smaller adjustments in tissue $\delta^{13}C$ values. When soil moisture supply was kept high, however, *P. sitchensis* displayed a superior balance between carbon gain and water loss (as evidenced by higher growth and $\delta^{13}C$ values) than *P. glauca*, a trait that was also displayed by the hybrids. Collectively, the hybrids appear to have inherited the contrasting superior performances of both parents to soil moisture supply, allowing relatively good growth under either moisture regime.

Figure 3 indicates that the two species and their hybrids attained different levels of $A$ at similar $c_i$ in a treatment-dependent manner. For a given $c_i$ in the well-watered treatment, populations of *P. sitchensis* had higher $A$ than populations of *P. glauca* while the hybrids were intermediate. This pattern was reversed under the drought treatment, where populations of *P. glauca* and the hybrids had higher levels of $A$ but at a lower $c_i$, than *P. sitchensis*.

Since $g_s$ was reduced to similar degree in the two species and their hybrids, but reductions in $A$ varied, it is likely that leaf-level factors other than $g_s$ may also have affected $A$ in *P. sitchensis* raised under the drought treatment. There may be species differences in ability to adjust carboxylating capacity in response to water supply. Such differences can result from changes in ribulose 1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) activity or RuBP regeneration (Sharkey 1985). A low carboxylating efficiency under water stress would result in further reductions to $A$, raising $c_i$ and reducing $\delta^{13}C$ (Farquhar et al. 1982). Reductions in foliar nitrogen concentration can also result in decreased carboxylating efficiency (Field and Mooney 1986; Schulze et al. 1998; Cordell et al. 1999). We did not measure nitrogen content in this study.

Similar to the observations of Wright et al. (1988), Sun et al. (1996) and Flanagan and Johnsen (1995), a strong positive correlation between total tissue $\delta^{13}C$ and total dry mass was found (Fig. 4). This relationship implies that variation in $A$, rather than $g_s$, is the predominant factor determining $c_i/c_a$, and thus $\delta^{13}C$ (Hubick et al. 1986; Wright et al. 1988, 1993; Ehleringer 1990). Consistent with this, a strong positive correlation between total tissue $\delta^{13}C$ and $A$ was observed within species (Fig. 5); individuals with the highest $\delta^{13}C$ also had the highest $A$. Thus, our results indicate that in *P. glauca*, *P. sitchensis* and their hybrids, variation in $\delta^{13}C$ (or WUE) is linked primarily to differences in photosynthetic capacity rather than stomatal conductance.

Gas exchange characteristics and $^{13}C$ discrimination in *P. sitchensis* and *P. glauca* reported in this study are similar to observations on populations of *Eucalyptus camaldulensis* (Gibson et al. 1991), *Banksia* spp. (Hubick and Gibson 1993) and *Nothofagus* spp. (Read and Farquhar 1991) in that, when grown in environments where soil moisture was not limiting, populations or species with the highest WUE and lowest $^{13}C$ discrimination originated from wetter habitats, while those from drier habitats had lower WUE. *P. glauca*, from a drier environment, displayed an opportunistic response with profligate water use resulting in a low WUE when soil moisture was abundant. Under the same conditions, *P. sitchensis* displayed a high WUE as a result of a higher $A$. Thus, the apparent high WUE displayed by *P. sitchensis* when soil moisture is not limited is likely to be an adaptation related to competition for other resources, and not water.

## Population-level variation in drought response

Population differences in $A$, $\delta^{13}C$, and dry mass production were found in seedlings of *P. glauca*, *P. sitchensis* and their hybrids (Fig. 6), similar to other reports on co-

nifers (e.g. Zhang and Marshall 1994; Flanagan and Johnsen 1995; Sun et al. 1996). Except for $\delta^{13}C$, however, these differences were either absent or much smaller when seedlings were raised under the drought treatment.

Consistent with other studies of genotypic variation in trees (Zhang and Marshall 1994; Flanagan and Johnsen 1995; Sun et al. 1996; Lauteri et al. 1997; Olivas-Garcia 2000), the ranking and degree of response in $\delta^{13}C$ of the different populations within each species generally remained the same under both watering regimes. Genotypic ranking in isotope discrimination, and presumably the associated underlying physiological mechanisms, is considered to be conservative in contrasting environments (Johnson et al. 1990; Comstock and Ehleringer 1992), reflecting little difference in plasticity of WUE among populations. This is in contrast to the changes in ranking, and therefore differences in plasticity, seen here between the species and their collective hybrids.

In conclusion, our results indicate distinct adaptive differences in the mechanisms by which *P. glauca*, *P. sitchensis* and their natural hybrid populations cope with variable soil moisture supply. When soil moisture was limited, populations of *P. glauca* were able to adjust their WUE at minimal cost to growth, thus displaying a more plastic WUE. This behaviour is consistent with the greater inter- and intra-annual variation in water supply typical of the continental interior. To some extent, the hybrid populations also displayed this trait. *P. sitchensis*, on the other hand, did not perform as well under similar conditions. However, when water was not limited, *P. sitchensis*, and the hybrid populations again, displayed a superior balance between carbon gain and water loss, a trait not associated here with adaptation to soil moisture deficit. Generally, the hybrid populations had intermediate behaviour, but also appeared to have inherited superior characteristics of both parents in response to soil moisture supply, allowing them to perform as well as the superior parental species under the prevailing soil moisture regime.

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