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**SEED-SOURCE VARIATION IN CARBON ALLOCATION AND CARBON
ISOTOPE DISCRIMINATION IN JUVENILE BLACK SPRUCE**

(*Picea mariana* (Mill.) B.S.P.)

by

MIKAÏLOU SY

B.Sc.A. (1991), M.Sc. (1993) Université Laval

**A thesis submitted to the Faculty of Graduate Studies
in partial fulfillment of
the requirements for the degree of**

Doctor of Philosophy

Carleton University

Ottawa, Ontario

13th October, 1998

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in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Chair, Department of Biology

Thesis Supervisor

External Examiner

Carleton University

January 1999

ABSTRACT

The covariance among growth, carbon allocation and leaf physiological characteristics was studied in black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings. Seedlots for 13 populations, from across the natural range of the species, and eight full-sib families, from three provenances within Ontario, were obtained. Seedlings from these populations and families were grown in two controlled environment chambers that simulated northern and southern environmental conditions. In addition, the 13 populations were grown together in a field experiment in Ottawa. The hypotheses tested were i) that populations allocating a higher proportion of carbon to roots also have a lower stomatal conductance in relation to photosynthetic capacity; ii) that more northerly populations exhibit a more conservative suite of characteristics (eg. higher root/shoot ratio, higher water-use efficiency); and iii) that strong genetic control on water conservation results in a consistent ranking of populations across environments for a given trait. The results indicate significant differentiation in a number of traits. The compromise in carbon allocation and that in leaf physiology appear to have evolved in response to different ecological components: the former responding to seed-source temperature and photoperiod while the latter responding to combined effects seed-source moisture and cultivation temperature. In general, populations of black spruce exhibit high plasticity at the leaf level, expressing more conservative traits in environments that least reflect their origin.

Accordingly, the two first hypotheses find support only under the simulated southern environment. Differentiation in carbon isotope discrimination (Δ) and thus in water-use efficiency, results primarily from variation in stomatal conductance when water is not limiting. However, there are families that conserve water via increased photosynthetic capacity and, consequently, may grow faster. There also is a threshold moisture below which black spruce populations shift from primary limitation to photosynthetic gas exchange caused by stomatal conductance to primary limitation caused by mesophyll biochemical capacity for CO₂ fixation. High plasticity of carbon allocation in time and leaf physiological characteristics across environments results in inconsistent rankings of populations. The use of Δ as a criterion when selecting for water conservation will come only at the cost of reduced growth.

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LIST OF ABBREVIATIONS

μmol	micromole
%	percent
^{12}C	lighter, most abundant stable isotope of carbon
^{13}C	heavier stable isotope of carbon
a	^{13}C fractionation constant during diffusion in air
A/g_s	assimilation-to-stomatal conductance ratio
A	leaf photosynthetic capacity
A_{gs}	conductance-weighted assimilation
Alt.	altitude
A'	measure of plant assimilatory apparatus
b	^{13}C fractionation constant for carboxylation by RuBP
BSP	Britton, N.L., Sterns, E.E, and Poggenburg, J.F.
C	carbon atom
C_3	plant metabolic pathway starting with 3-C compounds
C_a	ambient carbon dioxide concentration
CE	Carlo Erba, an elemental analyzer manufacturer
cf.	compare
C_i/C_{ags}	conductance-weighted C_i/C_a
C_i/C_a	leaf intercellular-to-ambient $[\text{CO}_2]$ ratio
C_i/C_{aA}	assimilation-weighted C_i/C_a
C_i	leaf intercellular carbon dioxide concentration
cm	centimetre
cm^2	squared centimetre
CMI_a	annual climatic moisture index
CO_2	carbon dioxide
CV	coefficient of variation
d	day

Δ	leaf carbon isotope discrimination
D/N	day/night
$\delta^{13}\text{C}$	leaf carbon isotopic composition
$\delta^{15}\text{N}$	leaf nitrogen isotopic composition
δ_a	carbon isotopic composition of source
$d\delta^{13}\text{C}$	leaf carbon isotope discrimination index
df	degrees of freedom
D_{ls}	seasonal (May-September) daylength
δ_p	carbon isotopic composition of plant material
E	transpiration rate
eg	<i>exempli gratia</i> (for example)
Eq.	equation
g	gram
g_s	stomatal conductance to water vapor
g_s^A	assimilation-weighted conductance
h	hour
HPS	high pressure sodium lamp
IAEA	international atomic energy agency
ie	<i>id est</i> (that is)
K	potassium
kPa	kilopascal
L	litre
LAR	leaf area ratio
Lat.	latitude
LED	light emitting diode
ln	natural logarithm
Long.	longitude
LSD	least significant difference

LWR	leaf weight ratio
m	metre
m ²	squared metre
mg	milligram
Mill.	Miller, P.
min	minute
ml	millilitre
mol	mole
MS	mean squares
n	number of replicates or observations
N	nitrogen atom
N1	standard for nitrogen isotope ratio analysis
N2	standard for nitrogen isotope ratio analysis
NAR	net assimilation rate
NBS	national bureau of standards
O	oxygen
σ_c	daily respiratory carbon loss
σ_w	proportional water loss independently of CO ₂ uptake
p	significance probability
P	phosphorus atom
PAR	photosynthetically active radiation
PDB	pee dee belemnite
PET	potential evapotranspiration
PET _a	annual potential evapotranspiration
PPFD	photosynthetic photon flux density
ppm	parts per million
P _{ra}	annual precipitation
r	coefficient of correlation
R	heavy-to-light isotope molar ratio

R^2	coefficient of determination
RGR	relative growth rate
RSR	root-to-shoot ratio
RuBP	ribulose biphosphate
RWR	root weight ratio
s	second
SAS	statistical analysis systems
sat.	saturation
SEM	standard error of the mean
SLA	specific leaf area ($\text{cm}^2 \text{g}^{-1}$)
spp.	species
std	standard
t	duration
t_1	time 1
t_2	time 2
T_{aa}	annual air temperature
T_{sa}	annual 10-cm depth soil temperature
USGS	united states geological survey
v/v	volume/volume ratio
vpd	saturation vapor pressure deficit
VPD_s	seasonal saturation vapour pressure deficit
W	total biomass
W_1	total biomass at time 1
W_2	total biomass at time 2
W_f	final biomass
W_i	initial biomass
wk	week
WUE	water-use efficiency

WUE_i	instantaneous water-use efficiency
WUE_l	long-term water-use efficiency
$[CO_2]$	carbon dioxide concentration
∇	leaf-to-air water vapor pressure gradient
'	minute of latitude or longitude
‰	per mil
°C	degree Celsius
°N	degree north
°W	degree west

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GENERAL INTRODUCTION

Context

The perspective of global climate change has prompted growing and joint efforts for not only predicting its outcome for terrestrial ecosystems but also understanding, in turn, the extent to which these biomes modulate our climate (Sellers *et al.* 1997). Although the magnitude and regional patterns vary, several predictions agree on greatest warming to occur at high latitudes, raising serious concerns for the boreal forest (Sellers *et al.* 1997). Here, the questions may be further complicated by forest management practices, which may impact on the genetic composition and structure of plant populations. To some extent, our ability to foresee potential shifts is intrinsically linked with that of predicting net primary productivity for dominant ecosystems. This challenge calls for a strong understanding of the plasticity in growth for most common species, including black spruce (*Picea mariana* (Mill.) B.S.P.). Black spruce occurs naturally over a broad latitudinal and longitudinal range and is considered as the main tree species of the North American boreal forest (Cauboue and Malenfant 1988, Woodward 1995). In addition to its wide distribution, the high quality of its fibre has made black spruce the most widely used tree in reforestation programs in Eastern Canada (Margolis and Brand 1986). Over its range, black spruce forests display great variation in growth and productivity, partly in association with complex gradients of both biotic and abiotic factors. Among others, the composition and dynamics of these forests are tightly linked with the recurrence of fire and outbreaks of

spruce budworm (Vincent 1965). Black spruce trees occupy soils ranging from almost completely saturated, with deep organic materials to dry, shallow or deep sands or gravel tills, and occasionally moss cap over bedrock (Vincent 1965). Pure stands, among which the greatest productivity is found, occur on moist loams or clay loams of till or lacustrine origin on well-drained uplands. Lowest productivity is observed in pure stands or with varying proportions of larch (*Larix* spp.) on very wet sites, with a deep peat layer. Along with changes in edaphic conditions, the variation in the length of the growing season, temperature and photoperiod are thought to account for a large part of the observable differences among black spruce populations. Little is known about the genetics of physiological variation in wild plant populations, and how it affects plant performance (Davis 1990, Geber and Dawson 1990, Schwarz 1991). However, given its large population size and great ecological amplitude, high levels of genetic variation should be expected in black spruce. Of particular interest is the evolution of an adaptive strategy that optimizes water use in relation to carbon gain.

Theory

In order to grow, plants must fix carbon. Carbon enters the leaf as CO_2 , diffusing mostly through stomata. The wider the stomata open, the greater the conductance to CO_2 but also the higher the water loss. As stomata open, there is a linear increase in transpiration rate, the magnitude of which is dependent on the driving force, the leaf-to-air vapor pressure gradient (Cowan 1982, Ehleringer 1991). In contrast, CO_2 assimilation initially shows a linear increase with stomatal opening and higher leaf intercellular $[\text{CO}_2]$ (C_i), but increase in photosynthesis usually starts to slow down at leaf C_i well below ambient level (C_a) (Farquhar and Sharkey 1982, Cowan 1986). Leaves tend, therefore, to have a specific operational range such that increasing stomatal opening any higher results in only marginal gain in carbon in relation to the cost of water lost (Cowan 1986, Ehleringer 1991). Leaf C_i can be used, therefore, as an index of water-use efficiency of photosynthesis. Leaf C_i can be measured by standard gas exchange techniques for an instantaneous assessment of metabolic activity. However, for extended time periods, C_i is better estimated by the stable carbon isotopic composition ($\delta^{13}\text{C}$) of the leaf which provides an integrated measure of the physiological activity over the lifetime of the leaf (Farquhar *et al.* 1989, Ehleringer 1991).

Leaf $\delta^{13}\text{C}$ (‰) is defined as:

$$\delta^{13}\text{C} = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \quad [\text{Eq. 1}]$$

where R_{sample} and R_{standard} are the molar ratios of heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$) in the sample and standard, respectively. The standard for carbon isotopes is the Pee Dee Belemnite (PDB) limestone.

The biological significance of $\delta^{13}\text{C}$ is better expressed by leaf carbon isotope discrimination (Δ) which is a measure of the amount of ^{13}C in the leaf relative to the atmosphere (Farquhar *et al.* 1989, Ehleringer 1991). Leaf Δ (‰) correlates negatively with $\delta^{13}\text{C}$ according to the following relationship (Farquhar *et al.* 1982):

$$\Delta = \frac{\delta a - \delta p}{1 + \delta p} \quad [\text{Eq. 2}]$$

where δa (‰) and δp (‰) are the stable carbon isotopic compositions of the source air and leaf tissue, respectively.

In C_3 plants, Δ is related to photosynthetic gas exchange through its relationship with C_i/C_a , the ratio of CO_2 concentrations in the leaf intercellular spaces to that in the atmosphere. Assuming no gradient in $[\text{CO}_2]$ between the leaf intercellular spaces and chloroplasts, Δ (‰) for C_3

leaves may reliably be estimated as (Farquhar *et al.* 1982):

$$\Delta = a + (b - a) \frac{C_i}{C_a} \quad [\text{Eq. 3}]$$

where a is the fractionation occurring due to diffusion of CO_2 in air (4.4‰), and b is the net fractionation caused by carboxylation (mainly by RuBP carboxylase, 27‰). Variations in C_i/C_a -hence in Δ - among plants result from variation in stomatal conductance (g_s) or variation in photosynthetic capacity (A) or both. Low Δ values result from low C_i/C_a , which also results from a higher CO_2 assimilation to stomatal conductance ratio (A/g_s), an instantaneous index of leaf water-use efficiency (Meinzer *et al.* 1990). For plants kept under constant vapor pressure gradient, both short- and long-term water-use efficiency are negatively correlated with Δ (Farquhar *et al.* 1989). This relationship offers the possibility of examining how some variations in leaf physiological characteristics might translate into growth patterns (Farquhar *et al.* 1989). For a constant g_s , increased A results in reduced Δ . All things being equal, this higher assimilation rate would translate into higher growth. Therefore, there should be a negative correlation between Δ and plant growth when variation in Δ results from changes in photosynthetic capacity (Farquhar *et al.* 1989). In contrast, Δ should be positively correlated with plant growth if changes in stomatal conductance are primarily driving variation in Δ (Farquhar *et al.* 1989). This

is because an increase in g_s will result in higher carbon assimilation, thereby increasing growth, and will also enhance Δ .

However, Cowan (1986) has pointed out the necessity of considering differences in carbon allocation pattern, in addition to leaf physiological characteristics, when linking leaf Δ and plant growth. Cowan (1986) hypothesized that the leaf-level compromise between water conservation and carbon assimilation should be coupled with that at the whole-plant level between allocation of carbon for the exploration of soil water and carbon allocation to leaves for increased growth rate. The relevance of Cowan's hypothesis is that differences in the patterns of carbon allocation may alter the relationship between Δ and growth when water supply is not limiting. For instance, when variation in photosynthetic capacity is primarily driving the variation in Δ , low- Δ populations/genotypes may grow slower if a higher proportion of carbon is allocated to root relative to shoot. Conversely, lack of water conservation may offset the expected positive relationship between plant growth and Δ when variation in the latter results from changes in stomatal conductance. Questions arise then on (1) the covariation between leaf physiology and whole-plant carbon allocation when water is not limiting and (2) the relationships among carbon allocation, leaf physiology and growth under contrasting environments. This study was aimed at a better understanding of these relationships at both the population and family levels in juvenile black spruce.

Specific objectives

The specific objectives of this study were to:

- (1) determine the extent to which wild populations of black spruce, from across the natural distribution range of the species, differ in carbon allocation patterns and leaf physiological characteristics.
- (2) determine the extent to which full-sib families of black spruce, from a much smaller range (Ontario), differ in carbon allocation patterns and leaf physiological characteristics.
- (3) determine how carbon allocation patterns relate to leaf physiological characteristics at different developmental stages and under contrasting environments.
- (4) determine how specific correlations among carbon allocation patterns and leaf physiological characteristics translate into growth of juvenile black spruce.
- (5) determine if the more conservative traits, in terms of water use relative to carbon gain, always co-occur in the same population or family within the juvenile period.

Hypotheses

Considering the strong interdependence between root and shoot growth and the cost to the plant, in terms of water loss, for acquiring carbon, it was hypothesized:

- (1) that black spruce populations/families allocating a higher proportion of carbon to roots -relative to shoot- also have a lower stomatal conductance in relation to photosynthetic capacity.
- (2) that more northerly populations/families, naturally subjected to more stressful conditions, exhibit a more conservative suite of characteristics (eg. higher root-to-shoot ratio, higher water-use efficiency).
- (3) that strong genetic control on water conservation results in a consistent ranking of populations/families across environments for a given trait.

CHAPTER ONE

LITERATURE REVIEW

1.1 CARBON ALLOCATION AND PLANT GROWTH

Over the long term, efficiency in carbon allocation among organs is as critical to plant development as is the maintenance of a positive carbon balance.

Carbon allocation, following Gower *et al.* (1995), refers to the distribution and accumulation of carbon into different parts within the plant. As plant tissues often exhibit an homogenous concentration of carbon (%C), carbon allocation is reliably quantified by the differential distribution and accumulation of dry matter into different parts within the plant. Carbon allocation can be seen as the end product of the process of partitioning which, in turn, refers to the carbon flow into and among tissues and organs (Kramer and Kowlowski 1979). This flow of carbohydrates among competing food sinks, largely or entirely as sucrose in most woody plants, involves a mass flow driven by a gradient of turgor pressure and proceeds via phloem loading and unloading. Carbon partitioning varies with the season, the relative strength of sinks and their proximity to source leaves, as well as the types and amount of plant growth hormones present.

Much interest has been placed on the variability in growth characteristics among individual plant species (Kvet *et al.* 1971). Techniques of plant growth analysis refer to growth as an increase in dry matter, an appropriate measure of which is the concept of mean relative growth rate (Evans 1972). Basically, the mean relative growth rate is an integration of all the processes resulting in an increase in dry weight of the plant (Evans 1972). Assuming continuity

in growth over a time period between t_1 to t_2 , the mean relative growth rate (RGR, d^{-1}) of an individual plant is defined as:

$$RGR = \frac{1}{(t_2 - t_1)} \int_{t_1}^{t_2} \frac{1}{W} \frac{dW}{dt} = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)} \quad [\text{Eq. 1.1}]$$

where W_1 and W_2 are the biomass at times t_1 and t_2 respectively.

The mean relative growth rate can be divided into two components describing either the efficiency of dry matter production or the relative size of the assimilatory apparatus. These components are commonly referred to as the mean net assimilation rate (NAR, $g\ cm^{-2}\ d^{-1}$) and the leaf area ratio (LAR, $cm^2\ g^{-1}$), respectively. Accordingly, the mean relative growth rate can be expressed as:

$$RGR = [NAR][LAR] = \left[\frac{1}{(t_2 - t_1)} \int_{t_1}^{t_2} \left[\frac{1}{A'} \frac{dW}{dt} \right] \left[\frac{A'}{W} \right] \right] \quad [\text{Eq. 1.2}]$$

where W is the total biomass of the plant whereas A' can be any measure of the size of the assimilatory apparatus (i.e. leaf area, leaf weight, leaf nitrogen, etc.). Further, the leaf area ratio can be expressed as the product of leaf weight ratio (LWR, $g\ g^{-1}$) and specific leaf area (SLA, $cm^2\ g^{-1}$).

Although the above growth characteristics are mutually dependent (Kvet *et al.* 1971), attempts are often made that seek which of the components of RGR is the most important in controlling growth. At the interspecific level, differences in NAR appear to be less important than LAR or SLA in

explaining the variation in RGR (Lambers and Poorter 1992). In contrast, Swanborough and Westoby (1996) found a greater influence of NAR than of leaf biomass. At the intraspecific level, plant growth potential and accompanying quantitative traits usually display geographic patterns in response to environmental heterogeneity, suggesting some adaptive significance (Grime 1977, Chapin *et al.* 1993, Bazzaz 1996). Understanding the distribution and variability of adaptive traits as well as the evolutionary forces that shaped them is a basic prerequisite to breeding programs (Muona *et al.* 1990). It may also prove useful to modelling plant response to changing environments.

From both agricultural and silvicultural standpoints, carbon allocation patterns are critical to plant economic value. However, a long-standing challenge in growth analysis has been to provide a sound basis for the allometric growth of plants. Different theories, although not mutually exclusive, have been developed to explain this balanced growth between roots and shoots. One current model assumes that substrate drives growth by mass action so that the mutual dependence of roots and shoots results, in the long-term, in a balanced growth (Brouwer 1983). Another explanation is based on the apical dominance theory and argues for a balance achieved through remote control of plant growth regulators (Brenner 1987). A third theory, and probably the most accepted one, suggests that the sucrose status is critical to the whole regulatory process (Farrar 1992). Accordingly, sucrose would act as

a messenger between source and sink and could regulate the expression of genes coding enzymes involved in the carbon fluxes (Farrar 1992).

1.2 WATER-USE EFFICIENCY AND PLANT GROWTH

The tight coupling between plant carbon acquisition and water loss has led to the development of the concept of water-use efficiency (WUE). However, because the efficiency in water use may be achieved through different ways - depending on the level at which it is determined- its relationship with plant productivity is not always predictable. At the leaf level, an instantaneous water-use efficiency (WUE_i), the molar ratio of carbon gain to water lost, can be calculated as:

$$WUE_i = \frac{A}{E} = \frac{C_a(1 - \frac{C_i}{C_a})}{1.6\nu} \quad [\text{Eq. 1.3}]$$

where A and E are rates of CO_2 assimilation and transpiration, respectively; C_a and C_i are ambient and intercellular CO_2 concentrations, respectively; ν is the leaf-to-air water vapor pressure gradient and 1.6 is the ratio of diffusivities of water vapor and CO_2 in air. For C_3 plants kept under a constant ν , greater WUE_i would be observed for plants exhibiting lower values of C_i/C_a (Farquhar *et al.* 1989, Ehleringer 1991). However whether this is achieved via increased photosynthetic capacity or decreased stomatal conductance may

have different consequences for plant productivity.

At the whole-plant level, long-term water-use efficiency (WUE_l) is calculated similarly, using leaf carbon isotope discrimination (Δ) as an index of C_i/C_a (Farquhar *et al.* 1989):

$$WUE_l = \frac{C_a \left(\frac{b - \Delta}{b - a} \right) (1 - \phi_c)}{1.6\nu(1 + \phi_w)} \quad [\text{Eq. 1.4}]$$

where ϕ_c is the daily respiratory carbon loss relative to assimilation; ϕ_w is the proportion of water lost independently of CO_2 uptake; a and b are ^{13}C fractionation constants due to diffusion in air (4.4‰) and carboxylation by RuBP carboxylase (27‰), respectively. For a constant ν , both WUE_l and WUE_i are negative functions of Δ (Farquhar *et al.* 1989). Variations in WUE_l are not only dependent on factors controlling Δ but also on carbon allocation patterns which determine ϕ_c . Optimum water conservation would be achieved in plants exhibiting a high WUE_l and concurrently allocating their carbon so as to reduce the likelihood of dessication (Cowan 1986). Such plants will have a lower Δ -that is a higher WUE- and allocate a higher proportion of carbon to roots. When water is not limiting, whether the plants in consideration are adaptively water-conservative will further determine the relationship between WUE_l and productivity.

In many C_3 plants, Δ -thus WUE- is genetically controlled (Farquhar and Richards 1984; Hubick *et al.* 1986; Virgona *et al.* 1990; Condon and Richards 1993; Flanagan and Johnsen 1995; Johnson *et al.* 1995). Such findings have prompted interest for the use of Δ as a potential screening criterion in plant breeding programs, providing at the same time some physiological basis for the differentiation in growth among and within populations. However the relationship between WUE and productivity also remains species-dependent, probably due to intrinsic differences in carbon allocation patterns. For instance, there was a positive correlation between wheat dry matter productivity and Δ (Condon and Richards 1993), suggesting a negative relationship with WUE. On the other hand, positive correlations have been found between WUE and dry matter productivity in peanut (Hubick *et al.* 1986), sunflower (Virgona *et al.* 1990), and white spruce (Sun *et al.* 1996).

1.3 ECOLOGICAL STUDIES OF BLACK SPRUCE

Studies of black spruce abound in the literature, a recent review of which has been provided by Lamhamedi and Bernier (1994). Although there is a general consensus on the existence of genetic variation within black spruce, the underlying evolutionary mechanisms remain unclear. In a series of provenance tests, Morgenstern (1969a,b; 1978) examined the phenology of shoot growth at juvenile stage and concluded that clinal variation existed for this trait. That is a gradual variation of traits in response to continuous ecological factors as opposed to discrete factors, which would lead to ecotypic

differentiation. Photoperiod and temperature were identified as the major ecological factors driving clinal variation among black spruce populations (Morgenstern 1978). The presence of photoperiodic ecotypes in tree species, including black spruce, has also been shown by Vaartaja (1959) and by Pollard and Logan (1974).

Free growth, the in-season initiation of cellular growth, may account for a large part of the juvenile performance of black spruce. Pollard and Logan (1974) reported reduced or no free growth whenever the cultivation photoperiod was shorter than that at the seed source. In the same vein, Johnsen and Seiler (1996) have recently examined the differentiation in juvenile growth, shoot phenology and physiology among populations of black spruce under elevated CO₂ concentration and extended photoperiod.

Northern populations were found to break and set buds earlier and to allocate more dry matter to root versus shoot, an allocation pattern that tended to alter under extended photoperiod. Interestingly, differences in growth, shoot phenology and physiology were similarly expressed among both seedlings and mature trees of the same provenances (Johnsen *et al.* 1996).

Traits related to seed germination and drought resistance, on the other hand, tend to exhibit patterns of ecotypic differentiation, suggesting some evolutionary significance for moisture and nutritional status at the seed source (Morgenstern 1969b). The importance of moisture conditions on vegetation zoning is well documented (Thornwhaite 1948, Sanderson 1948,

Bonan and Sirois 1992). The southern limit of the western Canadian boreal forest has been found to coincide with the zero isoline where potential evapotranspiration balances precipitation (Hogg 1994). Surprisingly though, several studies failed to support the existence of edaphic ecotypes in black spruce (Fowler and Mullin 1977; El Abidine *et al.* 1994, 1995). However, O'Reilly *et al.* (1985) argue that these may still exist for traits that have not been examined. Besides, it is possible to have different patterns of variation for adaptive and non-adaptive characters (Morgenstern 1978). On the one hand, El Abidine *et al.* (1994) found no differentiation in gas exchange characteristics among lowland and upland stands of black spruce. On the other hand, upland stands of black spruce were found to express greater heterozygosity than lowland stands at the electrophoretic level (O'Reilly *et al.* 1985). These observations, however, contrast with the general tendency for adaptive quantitative traits to be much more differentiated among populations than isozymes (Muona *et al.* 1990).

As the prospect of global climate change becomes more and more realistic, much interest is being put toward an adequate assessment of the potential consequences for boreal ecosystems (Margolis and Ryan 1997, Sellers *et al.* 1997). Warmer and drier environmental conditions as well as greater nitrogen deposition, among other predictions, are highly likely for the boreal forest. Because these environmental factors are critical to the distribution of species, significant shifts in plant communities are to be expected (Woodward

1995, Hogg 1997). Furthermore, concerns have been raised on the capacity of the currently-selected fast-growing genotypes to keep pace with the rapid changes in their environment (Kimmins 1987, Muona *et al.* 1990, Fowler and Loo-Dinkins 1991). In a series of climate-simulation tests, Wang *et al.* (1994, 1995) have examined this question with different families of black spruce to reach contrasting conclusions over time. While the data from first-season seedlings supported the idea of fast-growing families performing as well in future climate as they do in present-day climate (Wang *et al.* 1994), the opposite appeared highly likely by the end of the second growing season (Wang *et al.* 1995). Some more recent evidence on the same question indicates strong stability (Major and Johnsen 1998).

CHAPTER TWO

MATERIALS AND METHODS

2.1 SEED COLLECTIONS

Seedlots for 13 bulk populations and eight full-sib families of black spruce were obtained from either the Canadian Forest Service or the Quebec Ministry of Natural Resources. The bulk populations were of different latitudinal, longitudinal and altitudinal origins and selected to span much of the variability across the distribution range of the species (Figure 2.1, Appendix 1). The full-sib families were from three provenances selected along a North-South transect within Ontario (Figure 2.2, Appendix 2). These families are from controlled pollinations among grafted scions at the former Petawawa National Forestry Institute, now Petawawa Research Forest, (Chalk River, Ontario) . Male and female trees were from the same origin. Because seed size may both have an adaptive value (Morgenstern 1969a) and affect juvenile performance (Wang *et al.* 1994, 1995), three random samples of 100 seeds (25 seeds in the case of four full-sib families) per seedlot were measured to assess the variation in seed weight among populations or families.

2.2 CULTIVATION CONDITIONS

2.2.1 Controlled environments

Experiments were conducted at the McGill University Phytotron (Montreal, Canada). According to three time replicates, two growth chambers (Model PGW36 M10, Conviron, Winnipeg, Man., Canada) were set to simulate a four-week germination period followed by either a northern or a southern climate within the distribution range of black spruce (Table 2.1). These

climates were meant to reflect growing season (May-September) environmental conditions for Ottawa (45°19'N, 75°40'W) and Whitehorse (60°43'N, 135°04'W). Simulated conditions included seasonal and diurnal air temperature, relative humidity, ambient carbon dioxide concentration, photoperiod and light (Table 2.2). The daily photosynthetic photon flux density (PPFD) within growth chambers averaged about 460 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but was at 510 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the main part of light period. Diurnal temperature was ramped over the last two weeks of the germination period to match parameters for the first simulated month in either climate.

The 13 bulk populations and eight full-sib families were simultaneously grown in the same environments. Chamber area was split lengthwise in two and each portion was assigned to either populations or families. Up to 8 non-stratified seeds per population or family were sown into individual plastic pots (0.8 L, i.e. 4"x4"x5.25", Kord Products, Toronto) filled with a 2:2:1 (v/v) mixture of peat, vermiculite and sand. For a given replicate x environment combination, there were six pots per population and 12 per family. A no-damp treatment (Oxine Benzoate, Plants Products Co. Ltd., Brampton, Ontario, Canada) was applied 10 days after sowing to prevent the establishment of damping-off organisms. Pots were watered daily and seedlings were thinned to one per pot at the end of the germination period (week 4). From there on, weekly fertilization was applied providing 0.7 mg N, 0.6 mg P, and 0.6 mg K per pot (Calmé *et al.* 1993). The fertilizing solution

consisted of 1.75 g of All purpose fertilizer (20-20-20 N-P-K, Plants Products Co. Ltd., Brampton, Ontario, Canada) and 0.75 g of Monosodium phosphate (00-44-00, Farnam Companies, Inc., Phoenix, AZ) diluted in 10 L of distilled water. An automatic dispenser (Varistaltic Pump, Manostat®, New York) was set to deliver a 20-ml fertilizing aliquot per pot every week.

In order to test the plasticity of juvenile traits with time, half the seedlings of full-sib families from the second replicate were cold-hardened (Bigras and D'Aoust 1992, Calmé *et al.* 1993) and grown for a second growing season.

Seedlings from both environments were concurrently cold-hardened over a five-week period in a third growth chamber identical to those described above. This period is characterized by a short photoperiod (8 hours) and a ramped day/night temperature which went from 10°/5°C on day 1 to 0°/0°C on day 21. Seedlings were maintained under 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD during the first three weeks and complete darkness during last two weeks. Watering frequency was gradually reduced from every other day (1/2) to one day out of three (1/3) and to none. Two days before transferring the seedlings to their respective environment, the day/night temperature was also ramped back to 10°/5°C with similar supply of light. During the second growing season, seedlings received 40 ml of the fertilizing solution weekly.

Throughout the cultivation period, environmental conditions were continuously monitored using a computerized system connected to the chamber controller. Hourly averages were recorded from 1-minute interval scannings of air temperature, relative humidity and PPFD. Ambient carbon dioxide concentration was similarly obtained using an infra-red gas analyzer (Model 250E, Horiba Instruments, Inc., Ann Arbor, MI) connected to the chamber controller. Growth conditions were further characterized using fine-wire copper-constantan thermocouples (Omega Engineering, Inc., Stamford, CT) on an external data logger (CR10, Campbell Scientific, Logan, Utah). Substrate (at 6 cm-depth), air and leaf temperatures were recorded over a one-week period, one environment at a time (Appendix 3).

2.2.2 Field test

The bulk populations were also germinated and grown under greenhouse conditions at the Carleton University ELBA (Ottawa, Canada), cold-hardened and then transplanted in the field (Table 2.1). Pots and substrate were of the same kinds as in controlled environments. The day/night temperature within greenhouse was varied regularly to match, as much as possible, that of southerly simulated environment. The daily PPFD varied but remained above $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ on sunny days. Photoperiod was controlled by artificial light (HPS400, Duro-Test Electric Ltd., Rexdale, Ont., Canada). Watering and fertilization were as in controlled experiments. The seedlings were maintained under these conditions for 25 weeks (10/22/1996 to

4/16/1997) before being transferred to two growth chambers (Model E15, Conviron Products, Winnipeg, Man., Canada) for cold hardening. This period lasted six weeks (4/16/1997 to 5/28/1997) and differed from that in controlled environments only in the onset of the dark period (started at the end of week 4). The seedlings were then transplanted to a field plot located near Ottawa (Ontario, Canada).

There were three randomized complete blocks consisting of buried pots [3R66 (6.5"x6") Ranco pots] filled with local substrate. Inter-block distance was 2 m. An experimental unit consisted of a row of six pots 0.75 m apart. The field plot had previously been tilled both at the end of the preceding Fall and just prior to transplanting. Pots were also mulched at the start of the experiment and weeded regularly while in the field (5/28/1997 to 9/17/1997). Due to an exceptional drought spell following the transplantation, artificial watering was supplied occasionally. A meteorological station was set up that recorded, on a quarter-hourly basis, 12 cm-depth soil temperature, 2 m-height air temperature and leaf temperature, photosynthetically active radiation (PAR), relative humidity and precipitation. Seedlings were allowed to grow in the field until late in the growing season before they were measured for photosynthetic gas exchange and harvested (9/5/1997 to 9/17/1997).

2.3 DATA ACQUISITION PROCEDURES

2.3.1 Gas exchange measurements

At the end of the cultivation period, up to three seedlings per environment and per population (or family) were randomly selected and labelled. For each of these seedlings, a healthy portion of the shoot was identified and prepared for photosynthetic gas exchange measurements. Sample preparation was done at least 24 hours prior to measurement and consisted of removing needles (but keeping them in separate bags) on both sides of the target portion, avoiding at the same time to have any axial bud over that portion. Photosynthetic gas exchange measurements were made using a portable photosynthesis system equipped with a 1/4-liter chamber (Li-6200, LiCor Inc., Lincoln, NE). Operating parameters were set for three observations lasting 20 seconds each per measurement. Light-saturated rates of photosynthesis (photosynthetic capacity, A) were thus measured under respective environments, providing at the same time the stomatal conductance to water vapor (g_s) and the intercellular carbon dioxide concentration (C_i).

Under controlled environments, the leaf chamber was illuminated with an LED light source (QB6200, Quantum Devices Inc., Barnsvelt, WI) set at a constant photon fluence rate ($1060 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Brooks *et al.* 1997). In the field, gas exchange measurements were restricted to clear days (PAR above $700 \mu\text{mol m}^{-2} \text{s}^{-1}$) and taken only between 10:00 a.m. and 12:00 noon to avoid the diurnal depression in photosynthesis (Dang *et al.* 1991).

Gas exchange characteristics were expressed on projected leaf area basis, which was measured (triplicate measurements) on a Li-3100 leaf area meter (LiCor Inc., Lincoln, NE). Integrity of the gas exchange data was checked by performing leaf energy balance and using the recorded rates of change in CO₂ concentration and vapor pressure deficit within the leaf chamber during measurements (Leuning and Sands 1989).

2.3.2 Biomass data and laboratory analyses

Seedlings used for gas exchange were harvested and their roots isolated by gentle washing. In some bulk populations, small-sized seedlings that could not be measured for gas exchange were harvested whenever there were less than three measurable seedlings. Plant tissues were oven-dried at 65°C for at least 48 hours, separated and weighed. Needles from gas exchange samples or a subsample of the leaf tissue for the small-sized seedlings was first dried and weighed separately from the remainder of leaf material in order to estimate the specific leaf area. All leaf material was then combined and ground to fine powder using a mortar and pestle.

Subsamples (ca. 1.2 mg) of dried and ground leaf material were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the G.G. Hatch Isotope Laboratories at the University of Ottawa (Ottawa, Ontario). The system used consists of a Finnigan MAT Delta^{plus} Isotope Ratio Mass Spectrometer (Finnigan, Bremen, Germany) combined with a CE 1110 elemental analyzer (CE Instruments, Milan, Italy). Leaf isotopic compositions (‰) were expressed as:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \quad [\text{Eq. 2.1}]$$

where R_{sample} and R_{standard} are the molar ratios of heavy to light isotope in the sample and standard, respectively. The $\delta^{13}\text{C}$ was expressed relative to the Pee Dee Belemnite (PDB) limestone using working standards from the U.S. National Bureau of Standards (NBS-21, Spectrographic graphite) and the U.S. Geological Survey (USGS-24, Graphite). Nitrogen isotopic composition ($\delta^{15}\text{N}$), on the other hand, was expressed relative to atmospheric air, using Ammonium sulfate calibration standards USGS-25, N1 and N2. The last two nitrogen standards are from the International Atomic Energy Agency (IAEA). As an indication of the accuracy of data, replicate measurements of the NBS-21 and USGS-24 standards gave $\delta^{13}\text{C} = -28.09\text{‰}$ (std deviation = 0.186‰ , $n=18$) and $\delta^{13}\text{C} = -16.10\text{‰}$ (std deviation = 0.173‰ , $n=23$) in comparison with accepted value of -28.1‰ and -16.1‰ , respectively. Similarly, replicate measurements of the USGS-25, N1 and N2 standards gave $\delta^{15}\text{N} = -30.35\text{‰}$ (std deviation of 0.363‰ , $n=15$), $\delta^{15}\text{N} = 0.25\text{‰}$ (std deviation = 0.321‰ , $n=12$) and $\delta^{15}\text{N} = 20.37\text{‰}$ (std deviation = 0.346‰ , $n=15$) versus accepted values of -30.4‰ , 0.4‰ and 20.3‰ , respectively. Leaf %C and %N were determined on a CE NC2500 elemental analyzer (CE Instruments, Milan, Italy) at the University of Lethbridge (Lethbridge, Alberta).

2.4 STATISTICAL ANALYSIS

2.4.1 Experimental design

The growth chamber experiment was considered as a factorial split-plot with Environment as the mainplot factor and Population/Family as the subplot factor. Main experimental units were replicated in time and set in three randomized complete blocks. Environments were randomized within replicates and Populations or Families within Environments. A subplot unit consisted of a group of six or twelve individual pots for populations or families, respectively. Separate analyses were performed for populations and families in consideration of differences in their biological characteristics.

2.4.2 Simulated environmental conditions

In order to characterize the variation in simulated environmental conditions both within and between environments, data were subjected to variance analysis for repeated measurements (SAS Institute Inc. 1989). Daily means for air temperature ($^{\circ}\text{C}$), relative humidity (%), saturation vapour pressure deficit (kPa), photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and ambient carbon dioxide concentration (ppm) were taken as observational units ($n=140$).

Where Time $\times$ Environment interaction was significant, Fisher's protected LSD was performed to compare means (Steel and Torrie 1980) (Appendix 4).

2.4.3 Growth and carbon allocation traits

Data were first examined for variance homogeneity and normal distribution under the univariate procedure of SAS (SAS Institute Inc. 1989). Covariance analysis for a split-plot design was performed on log-transformed data according to the following linear model:

$$Y_{ijkl} = \mu + B_i + E_j + \alpha_{ij} + P_k + PE_{jk} + \beta_{yx}(x_{ijkl} - \bar{x}_{...}) + \varepsilon_{ijk} + \sigma_{ijkl} \quad [\text{Eq. 2.2}]$$

where Y_{ijkl} is the trait of interest for sample l from population (or family) k in block i under the environment j , μ is a fixed general mean, B_i is the fixed effect of block i , E_j is the fixed effect of the j^{th} environment, α_{ij} is the random error of i^{th} block in j^{th} environment, P_k is the fixed effect of k^{th} population (or family), PE_{jk} is the interaction between population (or family) k and environment j (fixed), β_{yx} is the fixed effect of the covariation of y with x (the covariate), x_{ijkl} is the value of covariate x for sample l of population (or family) k under environment j within block i (and $\bar{x}_{...}$ is the corresponding general mean), ε_{ijk} is the random error of i^{th} block in j^{th} environment for k^{th} population (or family) and σ_{ijkl} is the random error for sample l from population (or family) k under environment j in block i .

Individual observations were then adjusted for covariates wherever associated parameters (β_{yx} in the above model) were statistically different

from zero. Adjusted values were back-transformed, tested for variance homogeneity and subjected to variance analysis according to the model in Eq. 2.2 with omission of the covariation effect (Lison 1958, Steel and Torrie 1980). To avoid size confounding effects, carbon allocation traits were adjusted to the mean total biomass (as a covariate) achieved by seedlings. These traits included the leaf weight ratio (LWR, g g^{-1}), the root weight ratio (RWR, g g^{-1}) and correlated parameters such as the leaf area ratio (LAR, $\text{cm}^2 \text{g}^{-1}$) and the root-to-shoot ratio (RSR, g g^{-1}). Similarly, specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) was also adjusted for sample size (as a covariate: the average leaf biomass for gas exchange measurement). Leaf area ratio was determined as the product of SLA and LWR. The total plant leaf area (cm^2) achieved by an individual seedling was calculated as the product of SLA and total plant leaf weight. The relative growth rate (RGR, d^{-1}) of seedlings was estimated as the relative change in log-biomass over the whole growing period. That is,

$$\text{RGR} = \left[\frac{\ln W_f - \ln W_i}{t} \right] \quad [\text{Eq. 2.3}]$$

where W_i (g) and W_f (g) refer to initial and final biomass achieved by seedlings; t (d) is the duration of the period considered. The initial biomass was equated to population or family mean seed weight wherever the relative growth rate was considered over one growing season. The relative growth rate was also subjected to variance analysis.

For any of the examined traits, the mean latitudinal source (53.27°N) was used as a threshold when comparing southern and northern populations. Orthogonal contrasts were performed in the case of full-sib families to compare northern and southern provenances. The relative growth rate of two-season old seedlings (full-sib families) was estimated by equating the initial biomass to the mean total biomass achieved by first season-seedlings at the environment $\times$ family level (omission of block effect since no replication). Traits for families grown over two growing seasons were adjusted by means of linear regression (Lison 1958, Steel and Torrie 1980).

2.4.4 Leaf gas exchange characteristics

As in the growth analysis, gas exchange data were first examined for variance homogeneity and normal distribution under the univariate procedure of SAS (SAS Institute Inc. 1989). All variables but ambient (C_a) and leaf intercellular (C_i) carbon dioxide concentrations necessitated a log-transformation. Multiple covariance analysis for a split-plot design was then performed. The general linear model is similar to that in Eq. 2.2 with, however, as many covariation effects as there are covariates. Photosynthetic capacity (A), stomatal conductance (g_s) and leaf intercellular-to-ambient [CO_2] (C_i/C_a) were adjusted for C_a , leaf-to-air vapour pressure deficit and PPFD. Observations adjusted for covariates were back-transformed, tested for variance homogeneity and subjected to variance analysis.

By means of covariance analysis, variation in instantaneous water-use efficiency was examined as (1) the variation in A for a fixed g_s and conversely (2) the variation in g_s for a fixed A . These traits were referred to as conductance-weighted assimilation (A_{g_s}) and assimilation-weighted conductance (g_{sA}). Accompanying values for C_i/C_a were indexed accordingly (C_i/C_{ags} and C_i/C_{aA}).

As with the growth parameters, the mean latitudinal source (53.27°N) was used as a threshold when comparing southern and northern populations. Orthogonal contrasts were performed in the case of full-sib families to compare provenances (Steel and Torrie 1980). Data for families grown over two growing seasons were adjusted by means of multiple regression.

2.4.5 Leaf carbon isotope discrimination

As is often the case, the bottled CO_2 used to maintain the ambient levels within our growth chambers was found much more depleted in ^{13}C ($\delta^{13}C = -44.13\text{‰}$) than standard atmospheric CO_2 ($\delta^{13}C = -8\text{‰}$). Taking these CO_2 source differences into consideration, an index of leaf Δ ($d\delta^{13}C$) was expressed as the deviation from the population (x environment x replicate) mean $\delta^{13}C$. This index ($d\delta^{13}C$) correlates positively with Δ . In the field, the leaf $\delta^{13}C$ remained within the expected range of variation for C_3 plants. Leaf Δ was then calculated according to Farquhar *et al.* (1982). However, for sake of

harmony with controlled environments, carbon isotope discrimination was also expressed as $\delta^{13}\text{C}$. It is this index of Δ that was subjected the analysis of variance.

2.4.6 Correlations with climatic variables

In order to identify climatic variables that were best associated with the examined traits, principal components analysis was performed on the correlation matrix among selected plant traits and seed-source environmental conditions (Pimentel 1979, (SAS Institute Inc. 1989). Using published Canadian climate normals (Environment Canada 1982), the seed-source potential evapotranspiration (PET) was calculated following the Jensen-Haise model (Burman & Pochop 1994). Seed-source moisture was characterized as the annual balance between PET and precipitation. Environmental input variables for the principal components analysis are the mean annual soil temperature at 10 cm-depth, the annual climatic moisture index, the mean seasonal saturation vapor pressure deficit and the mean seasonal daylength. Growth and carbon allocation traits are RGR, SLA, LWR and RWR while leaf physiological characteristics comprise A , g_s , C_i/C_a and $\delta^{13}\text{C}$. Because populations or families might respond differently to contrasting climates, the principal components analysis was performed for separate environments. The four main components were interpreted according to the magnitude and sign of their respective loadings along variables (Pimentel 1979).

Table 2.1. Details of cultivation conditions. Simulated temperature and photoperiod were based on Canadian climate normals for 1951-80 period. For temperature, 2°C was added to the monthly normal and the day/night (D/N) differential temperature was set at 5°C.

Environment	Germination		Simulated period ¹			
	(2 wks)	(2 wks)	May (4 wks)	June (4 wks)	July (4 wks)	August (4 wks)
Northern: Whitehorse (60°43'N, 135°04'W)						
D/N temperature (°C)		ramped	11/06	16/11	19/14	17/12
Photoperiod (h:min)	21/21 16:00	16:00	17:00	19:00	18:00	16:00
Southern: Ottawa (45°19'N, 75°40'W)						
D/N temperature (°C)		ramped	18/13	23/18	26/21	24/19
Photoperiod (h:min)	21/21 16:00	16:00	15:00	16:00	15:30	14:00
Field: Ottawa	Seedling production¹ (25 wks)		Cold hardening² (3 x 2 wks)		Field (16 wks)	
D/N temperature (°C)		southern	10/05	03/03	00/00	natural
Photoperiod (h:min)		southern	08:00	08:00	00:00	natural
Fertilization			none	none	none	
Watering			1/2	1/3	none	

Note: 1 - Frequency for watering and fertilization was 1/1 and 1/7, respectively; 2 - 5 weeks for full-sib families.

Table 2.2. Environmental conditions during the cultivation and photosynthetic gas exchange measurements periods. Conditions are daily and leaf chamber means ($\pm$ S.E.M., $n=3$); Entries are ambient carbon dioxide concentration (CO_2), photosynthetic photon flux density (PPFD), air temperature, relative humidity and saturation vapor pressure deficit (vpd).

Period	Environment	CO_2 (ppm)	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Temperature ($^{\circ}\text{C}$)	R. humidity (%)	Sat. vpd (kPa)
Cultivation	Northern	358 $\pm$ 6	457 $\pm$ 2	14.9 $\pm$ 0.1	73.7 $\pm$ 0.1	0.46 $\pm$ 0.00
	Southern	361 $\pm$ 5	459 $\pm$ 0	20.5 $\pm$ 0.0	74.1 $\pm$ 0.0	0.65 $\pm$ 0.01
	Field	-----	787 $\pm$ 53	19.0 $\pm$ 0.5 ¹	72.2 $\pm$ 1.9	0.75 $\pm$ 0.07
Gas exchange	Northern	373 $\pm$ 2	1009 $\pm$ 1	22.4 $\pm$ 0.2	52.5 $\pm$ 1.3	1.30 $\pm$ 0.05 ²
	Southern	368 $\pm$ 5	1021 $\pm$ 12	25.0 $\pm$ 0.6	53.1 $\pm$ 1.0	1.49 $\pm$ 0.07 ³
	Field	349 $\pm$ 1	1489 $\pm$ 51	26.2 $\pm$ 0.9	50.0 $\pm$ 1.4	1.72 $\pm$ 0.04 ⁴

Note: 1 - Lower than measured in the simulated southern environment only because of september days; 2 - 3 - 4 - Add 5, 7 and 23% for leaf-to-air vapor pressure deficit, respectively.

Figure 2.1. Natural distribution range of black spruce (*Picea mariana* (Mill.) B.S.P. and origin of bulk populations in this study. This map is adapted from Cauboue et Malenfant (1988) by authorization of Les Publications du Québec.

Figure 2.2. Natural distribution range of black spruce (*Picea mariana* (Mill.) B.S.P. and origin of full-sib families in this study. This map is adapted from Cauboue et Malenfant (1988) by authorization of Les Publications du Québec.

CHAPTER THREE

DIFFERENTIATION IN GROWTH, CARBON ALLOCATION AND CARBON ISOTOPE DISCRIMINATION AMONG BLACK SPRUCE POPULATIONS

3.1 RESULTS

3.1.1 Controlled environments

3.1.1.1 Growth and carbon allocation traits

There was a strong Environment by Population interaction for relative growth rate (RGR) and specific leaf area (SLA) (Table 3.1). Irrespective of environment, there was a trend for northern populations (more northerly than 53°N, that is population 8 thru 13) of black spruce to grow slower and to grow even slower in the southern environment [Southern versus Northern seed sources: RGR (d^{-1})=0.041±0.001 versus 0.040±0.001 under northern climate, RGR (d^{-1})=0.041±0.001 versus 0.036±0.001 under southern climate] (Figure 3.1a). The more northerly populations also tended to express higher, but not statistically significant, specific leaf area in the warmer southern environment [Southern versus Northern seed sources: SLA ($\text{cm}^2 \text{g}^{-1}$)=92±3 versus 97±5] (Figure 3.1b). However, there were a number of populations (generally those more southerly than population 10, latitude 57°N) that did not significantly change neither RGR nor SLA between environments. For seedlings of a given size, the proportion of carbon allocated to leaves (LWR) and to roots (RWR) were determined by significant but separate main effects of Environment and Population (Table 3.1). However, much of the variation in LWR and RWR was associated with environmental effects (cf. mean squares in Table 3.1). Regardless of the environment, the farther north the population origin, the lower the proportion of carbon allocated to leaf

[Southern versus Northern seed sources: $LWR (g\ g^{-1}) = 0.632 \pm 0.010$ versus 0.564 ± 0.014 under northern climate, $LWR (g\ g^{-1}) = 0.490 \pm 0.013$ versus 0.395 ± 0.011 under southern climate] (Figure 3.1c) and the higher the proportion that was allocated to roots [Southern versus Northern seed sources: $RWR (g\ g^{-1}) = 0.258 \pm 0.008$ versus 0.322 ± 0.013 under northern climate, $LWR (g\ g^{-1}) = 0.381 \pm 0.013$ versus 0.458 ± 0.010 under southern climate] (Figure 3.1d). More importantly, regardless of the population, LWR was reduced ($P=0.0081$) but RWR was increased ($P=0.0199$) in response to the environmental shift from the cooler northern climate to the warmer southern environment (Table 3.1, Figures 3.1c,d). Overall, populations that exhibited higher values for RGR, LWR, or RWR in one environment tended to do so in the other (Table 3.2). However, the relative ranking of populations changed between environments for any of those traits. As a result of these intrinsic differences in growth and carbon allocation patterns, the total plant leaf area and the total plant biomass varied significantly among populations (Table 3.3, Figures 3.2a,b). Consistent with the variation in total plant leaf area and reflecting the combined patterns of both SLA and LWR (Tables 3.1, 3.3; Figures 3.1b,c, 3.2c), the plant leaf area ratio (LAR) varied significantly among populations (Table 3.3, Figure 3.2c). The root-to-shoot ratio (RSR), on the other hand, varied both between environments and among populations (Table 3.3, Figure 3.2d), a pattern consistent with that in RWR (Tables 3.1, 3.3; Figures 3.1d, 3.2d).

3.1.1.2 Leaf gas exchange characteristics

Populations of black spruce expressed highly variable (but not statistically significant) rates for photosynthetic capacity (A) and stomatal conductance (g_s), the combined patterns of which resulted in a significant Population differences in leaf intercellular-to-ambient [CO_2] ratio (C_i/C_a) (Table 3.4, Figure 3.3). Leaf photosynthetic capacity varied in tight correlation with both stomatal conductance ($r=0.57$, $P=0.0418$ under northern environment; $r=0.76$, $P=0.0039$ under southern environment) and leaf nitrogen concentration (%N) ($r=0.80$, $P>0.05$ for Northern.1; $r=0.83$, $P>0.05$ for Southern.1; $r=0.49$, $P=0.0042$ in the field) (Figure 3.4). A greater sensitivity of stomatal conductance to photosynthetic capacity [lower slope of their linear relationship: 6.84 ± 2.97 , $P=0.0418$ versus 19.14 ± 5.13 , $P=0.0039$] was observed under the cooler northern environment (Figure 3.4a).

There was a tendency for populations to express a higher A [Southern versus Northern seed sources: A ($\mu\text{mol m}^{-2} \text{s}^{-1}$) = 2.54 ± 0.16 versus 2.79 ± 0.22 under northern climate, A ($\mu\text{mol m}^{-2} \text{s}^{-1}$) = 4.24 ± 0.45 versus 3.54 ± 0.40 under southern climate] combined with a lower g_s [Southern versus Northern seed sources: g_s ($\text{mol m}^{-2} \text{s}^{-1}$) = 0.291 ± 0.015 versus 0.328 ± 0.024 under northern climate, g_s ($\text{mol m}^{-2} \text{s}^{-1}$) = 0.218 ± 0.017 versus 0.150 ± 0.013 under southern climate] (Figures 3.3a,b) under the warmer southern environment. Northern populations exhibited the greatest plasticity for g_s in response to the environmental shift. Overall, these populations had the highest g_s rates

under the cooler northern environment (0.328 ± 0.024) while they expressed the lowest rates under the warmer southern climate (0.150 ± 0.013) (Figures 3.3a,b). As a consequence of these patterns in A and g_s , higher values of C_i/C_a -lower instantaneous water-use efficiency- were observed under the cooler northern climate [C_i/C_a ($\mu\text{mol } \mu\text{mol}^{-1}$) = 0.930 ± 0.004 versus 0.901 ± 0.005 for southern populations; C_i/C_a ($\mu\text{mol } \mu\text{mol}^{-1}$) = 0.925 ± 0.005 versus 0.882 ± 0.009 for northern populations] (Figure 3.3c). While northern populations exhibited significantly lower C_i/C_a under southern climate, no clear latitudinal trend could be identified under the northern climate (Figure 3.3c). In order to determine which of photosynthetic capacity and stomatal conductance was dominant in the variation of instantaneous water-use efficiency, rates adjusted for one another were analyzed (Table 3.5). For a fixed stomatal conductance, there were only slight differences in photosynthetic capacity (A_{gs}) among populations (Table 3.5, Figure 3.5a) as indicated by a significant Population effect (Table 3.5) in the variance analysis of the g_s -weighted C_i/C_a (C_i/C_{ags}). There were trends for a northward increase in A_{gs} (not statistically significant) and for significantly higher values expressed under the warmer southern climate [Southern versus Northern seed sources: A_{gs} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) = 2.30 ± 0.15 versus 2.43 ± 0.20 under northern climate, A_{gs} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) = 4.19 ± 0.34 versus 4.35 ± 0.43 under southern climate]. Conversely, populations expressed much more differentiation in stomatal

conductance (g_{sA}) at a given photosynthetic capacity across environments (Table 3.5, Figure 3.5b). Indeed, there were nearly significant environmental ($P=0.0675$) and significant Population ($P=0.0065$) main effects on the assimilation-weighted C_i/C_a (C_i/C_{aA}). The latitudinal trends for g_{sA} were consistent with those of g_s [Southern versus Northern seed sources: g_{sA} ($\text{mol m}^{-2} \text{s}^{-1}$) = 0.293 ± 0.015 versus 0.319 ± 0.023 under northern climate, g_{sA} ($\text{mol m}^{-2} \text{s}^{-1}$) = 0.206 ± 0.012 versus 0.151 ± 0.012 under southern climate] (Figures 3.3b, 3.5b) and resulted in lower values for C_i/C_{aA} -higher water-use efficiency- in the warmer environment [C_i/C_{aA} ($\mu\text{mol } \mu\text{mol}^{-1}$) = 0.928 ± 0.003 versus 0.902 ± 0.004 for southern populations; C_i/C_{aA} ($\mu\text{mol } \mu\text{mol}^{-1}$) = 0.930 ± 0.004 versus 0.877 ± 0.005 for northern populations]. Environmental effects appear dominant in the variation of g_{sA} (Table 3.5).

3.1.1.3 Leaf carbon isotope discrimination

Leaf carbon isotope discrimination was indexed by $\delta\delta^{13}\text{C}$, the deviation from the population (x environment x replicate) mean $\delta^{13}\text{C}$. Bulk populations of black spruce expressed different values of leaf $\delta\delta^{13}\text{C}$ between environments as indicated by a significant Environment x Population interaction (Table 3.6, Figure 3.5c). There was a trend for populations to exhibit lower discrimination values -higher water-use efficiency- in the environment that least reflects their origin [Southern versus Northern seed sources: $\delta\delta^{13}\text{C}$ (‰)

$= -0.04 \pm 0.07$ versus 0.06 ± 0.05 under northern climate, $\delta^{13}\text{C} (\text{‰}) = 0.71 \pm 0.08$ versus -0.65 ± 0.30 under southern climate] (Figure 3.5c). Although there were few populations that did not vary in $\delta^{13}\text{C}$ values across environments, populations from farther north than population 10 (Latitude 57°N) exhibited significantly lower carbon isotope discrimination levels under the warmer southern environment (Figure 3.5c).

Consistent with theoretical expectations, leaf $\delta^{13}\text{C}$ was weakly but positively correlated with the measured ratio of leaf intercellular-to-ambient $[\text{CO}_2]$ (C_i/C_a) ($r=0.17$, $P>0.05$ under northern climate; $r=0.22$, $P>0.05$ under southern climate) (Figure 3.5d). There was no consistency across environments for leaf gas exchange characteristics nor leaf $\delta^{13}\text{C}$ (Table 3.2).

3.1.1.4 Water-use efficiency and growth

Under the southern environment, the relative growth rate (RGR) of black spruce populations was positively correlated with leaf carbon isotope discrimination ($\delta^{13}\text{C}$) ($r=0.93$, $P=0.0001$), indicating a negative relationship with long-term water-use efficiency (Figure 3.6a). This was accompanied by higher specific leaf area (SLA) ($r=-0.86$, $P=0.0001$), the least allocation of carbon to leaf (LWR) ($r=0.82$, $P=0.0007$) but the most to roots (RWR) ($r=-0.50$, $P=0.0794$) for low- $\delta^{13}\text{C}$ populations (Figure 3.6).

Under the northern environment, however, growth and carbon allocation

traits appeared poorly correlated with leaf $\delta^{13}\text{C}$. If any trend at all, there seemed to be a negative correlation between plant RGR and leaf $\delta^{13}\text{C}$ ($r=-0.41$, $P>0.05$), suggesting a positive relationship with long-term water-use efficiency (Figure 3.7a). Similarly, low- $\delta^{13}\text{C}$ populations tended to have higher SLA ($r=-0.04$, $P>0.05$) or RWR ($r=-0.24$, $P>0.05$), and lower LWR ($r=0.33$, $P>0.05$) (Figure 3.7).

3.1.2 Field test

3.1.2.1 Growth and carbon allocation traits

The relative growth rate (RGR) of populations of black spruce was lower in the field than measured under the simulated southern environment [RGR (d^{-1}) = 0.016 ± 0.0002 versus 0.041 ± 0.001 for southern populations; RGR (d^{-1}) = 0.015 ± 0.0003 versus 0.036 ± 0.001 for northern populations] and varied significantly among populations (Tables 3.1; 3.7, Figures 3.1a; 3.8a). A northward decrease in RGR was exhibited. No clear latitudinal trends could be seen for specific leaf area (SLA) (Southern seed sources versus Northern seed sources: $105 \pm 2 \text{ cm}^2 \text{ g}^{-1}$ versus $107 \pm 1 \text{ cm}^2 \text{ g}^{-1}$), the proportional carbon allocation to leaves (LWR) ($0.336 \pm 0.012 \text{ g g}^{-1}$ versus $0.348 \pm 0.012 \text{ g g}^{-1}$) or the proportional carbon allocation to roots (RWR) ($0.432 \pm 0.013 \text{ g g}^{-1}$ versus $0.454 \pm 0.011 \text{ g g}^{-1}$) (Figures 3.8b,c,d). While the SLA ($106 \text{ cm}^2 \text{ g}^{-1}$ versus $95 \text{ cm}^2 \text{ g}^{-1}$) and RWR (0.442 g g^{-1} versus 0.354 g g^{-1}) were higher in the field relative to controlled environments, LWR was decreased (0.341 g g^{-1} versus

0.521 g g⁻¹). Overall, populations that exhibited higher values for RGR, LWR or RWR under the simulated southern environment tended to express higher RGR or RWR but lower LWR in the field (Table 3.2).

The combined variations in RGR, SLA and LWR resulted in significant differences among populations for total plant leaf area and total plant biomass (Table 3.8). Both of these latter plant traits remained lower than observed under controlled environments [total plant leaf area (cm²): 18±2 versus 8±1; total plant biomass (g): 0.385±0.059 versus 0.208±0.021] and decreased in the more northerly populations [Southern versus Northern seed sources: Total plant leaf area (cm²)=9±1 versus 6±1; total plant biomass (g)=0.250±0.021 versus 0.157±0.019) (Figures 3.2a,b; 3.9a,b). Patterns of variation in plant leaf area and root-to-shoot ratios (LAR, RSR) were similar to those shown for LWR and RWR, respectively (Figures 3.7c,d; 3.9c,d).

3.1.2.2 Leaf gas exchange characteristics

Field-grown populations of black spruce expressed no significant differences in photosynthetic capacity (A) and stomatal conductance (gs), but the combined variations of those characteristics resulted in significant differences in leaf intercellular-to-ambient [CO₂] ratio (C_i/C_a) (Table 3.9, Figure 3.10).

Variation in leaf A was strongly correlated with both gs (r=0.75, P=0.0032) and leaf nitrogen concentration (%N) (r=0.49, P=0.0042) (Figure 3.4). In comparison with plants from controlled environments, field data exhibited the least sensitivity of gs to A [higher slope of their linear relationship:

27.81 ± 7.42 , $P=0.0032$ versus 19.14 ± 5.13 , $P=0.0039$] (Figure 3.4a).

Northern populations showed a trend for higher A ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (Southern versus Northern seed sources: 4.24 ± 0.28 versus 5.03 ± 0.41), slightly higher g_s ($\text{mol m}^{-2} \text{s}^{-1}$) (Southern versus Northern seed sources: 0.162 ± 0.007 versus 0.173 ± 0.012) but lower C_i/C_a ($\mu\text{mol } \mu\text{mol}^{-1}$) (Southern versus Northern seed sources: 0.835 ± 0.009 versus 0.814 ± 0.011), although the latitudinal pattern for C_i/C_a was less clear (Figure 3.10). In comparison with plants from controlled environments, leaf photosynthetic capacity (A) was generally higher in field-grown plants ($4.58 \mu\text{mol m}^{-2} \text{s}^{-1}$ versus $3.26 \mu\text{mol m}^{-2} \text{s}^{-1}$) whereas both stomatal conductance (g_s) ($0.17 \text{ mol m}^{-2} \text{s}^{-1}$ versus $0.25 \text{ mol m}^{-2} \text{s}^{-1}$) and leaf C_i/C_a ($0.83 \mu\text{mol } \mu\text{mol}^{-1}$ versus $0.91 \mu\text{mol } \mu\text{mol}^{-1}$) were lower in field-grown plants (Figures 3.3; 3.10).

For a fixed stomatal conductance, populations differed in photosynthetic capacity (A_{g_s}) as indicated by a significant Population effect on the g_s -weighted leaf intercellular-to-ambient [CO_2] ($C_i/C_{a_{g_s}}$) (Table 3.10, Figures 3.11a,b).

Northern populations tended to express higher A_{g_s} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) [Southern versus Northern seed sources: 4.20 ± 0.25 versus 4.87 ± 0.36] and, therefore, to exhibit lower $C_i/C_{a_{g_s}}$ ($\mu\text{mol } \mu\text{mol}^{-1}$) [0.834 ± 0.009 versus 0.814 ± 0.011]. In contrast, populations did not vary in their assimilation-weighted stomatal conductance (g_{s_A}) nor in their C_i/C_{a_A} (Table 3.10, Figures 3.11a,b).

3.1.2.3 Leaf carbon isotope discrimination

Leaf carbon isotope discrimination, either expressed as $\delta^{13}\text{C}$ or Δ , varied almost significantly ($P=0.0581$) among populations (Table 3.11, Figure 3.11c). Consistent with theory, leaf $\delta^{13}\text{C}$ correlated positively and almost significantly with the measured leaf C_i/C_a ($r=0.53$, $P=0.0613$) (Figure 3.11d). Overall, populations south of population 9 (Latitude 56°N) expressed lower $\delta^{13}\text{C}$ values in the field than was observed under the simulated southern environment, whereas the opposite held for populations north of population 9 (Figures 3.5c, 3.11c). In parallel with the pattern of C_i/C_a , the lowest discrimination values are observed in mid-latitudinal populations (Figures 3.10c, 3.11c). Between the field and the simulated southern environment, there was no consistency among populations for leaf gas exchange characteristics or $\delta^{13}\text{C}$ values (Table 3.2).

3.1.2.4 Water-use efficiency and growth

In contrast with the simulated southern environment, the relative growth rate (RGR) of field-grown populations of black spruce tended to correlate negatively, although weak, with leaf carbon isotope discrimination ($\delta^{13}\text{C}$) ($r=-0.46$, $P=0.1151$), indicating a positive relationship between RGR and long-term water-use efficiency (Figure 3.12a). However, relationships between leaf $\delta^{13}\text{C}$ and several carbon allocation traits remained even weaker (Figures

3.12b,c,d). Low- $\delta^{13}\text{C}$ populations tended to express lower specific leaf area (SLA) ($r=0.16$, $P>0.05$), to allocate less carbon to leaf (LWR) ($r=0.05$, $P>0.05$) but more carbon to roots (RWR) ($r=-0.05$, $P>0.05$) (Figure 3.12).

3.1.3 Correlations among seed-source climatic variables and seedling characteristics

3.1.3.1 Controlled environments

Principal components analysis was used to study relationships among seedling characteristics and climatic conditions in their original, natural environments. Four components or axes explained between 89-91% of total variance among variables (Tables 3.12, 3.13). The interpretation of these axes varied between the simulated southern and northern environments. Under both climates, the first axis, which accounted for 45% of total variation, was interpreted as "*Length of growing season*", since it was approximately equally correlated with temperature and photoperiod. Provided that the accumulation of degree-days is a reliable measure of the length of growing season, this first axis could equally be termed "*Temperature*". The second axis, which explained 23% of total variation, was termed "*Moisture*" in both environments as it basically reflected the climatic moisture index and the seasonal mean vapor pressure deficit. The third axis, with 12 to 15% of total variation, had different loading patterns between environments. While this component reflected "*Temperature*" under the cooler northern climate, it was considered to be an indicator of "*Moisture*" under the warmer southern

climate. Conversely, the fourth component, associated with 8 to 9% of total variance, indicated "*Moisture*" under northern climate but "*Temperature*" under southern climate.

The relative growth rate (RGR) and leaf $\delta^{13}\text{C}$ parameters were found to be strongly correlated to the length of growing season at seed source under southern climate and to temperature in the northern environment. Specific leaf area (SLA) consistently associated with seed-source temperature while carbon allocation to leaves (LWR) and carbon allocation to roots (RWR) invariably were correlated with the length of growing season. Leaf photosynthetic capacity (A) was correlated with seed-source moisture in the southern climate and length of growing season under the northern climate. In contrast, stomatal conductance (gs) and leaf intercellular-to-ambient [CO_2] (C_i/C_a) were associated with seed-source moisture in both environments. Length of growing season and temperature components were lumped together. Growth and carbon allocation traits were then plotted against soil temperature (Figure 3.13) while leaf physiological characteristics were related to seed source climatic moisture index (Figures 3.14). Observed trends indicated that populations from warmer habitats grew faster (RGR) ($r=0.45$, $P=0.1209$ under northern environment; $r=0.83$, $P=0.0003$ under southern environment) and allocated more carbon to leaves (LWR) ($r=0.88$, $P=0.0001$ under northern environment; $r=0.85$, $P=0.0002$ under southern environment) but less to roots (RWR) ($r=-0.76$, $P=0.0024$ under northern

environment; $r=-0.66$, $P=0.0138$ under southern environment) (Figure 3.13). These same populations (from warmer habitats) expressed lower specific leaf area (SLA) under the warmer environment ($r=-0.62$, $P=0.0226$) and but slightly higher SLA under the cooler northern climate ($r=0.22$, $P>0.05$) (Figure 3.13). Across environments, populations exhibited contrasting and non-linear responses of photosynthetic capacity (A), stomatal conductance (g_s) and leaf intercellular-to-ambient $[CO_2]$ (C_i/C_a) to seed-source climatic moisture index (Figures 3.14a,b,c). The patterns of g_s and C_i/C_a showed populations from drier (and cooler) habitats expressing great plasticity, shifting from larger values under the cooler northern environment to smaller ones in the warmer southern climate (Figures 3.14b,c). Low-moisture (cooler sources) populations expressed lower leaf $\delta^{13}C$ values under the warmer southern climate whereas high-moisture populations (warmer sources) did so under the cooler northern climate (Figure 3.14d).

3.1.3.2 Field test

For the data from field experiment, four components were identified which explained about 86% of total variance among selected variables (Table 3.14). The first axis, which accounted for 32% of total variation, was correlated with photoperiod, temperature and moisture and was interpreted as "*Length of growing season*". "*Temperature*" could be an alternative interpretation of this first axis. The second axis, which explained 26% of total variation, was also termed "*Temperature*", as it had the highest correlation with soil

temperature. The third and fourth axes, with 19% and 9% of total variation, respectively, reflected both the climatic moisture index and the seasonal mean vapor pressure deficit, were interpreted as "*Moisture*".

Relative growth rate (RGR) and carbon allocation to leaves (LWR) correlated most strongly to the length of growing season at seed source. Carbon allocation to roots (RWR) was correlated with both moisture and temperature at seed source. Specific leaf area (SLA), photosynthetic capacity (A) and stomatal conductance were all associated with temperature while leaf intercellular-to-ambient $[CO_2]$ (C_i/C_a) and leaf carbon isotope discrimination ($\delta^{13}C$) were most strongly correlated with moisture at seed source.

As in controlled environments, the components of length of growing season and temperature were lumped together. Growth and carbon allocation traits were then plotted against soil temperature while leaf physiological characteristics were related to the climatic moisture index at seed source.

Consistent with controlled environments, populations from warmer habitats grew faster in the field ($r=0.62$, $P=0.0242$) (Figure 3.13a). The field responses of specific leaf area (SLA) and carbon allocation to leaf (LWR) to seed-source temperature contrasted with those under the simulated southern environment. In the field, seed-source temperature tended to correlate positively with SLA ($r=0.14$, $P>0.05$) but negatively with LWR ($r=-0.53$, $P=0.0628$). The opposite trends were observed under the simulated southern environment (Figures 3.13b,c). Carbon allocation to roots (RWR) ($r=-0.43$,

$P=0.1407$), on the other hand, remained consistent across environments (Figure 3.13d).

Populations, particularly those from drier habitats (cooler sources), exhibited slightly higher leaf photosynthetic capacity (A) ($P>0.05$) (Figure 3.15a). The responses of both A and stomatal conductance (g_s) to moisture changed between the field and the simulated southern environment (Figures 3.14a,b; 3.15a,b). The pattern for C_i/C_a , on the other hand, remained similar with much lower values observed in the field (Figures 3.14c; 3.15c). Low-moisture populations (cooler sources) expressed lower leaf $\delta^{13}C$ when grown in the field (Fig 3.15d). However, these same populations expressed much lower leaf $\delta^{13}C$ when grown under the simulated southern environment (Fig 3.14d).

3.2 DISCUSSION

3.2.1 Growth and carbon allocation traits

Under controlled environments, populations from farther north than 57°N grew slower than more southerly populations, and they grew even slower when cultivated under the warmer southern climate (Figure 3.1a).

Differentiation in mean relative growth rate (RGR) arises through variation in either or both net assimilation rate (NAR) and leaf area ratio (LAR) (Kvet et al. 1971; Evans 1972). In turn, the latter is a unique function of specific leaf area (SLA) and leaf weight ratio (LWR). Among populations, faster growth was observed in plants with a higher proportion of carbon allocated to leaves (LWR) and a lower proportion to roots (RWR), which was consistent with observations by Johnsen and Seiler (1996). However, carbon allocation patterns could only partially explain the differences in RGR observed between environments. For instance, extreme northern populations differed in relative growth rate across environments despite a similar leaf area ratio while the opposite held for southern populations (Figures 3.1, 3.2). Therefore, across-environment differences in growth rate were more likely the result of an interaction between carbon allocation patterns and net assimilation rate. An increase in net assimilation rate may compensate for a reduced allocation of carbon to leaves.

In this study, carbon allocation traits were examined at the same developmental stage and for seedlings of a given size, the average total biomass achieved by populations. This allows comparisons among populations to be made based on their intrinsic differences (Evans 1972; Virgona and Farquhar 1996; Rice and Bazzaz 1997) both within and between environments. Independently of the cultivation environment, the proportion of carbon allocated to leaves and that to roots were negatively correlated -suggesting a strong tradeoff- determined by significant but separate main effects of Environment and Population. The absence of a significant interaction indicated that differences in those allocation traits were similarly expressed across environments. Accordingly, the farther north the population origin, the lower the proportion of carbon allocated to leaves but the higher allocated to roots (Figure 3.1).

Greater allocation to roots, which results in higher root-to-shoot ratio, is an intrinsic component of a stress resistance syndrome (Chapin *et al.* 1993) and may be explained by a number of factors. Northern populations of black spruce experience low water and nutrient availability, low temperature and short period for shoot growth, all of which may result in a strong selection pressure for greater allocation of carbon to roots. Low temperature increases the viscosity of water and reduces the permeability of root tissues (Kramer and Kozlovski 1979), which might combine with low water availability to select for an adaptive strategy for the specific carbon allocation pattern

observed here. Further, higher allocation of carbon to roots may come as a byproduct of a shorter period for shoot growth. Conversely, populations inhabiting warmer areas, in addition to experiencing longer periods for shoot growth, are naturally subjected to more acute competition and herbivory, which may select for greater allocation of carbon to shoot. In fact, conifer seedlings, black spruce in particular, are very sensitive to above-ground competition (Jobidon 1992, 1994). Spruce budworm outbreaks are also more intense over the southern part of the boreal forest (Levasseur and Morin 1998).

In response to the environmental shift, from the cooler northern climate to the warmer southern climate, populations allocated less carbon to leaves but more to roots (Figure 3.1c). This pattern in allocation of carbon, unlike the adaptive one within environments, is more likely an acclimation response. Higher evaporative demand for water in the warmer climate (Chapter 2) may have induced greater investment of carbon into roots.

Principal components analysis suggested that differences in relative growth rate and carbon allocation traits were most strongly correlated with seed-source mean annual temperature (Tables 3.12-14). Within the range of this study, soil and air temperatures are tightly coupled, both depending on the amount of solar energy received and on substrate thermal properties. In general, soil temperature lags behind solar radiation more than does air temperature, and this lag lengthens at greater depths in the soil (Kimmins

1987, Bazzaz 1996). In the Fall, root growth may proceed several weeks after bud set while the opposite pattern takes place in the Spring. This anachronistic growth of roots and shoots indicates that soil temperature may be more relevant than air temperature for carbon allocation. Furthermore, in consideration of the shallow rooting pattern of naturally-occurring black spruce, the soil temperature at 10 cm depth can reasonably be assumed as the average one experienced by below-ground tissues in first-year seedlings. Consistent with theory, populations from warmer habitats allocated more carbon to leaves, less to root and consequently grew faster under controlled environments. In the field, however, these same populations grew faster despite a lower investment of carbon in leaves. Such a pattern in growth rate could have been brought about by not only differential leaf shedding or herbivory (as seen in the field during gas exchange measurements) at the time of harvesting but also altered differentiation in net assimilation rate among populations.

Field-grown seedlings also differed from those under the simulated southern environment in other aspects: field seedlings are older (42 versus 20 weeks) but smaller (average total biomass 0.208 versus 0.385 g), probably as a result of less favorable growing conditions. In addition to relatively variable greenhouse conditions, the summer 1997 proved to be hotter and drier than normal for Ottawa (Appendix 5). Interestingly, the influence of seedling size on carbon allocation to leaves and roots did not vary significantly between the

two environments. On a logarithmic basis, 1 unit deviation from the average total biomass, was associated with 1.05 ± 0.03 and 1.08 ± 0.06 units change in leaf biomass for controlled and field conditions, respectively. As regards root biomass, these figures amounted to 0.93 ± 0.04 and 0.99 ± 0.06 , respectively.

Accordingly, as seedling development proceeds within the juvenile period, more and more carbon is allocated to shoot at the expense of root.

The stability in carbon allocation between the field and the simulated southern environment also helps decipher the variation pattern in specific leaf area. The increased specific leaf area for a reduced leaf weight ratio in northern populations should have come about through reduced carbon content (both structural and non structural) rather than expanded leaf blade. The analysis of leaf %C supports this idea. Although leaf %C did not vary significantly among populations (44 to 47% in the field), northern populations tended to exhibit lower values. In fact, these populations tended to exhibit chlorotic needles and to grow dwarf-like. On the one hand, chlorotic leaves are likely to contain lower non structural carbon. On the other hand, the dwarfness of these populations could be the basis of the least sensitivity of their relative growth rate between the field and the simulated southern environment (Figure 3.13a).

3.2.2 Leaf physiological characteristics

Gas exchange characteristics were measured under a constant photosynthetic photon flux density (PPFD) and adjusted for leaf-to-air vapour pressure deficit and ambient carbon dioxide concentration. Therefore, the observed patterns in leaf physiology are the sole effects of cultivation conditions and properties inherent to black spruce populations. Photosynthetic capacity (A) correlated with both stomatal conductance (g_s) and leaf %N (Figure 3.4). In addition, A tended to be higher in northern than southern populations (non-significant trend) and under warmer than cooler cultivation temperatures (significant trend). In common environment tests, populations from cooler habitats generally exhibited higher leaf %N (Reich *et al.* 1996; Johnsen and Seiler 1996; Johnsen *et al.* 1996) which is likely to correlate positively with N investment into key photosynthetic enzymes (Cowan 1986; Field and Mooney 1986). Higher leaf %N is thought to enhance the metabolic activity and growth under cooler growing conditions (Reich *et al.* 1996). In this study, the observed higher leaf %N under warmer environments (field and simulated southern climate), occurred through either increased uptake and allocation of N to leaves or as a byproduct of reduced allocation of carbon to leaves. The higher leaf %N content may account for higher A in warmer environments. In turn, the higher atmospheric demands for water under warmer temperatures could be the basis for the decreased sensitivity of g_s to A .

In contrast with the intra-environment patterns, stomatal conductance and photosynthetic capacity were less coupled across environments. A high plasticity for g_s was observed in response to changes in environmental conditions. Not only did populations express a higher (not statistically significant) g_s in the environment that best reflected their origin, but these rates also remained lower (but not statistically significant) under the warmer southern climate. These patterns resulted in significantly greater stomatal plasticity for northern populations.

The ratio of leaf intercellular-to-ambient $[CO_2]$ (C_i/C_a) is a reliable index of instantaneous water-use efficiency. At the intraspecific level, variation in C_i/C_a among populations is brought about by changes in either or both photosynthetic capacity and stomatal conductance. The higher the photosynthetic capacity to stomatal conductance ratio [A/g_s , an intrinsic measure of instantaneous water-use efficiency (Meinzer *et al.* 1990)], the lower the C_i/C_a . In order to determine which of the two leaf physiological characteristics (A or g_s) was dominant, rates adjusted for one another were analyzed. Under controlled environments, both populations and environments differed little in A for a fixed g_s (Figure 3.5a). Conversely, populations expressed more differentiation in g_s among themselves and between environments for a given A (Figure 3.5b). In the field, however, populations differed more in A than they did in g_s , partly due to greater water limitation (Figures 3.11a,b). Accordingly, variation in carbon isotope

discrimination (Δ , here indexed by $d\delta^{13}\text{C}$), thus in water-use efficiency (WUE), was primarily via changes in g_s under controlled environments but through changes in A under field conditions.

Assimilation-weighted stomatal conductance (g_{s_A}), in itself, is an inverse measure of instantaneous water-use efficiency. Since the effects of both photosynthetic capacity and measurement environmental conditions are accounted for, the patterns of g_{s_A} also reveal an intrinsic property of black spruce to express lower stomatal conductance rates (g_s) under warmer cultivation temperatures. Regardless of the underlying mechanisms, evolution of such a physiological response appears best expressed in northern populations. In turn, the latter populations showed the most plastic stomatal behaviour (Figures 3.5b), resulting in higher leaf carbon isotope discrimination ($d\delta^{13}\text{C}$) under the northern climate but lower leaf $d\delta^{13}\text{C}$ under the southern environment (Figure 3.5c).

Leaf carbon isotope discrimination (Δ), here indexed by $d\delta^{13}\text{C}$, is a time-integrated measure of internal plant physiological and environmental properties influencing photosynthetic gas exchange (Meinzer *et al.* 1990; Ehleringer 1991). Leaf Δ reliably predicts long-term water-use efficiency (WUE) and for C_3 plants, Δ is a linear function of C_i/C_a (Farquhar *et al.* 1989). In fact, the weak but positive correlations between leaf C_i/C_a and leaf $d\delta^{13}\text{C}$ indicate some coupling between short- and long-term WUE in black spruce

(Figures 3.5d, 3.11d). However, the mechanism for the variation in leaf $\delta^{13}\text{C}$ varied across environments. While differentiation in leaf $\delta^{13}\text{C}$ arose primarily through stomatal physiology under controlled environments, it was via variation in mesophyll capacity for CO_2 fixation in the field (photosynthetic capacity). The relationships between leaf $\delta^{13}\text{C}$ and relative growth rate under the simulated southern (Figure 3.6a) and field (Figure 3.12a) environments provided further support for the observed sources of variation in leaf $\delta^{13}\text{C}$ while the negative relationship under the northern climate (Figure 3.7a) was more likely the result of greater sensitivity of g_s to A (Figure 3.4a).

Lately, there has been growing interest in the potential use of Δ as screening criterion for improved WUE and productivity (Virgona *et al.* 1990; Meinzer *et al.* 1990; Flanagan and Johnsen 1995; Virgona and Farquhar 1996; Anderson *et al.* 1996; Sun *et al.* 1996). In that perspective, theoretical relationships have been suggested that describe how some specific variations in leaf physiological characteristics might translate into growth patterns. All things being equal, there should be a negative correlation between Δ and plant growth when variation in Δ results from changes in photosynthetic capacity (Farquhar *et al.* 1989). In contrast, conductance-driven variation in Δ should lead to a positive correlation between Δ and plant growth (Farquhar *et al.* 1989). However, because optimum water conservation requires matching

compromises in leaf photosynthetic gas exchange and whole-plant carbon allocation (Cowan 1986), differences in allocation patterns may offset the above theoretical relationships when water is not limiting. For instance, where variation in Δ is primarily driven by photosynthetic capacity, low- Δ populations may grow slower if a higher proportion of carbon is allocated to roots relative to shoots. Conversely, lack of water conservation may offset the expected positive relationship between plant growth and Δ where variation in the latter results from changes in stomatal conductance.

Although, observations in this study are in agreement with the above theory, the field case deserves further comments. Plant responses to water stress include resistance (closure of stomata, abscission of leaves), avoidance (cuticle thickness, greater allocation of carbon to roots) or tolerance (osmoregulation, cell wall elasticity) (Kramer and Boyer 1995). Often, alternative use of these mechanisms results in complex interrelationships among plants traits. In the field, for instance, low- $\delta^{13}\text{C}$ populations grew faster despite a lower leaf area ratio and a slightly higher root-to-shoot ratio. This observation indicates that both morphological and physiological adjustments were used by populations under harsh conditions. It is highly likely, however, that the low leaf area ratio was brought about by differential leaf shedding (or herbivory, as seen in the field during gas exchange measurements). Partial defoliation may have increased A in the remaining leaves (Carmi and van Staden 1983), and therefore lowered $\delta^{13}\text{C}$, but also enlarged the short-term root/shoot ratio.

The observation that change photosynthetic capacity (A) was the primary source of variation in leaf $\delta^{13}\text{C}$ under water-limiting (and much warmer) field conditions was in agreement with findings among mature full-sib families of black spruce (Flanagan and Johnsen 1995; Johnsen and Major 1995) and among juvenile white spruce crosses (Sun *et al.* 1996). Under high water availability, variation in A also accounted for genotypic variation in Δ for sunflower genotypes (Virgona *et al.* 1990; Virgona and Farquhar 1996), for Eucalypt taxa (Anderson *et al.* 1996) and for white spruce crosses (Sun *et al.* 1996). The latter findings contrast with the conductance-driven variation in leaf $\delta^{13}\text{C}$ observed in the present study under non-water limiting conditions. Variation in Δ among well-watered coffee genotypes also resulted from changes in stomatal conductance (g_s) (Meinzer *et al.* 1990). Sun *et al.* (1996) reported a negative relationship between Δ and biomass production in juvenile white spruce independent of soil moisture conditions. To the contrary, only on a drier site could such a relationship be established (Flanagan and Johnsen 1995) or inferred (Johnsen and Major 1995) among mature full-sib families of black spruce. Thus, there should be a threshold moisture level (edaphic or atmospheric, or a correlated factor) below which black spruce populations shift from differing in g_s to varying in A . Because the two types of physiological control have different implications as regards plant growth and carbon allocation, further knowledge about this critical moisture level may prove useful in matching populations with specific

environments.

From principal components analysis, leaf physiological characteristics appeared to respond to seed source moisture conditions. Similar adaptive responses have been reported. Leaf physiological characteristics of *Hymenoclea salsola* ecotypes (Comstock and Ehleringer 1992) and of Eucalypt genotypes (Anderson *et al.* 1996) were correlated with the mean vapour pressure deficit at their native habitat. In this study, an annual climatic moisture index at seed source was devised as the balance between precipitation and potential evapotranspiration (Thornwaithe 1948, Hogg 1994). Among seed sources, this index was highly and negatively correlated with saturation vapour pressure deficit ($r=-0.76$, $P<0.05$). Regardless of the cultivation environment in this study, leaf physiological characteristics responded non-linearly to the climatic moisture index (Figures 3.14, 3.15). However, the contrasting response patterns, particularly under controlled environments, indicate rather an interaction between seed-source moisture and cultivation temperature (Figure 3.14).

Interestingly, low-moisture (cooler sources) populations appear much more differentiated among themselves than high-moisture as regards C_i/C_a and $\delta^{13}C$ (Figures 3.14, 3.15). *Picea* spp., black spruce included, generally have high outcrossing rates (Muona *et al.* 1990). Such a mating behavior may allow the presence of unfit genotypes within populations prior to complete stabilizing selection. One might also think of a behavior that could help

populations cope with unstable climates. Regardless of the underlying phenomenon, high differentiation among low-moisture (cooler sources) populations, particularly in the field, contributes to weakening the response of leaf physiological characteristics to seed-source moisture index.

3.23 Addressing the hypotheses

Grown under the cooler northern climate, low-moisture (cooler sources) populations express a higher g_s -to- A ratio, resulting in a higher C_i/C_a and therefore a higher $\delta^{13}C$ (Figure 3.14). Since these same populations, which naturally occur in cooler areas, also allocate a higher proportion of carbon to roots than to shoots (Figure 3.13), the leaf and whole-plant tend to be decoupled in terms of water conservation. In contrast, strong water conservation was observed under the warmer southern environment. Therein, not only do low-moisture (cooler sources) populations have a lower g_s in relation to A -thus a lower C_i/C_a and a lower $\delta^{13}C$ (Figure 3.14)- but they also allocate a higher proportion of carbon to roots relative to shoots (Figure 3.13). These contrasting observations provide evidence for the rejection of the first two hypotheses under the northern climate but for their acceptance under the southern one.

In the field, low-moisture (cooler sources) populations expressed higher photosynthetic capacities -thus lower C_i/C_a or $\delta^{13}C$ (Figure 3.15). As low-moisture (cooler sources) populations also allocated more carbon to root

relative to shoot (Figure 3.13), water conservation at both leaf and whole-plant levels tended to be coordinated under field conditions. However, the most conservative suite of traits occurred in populations from upper mid-latitudes. These observations provide support for the acceptance of the first hypothesis but for the rejection of the second hypothesis under field conditions.

The ranking of populations for growth rate and carbon allocation traits was generally consistent across controlled environments but that ranking was slightly modified under the drier and warmer field conditions (Table 3.2). Leaf physiological characteristics, on the other hand, were inconsistently expressed across all environments (Table 3.2). This divergence in expression of plant traits suggests a more prominent adaptation or correlated response in carbon allocation than in leaf physiology for black spruce. These observations also constitute a basis for the rejection of the third hypothesis at the population level.

3.3 CONCLUSIONS

- Black spruce populations are differentiated in growth rate, carbon allocation traits and leaf physiological characteristics. Optimum expression of water conservation was not only environment dependent but it also occurred via distinct mechanisms. For example, in the field experiment leaf physiological characteristics and whole-plant carbon allocation traits were coordinated. However in controlled environment studies, such a coordination held only under the warmer southern climate. Further, while leaf physiology was primarily driven by stomatal conductance under controlled environments, it was driven by photosynthetic capacity under field conditions.
- Congruence of warmer temperatures and higher water availability appears to be a *sine qua non* condition for the veracity of the first two hypotheses. Under such climates, populations allocating a higher proportion of carbon to roots relative to shoots also express a lower stomatal conductance (g_s) in relation to photosynthetic capacity (A). These populations have therefore a low carbon isotope discrimination (Δ) and are generally of northern origin.

- Increased net assimilation rate, under warmer well-watered environments, may compensate for reduced allocation of carbon to shoots and help maintain biomass yield of juvenile black spruce. However, such an apportionment of carbon may have important ecological implications as regards seedling establishment. From a silvicultural standpoint and in view of the high sensitivity of juvenile black spruce to above-ground competition, this pattern of carbon allocation may translate into more intensive vegetation management practices.

- While differences in growth and carbon allocation were similarly expressed across environments, those in leaf physiology remained inconsistent, precluding therefore the veracity of the third hypothesis at the population level. This divergence among plant traits suggests a more prominent adaptation or correlated response in carbon allocation than in leaf physiology for black spruce.

- There appears to be a threshold moisture (edaphic or atmospheric) below which black spruce populations shift from primary limitation to photosynthetic gas exchange caused by stomatal conductance to primary limitation caused by mesophyll biochemical capacity for CO₂ fixation. Since the two types of physiological control have different implications for carbon gain and carbon allocation characteristics, further knowledge of the interrelationships among gas exchange and carbon allocation may prove useful in matching populations with specific environments.

Table 3.1. Variance analysis of growth rate and primary carbon allocation traits in bulk populations of black spruce at juvenile stage under controlled environments.

Source of variation	Relative growth rate			Specific leaf area			Leaf weight ratio			Root weight ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	0.00230	0.0060	2	51344	0.0175	2	0.044	0.1519	2	0.040	0.2440
Environment (E)	1	0.00036	0.0366	1	80	0.7954	1	0.965	0.0081	1	0.623	0.0199
Main plot Error	2	0.00001		2	917		2	0.008		2	0.013	
Population (P)	12	0.00022	0.0001	12	458	0.0022	12	0.070	0.0001	12	0.046	0.0001
E x P	12	0.00008	0.0001	12	309	0.0287	12	0.004	0.6031	12	0.005	0.5554
Sampling error	122	0.00001		116	128		122	0.003		122	0.003	
Subplot error	40	0.00001		39	138		40	0.005		40	0.005	
Mean=0.039			Mean=95			Mean=0.521			Mean=0.354			
C.V.=7%			C.V.=12%			C.V.=11%			C.V.=16%			

Table 3.2. Between-environment linear correlation coefficients (significance level shown in parentheses) for different traits determined for bulk populations of black spruce (n=13) at the juvenile stage. Environments are simulated northern and southern climates, and a southern field plot; Traits are: mean relative growth rate (RGR, d⁻¹), specific leaf area (SLA, cm² g⁻¹), leaf weight ratio (LWR, g g⁻¹), root weight ratio (RWR, g g⁻¹), photosynthetic capacity (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), intercellular-to-ambient [CO₂] (C_i/C_a, $\mu\text{mol } \mu\text{mol}^{-1}$), and carbon isotope discrimination index (d $\delta^{13}\text{C}$, ‰).

	RGR	SLA	LWR	RWR	A	gs	C _i /C _a	d $\delta^{13}\text{C}$
Between controlled environments	0.78 (0.0018)	0.05 (0.8650)	0.92 (0.0001)	0.83 (0.0004)	0.27 (0.3950)	0.08 (0.8133)	0.06 (0.8547)	0.17 (0.5795)
Between the field and the simulated southern environment	0.73 (0.0046)	0.06 (0.8452)	-0.51 (0.0736)	0.54 (0.0572)	0.33 (0.2894)	0.20 (0.5351)	-0.09 (0.7717)	0.14 (0.6376)

Table 3.3. Analysis of variance for secondary carbon allocation traits and biomass of bulk populations of black spruce at the juvenile stage under controlled environments.

Source of variation	Total leaf area			Total biomass			Leaf area ratio			Root-to-shoot ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	8303	0.0235	2	6.044	0.0052	2	16075	0.1068	2	0.230	0.3021
Environment (E)	1	643	0.2146	1	0.014	0.5712	1	8849	0.1525	1	3.991	0.0240
Main plot Error	2	200		2	0.032		2	1921		2	0.099	
Population (P)	12	325	0.0004	12	0.204	0.0013	12	707	0.0001	12	0.374	0.0001
E x P	12	52	0.7729	12	0.059	0.4506	12	89	0.6008	12	0.031	0.6490
Sampling error	116	48		122	0.020		116	88		122	0.023	
Subplot error	39	79		40	0.058		39	113		40	0.039	
Mean=18			Mean=0.385			Mean=50			Mean=0.594			
C.V.=38%			C.V.=37%			C.V.=19%			C.V.=26%			

Table 3.4. Analysis of variance for leaf gas exchange characteristics in bulk populations of black spruce at the juvenile stage under controlled environments. These traits are: photosynthetic capacity (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), intercellular-to-ambient $[\text{CO}_2]$ (C_i/C_a , $\mu\text{mol } \mu\text{mol}^{-1}$).

Source of variation	d.f.	A		g_s		C_i/C_a	
		M.S.	p	M.S.	p	M.S.	p
Bloc	2	75.82	0.3991	0.1284	0.3466	0.0097	0.3790
Environment (E)	1	100.69	0.2930	0.2221	0.2127	0.0451	0.1106
Main plot Error	2	50.36		0.0681		0.0060	
Population (P)	12	3.30	0.1501	0.0171	0.1266	0.0022	0.0301
E x P	11	2.59	0.3082	0.0113	0.4013	0.0017	0.0870
Sampling error	86	1.27		0.0069		0.0007	
Subplot error	33	2.11		0.0104		0.0009	
		Mean=3.26		Mean=0.25		Mean=0.91	
		C. V.=35%		C. V.=33%		C. V.=3%	

Table 3.5: Analysis of variance for weighted gas exchange characteristics in bulk populations of black spruce at the juvenile stage under controlled environments. Traits are: gs-weighted assimilation (A_{gs} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and C_i/C_a (C_i/C_{ags} , $\mu\text{mol } \mu\text{mol}^{-1}$), A-weighted stomatal conductance (gs_A , $\text{mol m}^{-2} \text{s}^{-1}$) and C_i/C_a (C_i/C_{aA} , $\mu\text{mol } \mu\text{mol}^{-1}$).

Source of variation	d.f.	A_{gs}		C_i/C_{ags}		gs_A		C_i/C_{aA}	
		M.S.	p	M.S.	p	M.S.	p	M.S.	p
Bloc	2	40.15	0.3532	0.0293	0.3147	0.0665	0.3830	0.0004	0.8530
Environment (E)	1	134.48	0.1316	0.0091	0.4978	0.3065	0.0979	0.0290	0.0675
Main plot Error	2	21.93		0.0135		0.0351		0.0022	
Population (P)	12	2.68	0.2156	0.0018	0.0171	0.0153	0.2378	0.0012	0.0065
E x P	11	2.99	0.1572	0.0012	0.1077	0.0140	0.3063	0.0006	0.1619
Sampling error	86	1.60		0.0007		0.0059		0.0004	
Subplot error	33	1.92		0.0007		0.0114		0.0004	
		Mean=3.23		Mean=0.91		Mean=0.25		Mean=0.91	
		C.V.=39%		C.V.=3%		C.V.=31%		C.V.=2%	

Table 3.6. Analysis of variance for leaf carbon isotope discrimination index in bulk populations of black spruce at the juvenile stage under controlled environments. Expressing the leaf carbon isotope discrimination as the deviation from population (x environment x replicate) mean $\delta^{13}\text{C}$ makes the coefficient of variation to reach very high values since the average of deviations tends toward zero.

Source of variation	d.f.	M.S.	p
Bloc	2	1.08	0.5274
Environment (E)	1	3.16	0.2473
Main plot Error	2	1.21	
Population (P)	12	8.88	0.0001
E x P	12	8.27	0.0001
Sampling error	122	0.34	
Subplot error	40	0.78	
			Mean=0.01
			C.V.=4748%

Table 3.7. Analysis of variance for growth rate and primary carbon allocation traits in field-grown bulk populations of black spruce at the juvenile stage.

Source of variation	Relative growth rate			Specific leaf area			Leaf weight ratio			Root weight ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	<10 ⁻⁵	0.1139	2	181	0.1933	2	0.005	0.5781	2	0.001	0.8279
Population	12	2x10 ⁻⁵	0.0001	12	168	0.1511	12	0.008	0.5596	12	0.009	0.3179
Sampling error	60	<10 ⁻⁵		58	136		60	0.006		60	0.007	
Error	22	<10 ⁻⁵		22	102		22	0.009		22	0.007	
Mean=0.016			Mean=106			Mean=0.341			Mean=0.442			
C.V.=9%			C.V.=11%			C.V.=22%			C.V.=19%			

Table 3.8. Analysis of variance for secondary carbon allocation traits and biomass in field-grown bulk populations of black spruce at the juvenile stage.

Source of variation	Total leaf area			Total biomass			Leaf area ratio			Root-to-shoot ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	27	0.0372	2	0.006	0.4913	2	100	0.2129	2	0.007	0.9582
Population	12	85	0.0001	12	0.068	0.0001	12	131	0.0547	12	0.114	0.2711
Sampling error	58	30		60	0.017		58	90		60	0.075	
Error	22	7		22	0.008		22	60		22	0.086	
Mean=8			Mean=0.208			Mean=37			Mean=0.832			
C.V.=72%			C.V.=63%			C.V.=26%			C.V.=33%			

Table 3.9. Analysis of variance for leaf gas exchange characteristics in field-grown bulk populations of black spruce at the juvenile stage. These traits are: photosynthetic capacity (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), intercellular-to-ambient $[\text{CO}_2]$ (C_i/C_a , $\mu\text{mol } \mu\text{mol}^{-1}$).

Source of variation	d.f.	A		g_s		C_i/C_a	
		M.S.	p	M.S.	p	M.S.	p
Bloc	2	3.24	0.6560	0.0219	0.0020	0.0014	0.7150
Population	12	3.71	0.8960	0.0041	0.1759	0.0105	0.0329
Sampling error	44	3.19		0.0027		0.0024	
Error	21	7.53		0.0026		0.0042	
		Mean=4.58		Mean=0.17		Mean=0.83	
		C.V.=39%		C.V.=31%		C.V.=6%	

Table 3.11. Analysis of variance for leaf carbon isotope discrimination (Δ) and its index ($d\delta^{13}\text{C}$) in field-grown bulk populations of black spruce at the juvenile stage. Expressing leaf carbon isotope discrimination as the deviation from the population mean $\delta^{13}\text{C}$ makes the coefficient of variation to reach very high values since the average of deviations tends toward zero.

Source of variation	d.f.	$d\delta^{13}\text{C}$		Δ	
		MS	p	MS	p
Bloc	2	2.69	0.1109	2.67	0.1091
Population	12	2.37	0.0581	2.33	0.0580
Sampling error	60	0.67		0.66	
Error	22	1.10		1.09	
		Mean=0.01 C. V.=10000%		Mean=24.67 C. V.=3%	

Table 3.12. Principal components analysis among seed-source climatic variables and seedling traits in bulk populations of black spruce under a simulated northern climate.

Component	1	2	3	4
Eigenvalue	5.43	2.82	1.44	1.12
Percent of total variance	0.45	0.23	0.12	0.09

Variables	Loadings			
Annual soil temperature	<u>0.38</u>	0.01	<u>-0.18</u>	-0.06
Annual climatic moisture index	0.18	<u>-0.44</u>	<u>0.14</u>	<u>0.42</u>
Growing season vapor pressure deficit	-0.03	<u>0.45</u>	-0.03	<u>-0.50</u>
Growing season photoperiod	<u>-0.39</u>	0.16	-0.01	-0.10
Relative growth rate	0.29	-0.13	<u>0.40</u>	-0.41
Specific leaf area	0.18	0.41	<u>0.41</u>	0.04
Leaf weight ratio	<u>0.41</u>	0.10	-0.15	0.06
Root weight ratio	<u>-0.35</u>	-0.18	0.06	-0.24
Photosynthetic capacity	<u>-0.39</u>	-0.02	0.08	0.18
Stomatal conductance	-0.25	<u>0.34</u>	0.20	0.42
Intercellular-to-ambient CO ₂ ratio	0.20	<u>0.42</u>	0.23	0.32
Carbon isotope discrimination index	0.04	0.24	<u>-0.70</u>	0.13

Component interpretation	Length of growing season	Moisture	Temperature	Moisture
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Table 3.13. Principal components analysis among seed-source climatic variables and seedling traits in bulk populations of black spruce under a simulated southern climate.

Component	1	2	3	4
Eigenvalue	5.46	2.78	1.79	1.01
Percent of total variance	0.45	0.23	0.15	0.08
Variables	Loadings			
Annual soil temperature	<u>0.38</u>	-0.20	0.01	0.17
Annual climatic moisture index	<u>0.19</u>	<u>0.32</u>	<u>-0.50</u>	<u>-0.02</u>
Growing season vapor pressure deficit	-0.04	<u>-0.47</u>	<u>0.36</u>	<u>0.05</u>
Growing season photoperiod	<u>-0.39</u>	-0.17	0.07	-0.18
Relative growth rate	<u>0.36</u>	-0.18	0.13	-0.24
Specific leaf area	-0.15	0.07	0.23	<u>0.83</u>
Leaf weight ratio	<u>0.40</u>	0.04	0.25	<u>0.06</u>
Root weight ratio	<u>-0.39</u>	-0.07	-0.29	-0.09
Photosynthetic capacity	-0.09	<u>0.47</u>	0.38	0.07
Stomatal conductance	0.07	<u>0.55</u>	0.08	0.37
Intercellular-to-ambient CO ₂ ratio	0.20	-0.20	<u>-0.48</u>	0.42
Carbon isotope discrimination index	<u>0.39</u>	0.03	0.09	-0.16
Component interpretation	Length of growing season	Moisture	Moisture	Temperature

Table 3.14. Principal components analysis among seed-source climatic variables and seedling traits in bulk populations of black spruce under southern field conditions.

Component	1	2	3	4
Eigenvalue	3.82	3.17	2.24	1.06
Percent of total variance	0.32	0.26	0.19	0.09

Variables	Loadings			
Annual soil temperature	<u>-0.34</u>	<u>-0.33</u>	-0.23	-0.05
Annual climatic moisture index	<u>-0.35</u>	<u>-0.10</u>	<u>0.39</u>	<u>0.11</u>
Growing season vapor pressure deficit	0.20	-0.00	<u>-0.51</u>	<u>-0.36</u>
Growing season photoperiod	<u>0.45</u>	<u>0.22</u>	0.05	<u>-0.13</u>
Relative growth rate	<u>-0.46</u>	0.07	-0.19	0.18
Specific leaf area	0.20	<u>-0.33</u>	-0.26	0.18
Leaf weight ratio	<u>0.43</u>	0.00	-0.09	0.36
Root weight ratio	-0.04	0.34	0.31	<u>-0.58</u>
Photosynthetic capacity	-0.08	<u>0.50</u>	-0.13	0.18
Stomatal conductance	-0.01	<u>0.45</u>	0.09	0.42
Intercellular-to-ambient CO ₂ ratio	0.22	-0.23	<u>0.40</u>	0.26
Carbon isotope discrimination index	0.13	-0.30	<u>0.37</u>	-0.15

Component interpretation	Length of growing season	Temperature	Moisture	Moisture
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Figure 3.1. Relative growth rate and primary carbon allocation traits in bulk populations of black spruce at the juvenile stage under controlled environments. Values are means $\pm$ S.E.M., n=3. Bars are: open, simulated northern climate; filled, simulated southern climate. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.2. Biomass and secondary carbon allocation traits in bulk populations of black spruce at the juvenile stage under controlled environments. Values are means $\pm$ S.E.M., n=3. Bars are: open, simulated northern climate; filled, simulated southern climate. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.3. Leaf gas exchange characteristics in bulk populations of black spruce at the juvenile stage under controlled environments. Values are means $\pm$ S.E.M., n=3. Bars are: open, simulated northern climate; filled, simulated southern climate. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.4. Sensitivity of stomatal conductance to photosynthetic capacity (a) and relationships between leaf %N and photosynthetic capacity (b) for black spruce seedlings grown under different environments. Values are means $\pm$ S.E.M., n=3. In (a), symbols are: open circle, simulated northern climate; closed circle, simulated southern climate, and closed triangle, southern field plot. In (b), symbols are: open circle (one season) or triangle (two seasons) for simulated northern climate, closed circle (one season) or triangle (two seasons) for simulated southern climate, and hatched circle, southern field plot. Data in (a) as well as those under field conditions in (b) refer to bulk populations. Data under controlled environments in (b) are from full-sib families of black spruce.

Figure 3.5. Source of variation in carbon isotope discrimination (a, b), its variation among (c) and relationship with leaf photosynthetic gas exchange in (d) bulk populations of black spruce grown under controlled environments.

Leaf carbon isotope discrimination was expressed as the deviation from the Population (× Environment × Replicate) mean $\delta^{13}\text{C}$. Values are means $\pm$ S.E.M., n=3. Bars are: open, simulated northern climate; filled, simulated southern climate. In (c, d), symbols are: open circle, simulated northern climate; closed circle, simulated southern climate. Population number is an increasing function of latitudinal source ($^{\circ}\text{N}$).

Figure 3.6. Leaf carbon isotope discrimination in relation to growth and primary carbon allocation traits in bulk populations of black spruce grown under the simulated southern environment. Leaf carbon isotope discrimination was expressed as the deviation from the Population (x Environment x Replicate) mean $\delta^{13}\text{C}$. Values are means, n=3.

Figure 3.7. Leaf carbon isotope discrimination in relation to growth and primary carbon allocation traits in bulk populations of black spruce grown under the simulated northern environment. Leaf carbon isotope discrimination was expressed as the deviation from the Population (x Environment x Replicate) mean $\delta^{13}\text{C}$. Values are means, n=3.

Figure 3.8. Relative growth rate and primary carbon allocation traits in field-grown bulk populations of black spruce at the juvenile stage. Values are means $\pm$ S.E.M., n=3. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.9. Biomass and secondary carbon allocation traits in field-grown bulk populations of black spruce at the juvenile stage. Values are means $\pm$ S.E.M., n=3. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.10. Leaf gas exchange characteristics in field-grown bulk populations of black spruce at the juvenile stage. Values are means $\pm$ S.E.M., n=3. Population number is an increasing function of latitudinal source ($^{\circ}$ N).

Figure 3.11. Source of variation in carbon isotope discrimination (a, b), its variation among (c) and relationship with leaf photosynthetic gas exchange in (d) field-grown bulk populations of black spruce. Leaf carbon isotope discrimination was expressed as the deviation from the Population (x Block) mean $\delta^{13}\text{C}$. Values are means $\pm$ S.E.M., n=3. Population number is an increasing function of latitudinal source ($^{\circ}\text{N}$).

Figure 3.12. Leaf carbon isotope discrimination in relation to growth and primary carbon allocation traits in field-grown bulk populations of black spruce. Leaf carbon isotope discrimination was expressed as the deviation from the Population (x Block) mean $\delta^{13}\text{C}$. Values are means, n=3.

Figure 3.13. Across-environment responses to seed-source mean annual temperature (at 10 cm depth) for growth rate and primary carbon allocation traits in bulk populations of black spruce. Values are means, n=3. Symbols are: open circle, simulated northern climate; closed circle, simulated southern climate, and closed triangle, southern field plot.

Figure 3.14. Responses to seed-source annual climatic moisture index for leaf physiological characteristics in bulk populations of black spruce grown under controlled environments. Leaf carbon isotope discrimination was expressed as the deviation from the Population ($\times$ Environment $\times$ Replicate) mean $\delta^{13}\text{C}$. The moisture index was determined as the balance between precipitation and potential evapotranspiration. Values are means, $n=3$. Symbols are: open circle, simulated northern climate; closed circle, simulated southern climate.

Figure 3.15. Responses to seed-source annual climatic moisture index for leaf physiological characteristics in field-grown bulk populations of black spruce. Leaf carbon isotope discrimination was expressed as the deviation from the Population (x Block) mean $\delta^{13}\text{C}$. The moisture index was determined as the balance between precipitation and potential evapotranspiration. Values are means, n=3.

CHAPTER FOUR

DIFFERENTIATION IN GROWTH, CARBON ALLOCATION AND CARBON ISOTOPE DISCRIMINATION AMONG FULL-SIB FAMILIES OF BLACK SPRUCE

4.1 RESULTS

4.1.1 Growth and carbon allocation traits

During the first growing season, relative growth rate (RGR) varied significantly ($P=0.0006$) and specific leaf area (SLA) varied nearly significantly ($P=0.0523$) among full-sib families of black spruce (Table 4.1; Figures 4.1a,b). Families of northern origin grew faster (significant contrast Southern seed-source versus Northern seed-source: $M.S.=0.00010$, $F=18.81$, $P=0.0002$) and exhibited slightly lower SLA (significant contrast Southern seed-source versus Northern seed-source: $M.S.=663$, $F=5.981$, $P=0.0210$). Although not statistically significant, both growth rate and specific leaf area tended to be higher under the warmer southern environment regardless of family. Over time, however, not only was RGR reduced but its pattern was also reversed both among family provenances and between environments (Figure 4.1a). For the two-season old seedlings, growth rate tended to be lower (although not statistically significant) in the warmer southern environment as well as in northern families. Specific leaf area, on the other hand, increased with seedling age, but differences were maintained both among family provenances and between environments (Figure 4.1b).

At the average seedling size, carbon allocation to leaves (LWR) and carbon allocation to roots (RWR) were determined by a significant main effects of Family (Table 4.1). Although not statistically significant, there was a trend for lower LWR but a higher RWR in plants grown under the warmer southern

environment, regardless of age class and family (Figures 4.1c,d). Irrespective of environment and family, LWR decreased but RWR increased significantly over time (Figures 4.1c,d). Full-sib families of northern origin also consistently allocated less carbon to leaves (significant contrast Southern seed-source versus Northern seed-source: $M.S.=0.046$, $F=10.95$, $P=0.0026$) but more to roots (significant contrast Southern seed-source versus Northern seed-source: $M.S.=0.028$, $F=7.11$, $P=0.0126$) irrespective of age classes and environment (Figures 4.1c,d).

These patterns in growth and carbon allocation resulted in no differentiation in total plant leaf area and total plant biomass, either among families or between environments (Table 4.2, Figures 4.2a,b). The variation patterns of these two traits differed in other aspects. Increased specific leaf area under the warmer southern environment compensated for the lower LWR, resulting in slightly higher total plant leaf area than seen under the cooler northern climate (Figures 4.1b, 4.2a). While this trend held particularly well for first-season seedlings in all families, it seemed to alter in few families over time (Figure 4.2a). For first-season seedlings, the variation in total plant biomass was consistent with patterns of relative growth rate, which tended to be higher in northern families and under the warmer climate (Figures 4.1a, 4.2b). Over time, however, there seemed to be an across-environment uncoupling between the growth rate and the total plant biomass achieved by seedlings (Figures 4.1a, 4.2b).

Despite these contrasting patterns of total plant leaf area and total plant biomass, leaf area and root-to-shoot ratios remained consistent with variations in the proportional allocation of carbon to leaves (LWR) and to roots (RWR), respectively (Table 4.2; Figures 4.1c,d; 4.2c,d). In first-season seedlings, families that exhibited a higher RGR, SLA, LWR or RWR in one climate also tended to do so in the other (Table 4.3). However, these between-environment correlations for individual traits seemed to alter in two-season old seedlings (Table 4.3).

4.1.2 Leaf gas exchange characteristics

Full-sib families of black spruce expressed no significant differences, among themselves or across environments, in leaf photosynthetic capacity (A) and in stomatal conductance (g_s) (Table 4.4, Figure 4.3a,b). However, the combined variations in A and g_s resulted in nearly significant environmental effects ($P=0.0524$) on leaf intercellular-to-ambient [CO_2] ratio (C_i / C_a) (Table 4.4, Figure 4.3c). In first-season seedlings, A tended to correlate positively with both g_s [$r=0.74$, $P=0.0371$ under northern climate; $r=0.56$, $P=0.1504$ under southern climate] and leaf nitrogen concentration (%N) [$r=0.21$, $P>0.05$ under northern climate; $r=0.58$, $P>0.05$ under southern climate] (Figure 4.4). However, these correlations were significantly altered among older seedlings (Figure 4.4). Regardless of environment and family, A decreased in time but tended to remain consistently higher under the warmer southern environment. No definite latitudinal patterns could be seen neither in A (Figure 4.3a) nor in

stomatal conductance (Figure 4.3b). However between-environment differences in g_s seemed to appear with time, where greater conductance rates would be observed under the warmer southern environment (Figure 4.3b). Lower values of C_i/C_a -higher instantaneous water-use efficiency- were observed in the warmer southern climate for all age classes and all families (Figure 4.3c). For a given combination of environment and age class, northern families tended to have higher (but not statistically significant) values for C_i/C_a (Figure 4.3c). In order to determine the relative control of A and g_s on the variation of water-use efficiency, rates adjusted for one another were analyzed. There was a nearly significant environmental effect ($P=0.0585$) on A for a fixed g_s (Table 4.5, Figures 4.5a, 4.6a). Further, the variance analysis of the g_s -weighted C_i/C_a ($C_i/C_{a_{gs}}$) revealed a significant Family $\times$ Environment interaction (Table 4.5). Conversely, families expressed no differentiation in g_s for a fixed A (Table 4.5, Figures 4.5b, 4.6b).

4.1.3 Leaf carbon isotope discrimination

Leaf carbon isotope discrimination, expressed as $\delta\delta^{13}\text{C}$, varied significantly among full-sib families of black spruce but not between environments (Table 4.6, Figures 4.5c, 4.6c). For both age classes, families of northern origin tended to have lower leaf $\delta\delta^{13}\text{C}$ values under the warmer southern environment but higher values under the cooler northern climate (Figures 4.5c, 4.6c). Over time, there also was a decreasing trend for leaf $\delta\delta^{13}\text{C}$ in the more northerly

families versus an increasing one for the southern families. Except for second-season seedlings under southern climate ($r=-0.57$, $P>0.05$), leaf C_i/C_a tended to correlate positively with leaf $\delta^{13}C$ ($r=0.66$, $P>0.05$ for first-season seedlings under northern climate; $r=0.52$, $P>0.05$ for first-season seedlings under southern climate; $r=0.09$, $P>0.05$ second-season seedlings under northern climate) (Figures 4.5d, 4.6d). Leaf gas exchange characteristics and $\delta^{13}C$ appeared more consistent in time within climates than between environments at a given time (Table 4.3).

4.1.4 Water-use efficiency and growth

Under the northern climate, the relative growth rate (RGR) of first-season seedlings tended to correlate positively with leaf carbon isotope discrimination ($\delta^{13}C$) ($r=0.46$, $P>0.05$), which indicated a negative relationship with long-term water-use efficiency (Figure 4.7a). However, this relationship was reversed over time ($r=-0.52$, $P>0.05$) (Figure 4.7a). Similarly, while low- $\delta^{13}C$ families tended to express higher specific leaf area (SLA) during the first growing season ($r=-0.13$, $P>0.05$), they tended to exhibit lower SLA over time ($r=0.35$, $P>0.05$) (Figure 4.7b). Low- $\delta^{13}C$ families tended to allocate less carbon to leaves (LWR) during the first growing season ($r=0.38$, $P>0.05$) but more carbon to leaves subsequently ($r=-0.09$, $P>0.05$) (Figure 4.7c). On the other hand, low- $\delta^{13}C$ families consistently tended to allocate more carbon to roots (RWR) ($r=-0.31$, $P>0.05$ for first-season seedlings; $r=-0.04$,

$P > 0.05$ for second-season seedlings). (Figure 4.7d).

Under the southern environment, the RGR of first-season seedlings tended to correlate negatively with leaf $\delta^{13}\text{C}$ ($r = -0.51$, $P > 0.05$), which indicated a positive relationship with long-term water-use efficiency. However, this relationship was reversed over time ($r = 0.36$, $P > 0.05$) (Figure 4.7a). Low- $\delta^{13}\text{C}$ families consistently tended to express lower SLA ($r = 0.12$, $P > 0.05$ for first-season seedlings; $r = 0.53$, $P > 0.05$ for second-season seedlings), lower LWR ($r = 0.26$, $P > 0.05$ for first-season seedlings; $r = 0.74$, $P > 0.0371$ for second-season seedlings) but higher RWR ($r = -0.07$, $P > 0.05$ for first-season seedlings; $r = -0.50$, $P > 0.05$ for second-season seedlings) (Figures 4.7b,c,d).

4.1.5 Correlations between seedling characteristics and seed-source climatic variables

Using the first-season data, four components or axes, explained approximately 96 to 97% of the total variance among selected variables (Tables 4.7,4.8). The interpretation of these axes proved very difficult and varied between environments. The main components tended to load equally on both temperature and moisture, more likely, because the gradient of water availability among the provenances is reversed (northward increase of moisture availability). Under both climates, the first axis, which accounted for between 48 and 60% of total variation and was equally correlated with all climatic variables, was interpreted as "*Length of growing season*". The second axis, which explained between 18 and 21% of total variation, was undefined

under the northern environment but was termed "*Moisture*" under southern environment. The third and fourth axes, with 6 to 16% of total variation, reflected "*Moisture*" under the cooler northern climate but remained undefined under the warmer southern climate. Plant traits exhibited their highest loadings on either moisture or undefined components. However, in consideration of the results obtained with bulk populations of black spruce, leaf physiological characteristics were plotted against moisture while growth and carbon allocation traits were related to soil temperature at seed source.

For first-season seedlings, full-sib families of black spruce from cooler habitats had the fastest growth rates in both environments ($r=-0.74$, $P=0.0356$ and $r=-0.60$, $P=0.1149$ under northern and southern climates, respectively), but this trend was reversed over time ($r=0.31$, $P=0.4609$ and $r=0.82$, $P=0.0120$ under northern and southern climates, respectively) (Figure 4.8a). Low-temperature families tended to express low specific leaf area (SLA) [except for first-season seedlings under southern climate ($r=0.09$, $P>0.05$)] [$r=0.77$, $P>0.05$ for first-season seedlings under northern climate; $r=0.52$, $P>0.05$ and $r=0.56$, $P>0.05$ for second-season seedlings under northern and southern climates, respectively] and to allocate less carbon to leaves (LWR) [$r=0.65$, $P=0.0829$ and $r=0.51$, $P=0.1961$ for first-season seedlings under northern and southern climates, respectively; $r=0.87$, $P=0.0053$ and $r=0.62$, $P=0.1016$ for second-season seedlings under northern and southern climates, respectively] but to allocate more

carbon to roots (RWR) [$r=-0.63$, $P=0.0908$ and $r=-0.49$, $P=0.2182$ for first-season seedlings under northern and southern climates, respectively; $r=-0.79$, $P=0.0199$ and $r=-0.39$, $P=0.3395$ for second-season seedlings under northern and southern climates, respectively] (Figures 4.8b,c,d). Under the northern climate, low-temperature families also exhibited a slightly higher shift in LWR or RWR over time (Figures 4.8c,d).

Patterns of leaf physiological characteristics, although not statistically significant, changed over time and between environments. Over time, full-sib families of black spruce tended to express lower photosynthetic capacity (A) and stomatal conductance (gs), which resulted in higher ratios of leaf intercellular-to-ambient [CO_2] (C_i/C_a). Under the cooler northern climate, low-moisture families tended to express slightly higher A ($r=-0.58$, $P>0.05$ and $r=-0.38$, $P>0.05$ for first- and second-season seedlings, respectively) slightly higher gs [except for lower gs for two-season seedlings ($r=0.60$, $P>0.05$)] ($r=-0.13$, $P>0.05$), lower C_i/C_a ($r=0.60$, $P>0.05$ and $r=0.50$, $P>0.05$ for first- and second-season seedlings, respectively) and, therefore, slightly lower leaf carbon isotope discrimination ($\delta^{13}\text{C}$) ($r=0.27$, $P>0.05$ and $r=-0.03$, $P>0.05$ for first- and second-season seedlings, respectively) (Figure 4.9). In contrast, under the warmer southern climate, low moisture at seed source tended to associate consistently with lower A ($r=0.50$, $P>0.05$ and $r=0.23$, $P>0.05$ for first- and second-season seedlings, respectively), lower gs ($r=0.60$, $P>0.05$ and $r=0.68$,

$P > 0.05$ for first- and second-season seedlings, respectively), lower C_i/C_a ($r = 0.04$, $P > 0.05$ and $r = 0.23$, $P > 0.05$ for first- and second-season seedlings, respectively) but higher leaf $\delta^{13}\text{C}$ ($r = -0.54$, $P > 0.05$ and $r = -0.44$, $P > 0.05$ for first- and second-season seedlings, respectively) (Figure 4.9).

4.2 DISCUSSION

4.2.1 Growth and carbon allocation traits

Differentiation in mean relative growth (RGR) among full-sib families of black spruce reflects a strong interaction between patterns of carbon allocation and net assimilation rate (NAR). For instance, first-season seedlings from families of northern origin grew faster than families of southern origin (Figure 4.1). The northern families tended to grow even faster (not statistically significant, $P = 0.1448$) under the warmer southern environment despite the associated lower allocation of carbon to leaves (LWR, $P = 0.0530$) and higher allocation of carbon to roots (RWR, $P = 0.0995$) (Figure 4.1). While NAR appears prominent in differentiating first-season seedlings, the reversal of growth patterns over time was largely attributable to changes in carbon allocation patterns. In fact, although differences in LWR and RWR were similarly expressed between climates -as indicated by the lack of a significant Family $\times$ Environment interaction- the sensitivity of those traits to seedling size changed over time.

For first-season seedlings, one unit deviation from the average total biomass (on a logarithmic basis) was associated with 1.04 ± 0.03 and 0.83 ± 0.07 units change in leaf and root biomass, respectively. In contrast, during the second growing season, these figures were to 0.81 ± 0.10 and 1.20 ± 0.10 . At some point in time, full-sib families of black spruce shifted from a preferential allocation of carbon to leaves to a greater investment in roots. Consequently, both root

weight and root-to-shoot ratios increased with seedling age at the expense of leaf weight and leaf area (LAR) ratios. Moreover, these developmental differences in carbon allocation varied not only between environments but also among family provenances.

While LWR was consistently diminished over time by -24% in either environment, RWR was increased by 28% and 52% under southern and northern climates, respectively. Similarly, the more northerly families grown under the northern climate exhibited a greater reduction in LWR (-28% versus -27%) or increase in RWR (59% versus 51%) than the southern families. The more pronounced shift in carbon allocation traits under the northern climate was associated with a shorter period for shoot growth. In fact, root growth may proceed several weeks after bud set, benefiting from late-season carbon gain. The potential advantages of the slightly higher LAR ($P=0.0708$) under the northern climate may then vanish with time. In addition, the higher maintenance costs may offset the higher NAR of the northern families.

The patterns of growth and carbon allocation, especially those of second-season seedlings, were in agreement with earlier observations on bulk populations of black spruce grown under the same conditions (Chapter 3). Regardless of environment and age class, the farther north the family origin, the lower the LWR but the higher the RWR. Greater allocation of carbon to roots is an intrinsic component of a stress resistance syndrome (Chapin *et al.*

1993) and may be explained a number of factors. In fact, northern populations of black spruce experience low water and nutrient availability, low temperature and a short period for shoot growth. A greater investment of carbon into roots may be of selective advantage for all of these environmental conditions. Noteworthy, however, the northern family provenances examined here were likely experiencing higher water availability than southern provenances. Seed-source moisture was characterized as the balance between annual precipitation and potential evapotranspiration. However, the influence of the Hudson Bay on the climate of Northern Ontario contributes to lowering the potential evapotranspiration at the northern sampling site, driving its moisture balance toward higher values than determined for the southern sites. As low temperature and high moisture tend to have opposite effects on carbon allocation patterns, the principal components analysis could not dissociate them (Tables 4.7, 4.8). Yet the agreement of the present observations, with those for bulk populations, supports the idea that mean relative growth rate and carbon allocation traits responded to seed-source temperature. Accordingly, full-sib families of black spruce from warmer habitats tend to grow faster and to express higher specific leaf area. These same families also allocate more carbon to leaves but less to roots.

4.2.2 Leaf physiological characteristics

In order to compare full-sib families based on their intrinsic differences, leaf gas exchange characteristics, measured under constant photosynthetic photon flux density (PPFD), were also adjusted for leaf-to-air vapour pressure deficit and ambient $[\text{CO}_2]$. Leaf photosynthetic capacity (A) varied in tight correlation with both stomatal conductance (g_s) and leaf nitrogen concentration (%N). In addition, A tended to be higher under the warmer climate (Figure 4.3), mainly responding to changes in leaf %N (Figure 4.4). During the first growing season, for instance, while g_s remained within the same range of variation in either environment, higher leaf %N in the warmer climate drove A toward higher values. That the more northerly families exhibit higher leaf %N is in agreement with findings by Reich *et al.* (1996), Johnsen and Seiler (1996) and Johnsen *et al.* (1996). Higher leaf %N is likely to correlate positively with N investment into key photosynthetic enzymes (Cowan 1986, Field and Mooney 1986) and is also thought to enhance the metabolic activity and growth under cooler growing conditions (Reich *et al.* 1996). Higher leaf %N under the warmer environment (in northern families as well), either through increased uptake and allocation of N to leaf or as a byproduct of reduced allocation of carbon to leaves, may also account for the variation in A .

However, as development proceeded, nitrogen dilution, brought about by increasing biomass (Wikström 1994), not only shifted A to lower values but also tended to uncouple A with g_s (Figure 4.4). In fact, lower sensitivity of g_s to A was observed in second-season seedlings. Moreover, families exhibited different ranges of variation for g_s , which also tended to become higher under the warmer southern climate. This latter trend, as well as the slightly higher sensitivity of g_s to A in first-season seedlings under the warmer southern climate, contrasts with patterns exhibited by the bulk populations of black spruce.

The patterns of A and g_s resulted in slightly higher (but not statistically significant, contrast Southern seed-source versus Northern seed-source: $M.S.=0.0033$, $F=1.96$, $P=0.1721$) leaf intercellular-to-ambient [CO_2] ratio (C_i/C_a) - lower instantaneous water-use efficiency (Meinzer *et al.* 1990)- for northern families (moister seed source) in either environment and for both age classes (Figure 4.9c). In addition, C_i/C_a increased with time and remained consistently and almost significantly higher under the cooler northern environment ($P=0.0524$). The relative influence of changes in A and g_s on C_i/C_a was assessed by analyzing rates adjusted for one another. It follows that these full-sib families of black spruce differed more in A (for a fixed g_s) than they did in g_s (for a fixed A) (Figures 4.5a,b; 4.6a,b). Accordingly, variation in leaf carbon isotope discrimination (Δ) arises primarily via changes in A .

Leaf Δ ($\delta^{13}\text{C}$) is a time-integrated measure of internal plant physiological and environmental properties influencing photosynthetic gas exchange (Meinzer *et al.* 1990, Ehleringer 1991). Leaf Δ reliably predicts long-term water-use efficiency (WUE) and for C_3 plants under constant vapour pressure deficit, Δ should be linearly and positively related to C_i/C_a (Farquhar *et al.* 1989). In fact, except for second-season seedlings under southern climate, positive but weak correlations were found between leaf C_i/C_a and leaf $\delta^{13}\text{C}$ (Figure 4.5d). Despite a doubling of its supply, leaf N content tended to limit growth under southern climate, and it also tended to uncouple short- and long-term WUE in black spruce.

That changed photosynthetic capacity (A) was the primary source of variation in leaf $\delta^{13}\text{C}$, contrasts with the conductance-driven variation in $\delta^{13}\text{C}$ in coffee genotypes (Meinzer *et al.* 1990) as well as in bulk populations of black spruce (Chapter 3). Under similar water availability, changes in A also accounted for genotypic variation in Δ for vegetatively growing sunflower genotypes (Virgona *et al.* 1990, Virgona and Farquhar 1996), for Eucalypt taxa (Anderson *et al.* 1996) and for white spruce genotypes (Sun *et al.* 1996). The A-driven variation in Δ finds further support in the relationships between mean relative growth rate (RGR) and leaf $\delta^{13}\text{C}$ (Figures 4.5, 4.6). All things being equal, there should be a negative correlation between Δ and plant growth when variation in Δ results from changes in A (Farquhar *et al.* 1989).

However, because optimum water conservation requires matching compromises in leaf photosynthetic gas exchange and whole-plant carbon allocation (Cowan 1986), differences in allocation patterns may offset the above theoretical relationship when water is not limiting. For instance, where variation in Δ is primarily driven by A , low- Δ genotypes may grow slower if a higher proportion of carbon is allocated to roots relative to shoots. All of these possibilities are exhibited by full-sib families of black spruce.

Under the southern environment, first-season seedlings with low- $\delta^{13}\text{C}$ values tended to grow faster ($r=-0.51$, $P>0.05$) despite less carbon allocated to leaves (LWR, $r=0.26$, $P>0.05$) and more carbon allocated to roots (RWR, $r=-0.07$, $P>0.05$) (Figure 4.7). This group of traits allows the plants to both conserve water and grow faster. Such a pattern, in accord with expectations (Farquhar *et al.* 1989), follows only a greater proportional change in A than in RWR. In contrast, water conservation during the second growing season came only at the expense of reduced growth. In fact, second-season seedlings with low- $\delta^{13}\text{C}$ values tended to grow slower ($r=0.36$, $P>0.05$) as higher RWR ($r=-0.50$, $P>0.05$) offset the theoretical negative relationship between leaf $\delta^{13}\text{C}$ and plant growth (Figure 4.7).

Under the northern climate, first-season seedlings with low- $\delta^{13}\text{C}$ values conserved water only at the expense of reduced growth through higher RWR ($r=-0.31$, $P>0.05$) and lower LWR ($r=0.38$, $P>0.05$) (Figure 4.7). In contrast, lack of water conservation in second-season seedlings, by less investment of carbon into root tissues (versus $\delta^{13}\text{C}$: $r=-0.04$, $P>0.05$), restored the expected negative relationship between leaf $\delta^{13}\text{C}$ and plant growth (Figure 4.7).

Principal components analysis could not clearly indicate what seed-source ecological factor leaf physiological characteristics were responding to the most. However, the between-environment contrasting responses of leaf physiological traits to seed-source moisture were in agreement with results with bulk populations (Figure 4.9). The trends, particularly those of A , g_s and leaf $\delta^{13}\text{C}$, indicated an interaction between seed-source moisture and cultivation temperature on the expression of leaf physiological traits. Under the northern environment, low-moisture families, which were of southern origin in this study, expressed higher A ($r=-0.58$, $P>0.05$ and $r=-0.38$, $P>0.05$ for first- and second-season seedlings respectively) than high-moisture families and, therefore, exhibited a lower leaf $\delta^{13}\text{C}$ ($r=0.27$, $P>0.05$ for first-season seedlings) (Figure 4.9). In contrast, low-moisture families had a lower A ($r=-0.50$, $P>0.05$ and $r=0.23$, $P>0.05$ for first- and second-season seedlings respectively) than was measured for high-moisture families under the southern environment. This latter pattern of variation in A resulted in a

higher leaf $\delta^{13}\text{C}$ for low-moisture families ($r=-0.54$, $P>0.05$ and $r=-0.44$, $P>0.05$ for first- and second-season seedlings respectively) under the southern environment in comparison with high-moisture families under the same cultivation conditions (Figure 4.9).

4.2.3 Addressing the hypotheses

The first hypothesis tested was that full-sib families that allocated a higher proportion of carbon to roots (RWR), relative to shoot (LWR), would also have a lower stomatal conductance (g_s) in relation to photosynthetic capacity (A). This hypothesis was shown to be true in either environment for first-season seedlings, as well as under the southern climate for second-season seedlings. Under those conditions, full-sib families of black spruce that conserved water by having low- $\delta^{13}\text{C}$ values (higher A/ g_s) also allocated more carbon to roots relative to shoots (negative correlations between RWR and $\delta^{13}\text{C}$ although $P>0.05$, Figure 4.7). However, the hypothesis did not hold for second-season seedlings under the cooler northern climate.

The second hypothesis tested was that more northerly full-sib families exhibited a more conservative suite of characteristics (eg. higher RWR, lower leaf $\delta^{13}\text{C}$). The veracity of this hypothesis was environment-dependent.

While the hypothesis held for either age class in the warmer, southern climate, it was false under the northern climate for either age class. When grown under the warmer southern environment, full-sib families of

northern origin (cooler and moister) were more water-conservative, and tended to display higher RWR (but $P > 0.05$) (Figure 4.8d) and lower leaf $\delta^{13}\text{C}$ (but $P > 0.05$) (Figure 4.9d).

The third hypothesis tested was that the ranking of full-sib families remained consistent across environments and in time for a given trait. In no circumstance did this hypothesis find support. However, tendencies were shown for growth and carbon allocation traits to be consistent between environments at a given time, and for leaf physiological characteristics to be consistent in time within environments (Table 4.3).

4.3 CONCLUSIONS

- Fast-growing full-sib families of black spruce, from within Ontario, were differentiated in growth, carbon allocation traits and leaf physiological characteristics. In contrast with bulk populations of the same species, these genotypes exhibited a highly efficient feature for water conservation at the leaf level: increasing photosynthetic capacity (A) rather than decreasing stomatal conductance (gs). In addition, these genotypes allocated much lower proportions of carbon to roots relative to shoots in comparison with the bulk populations within the same latitudinal range (RWR=0.233±0.006 versus 0.249±0.009 under southern climate, RWR=0.324±0.010 versus 0.368±0.014 under northern climate). Because the compromise in carbon allocation and that in leaf physiology have likely evolved in response to different ecological factors, optimum water conservation was not only environment-dependent but it also resulted in different costs for growth over time.
- Congruence of warmer temperatures and higher water availability appears to be a *sine qua non* condition for the veracity of the first two hypotheses. Under such climates, families allocating a higher proportion of carbon to roots relative to shoots are likely to express a higher A in relation to gs. These families have, therefore, a lower carbon isotope discrimination ($\delta^{13}\text{C}$) and are generally of northern origin.

- Differences in carbon allocation patterns may offset the theoretical relationships between leaf $\delta^{13}\text{C}$ and plant growth. A high net assimilation rate of first-season seedlings, under warmer, well-watered environments, may buffer the slightly higher allocation of carbon to roots by low- $\delta^{13}\text{C}$ genotypes and help maintain biomass yield. However, because such a pattern was less likely to hold in the long term, water conservation arose only at cost of reduced growth.
- While growth and carbon allocation patterns were inconsistent in time, patterns of variation in leaf physiology remained inconsistent between climates, precluding the veracity of the third hypothesis.

Table 4.1. Analysis of variance for growth rate and primary carbon allocation traits in first-season full-sib families of black spruce grown under controlled environments.

Source of variation	Relative growth rate			Specific leaf area			Leaf weight ratio			Root weight ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	0.00049	0.1082	2	82213	0.0305	2	0.046	0.3939	2	0.104	0.2352
Environment (E)	1	0.00033	0.1448	1	3483	0.3655	1	0.517	0.0530	1	0.274	0.0995
Main plot Error	2	0.00006		2	2584		2	0.030		2	0.032	
Family (F)	7	0.00003	0.0006	7	258	0.0523	7	0.019	0.0020	7	0.011	0.0214
E x F	7	<10 ⁻⁵	0.9504	7	124	0.3812	7	0.002	0.8851	7	0.002	0.8644
Sampling error	92	<10 ⁻⁵		92	129		92	0.002		92	0.002	
Subplot error	28	0.00001		28	111		28	0.004		28	0.004	
Mean=0.040			Mean=103			Mean=0.593			Mean=0.279			
C.V.=5%			C.V.=11%			C.V.=8%			C.V.=18%			

Table 4.2. Analysis of variance for secondary carbon allocation traits and biomass in first-season full-sib families of black spruce grown under controlled environments.

Source of variation	Total leaf area			Total biomass			Leaf area ratio			Root-to-shoot ratio		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	12907	0.2342	2	1.679	0.1580	2	27661	0.0055	2	0.449	0.2968
Environment (E)	1	4516	0.3968	1	1.377	0.1717	1	1938	0.0708	1	1.243	0.1247
Main plot Error	2	3947		2	0.315		2	153		2	0.190	
Family (F)	7	107	0.6517	7	0.009	0.8866	7	412	0.0033	7	0.043	0.0219
E x F	7	79	0.8031	7	0.012	0.7497	7	74	0.6406	7	0.008	0.8106
Sampling error	92	146		92	0.021		92	77		92	0.011	
Subplot error	28	148		28	0.021		28	100		28	0.015	
Mean=30			Mean=0.478			Mean=61			Mean=0.407			
C.V.=41%			C.V.=30%			C.V.=14%			C.V.=26%			

Table 4.3. Environmental and temporal correlation coefficients (significance level shown in parentheses) for different traits determined for full-sib families of black spruce. Environments are simulated (1 or 2 seasons)

northern and southern climates; traits are: relative growth rate (RGR, d⁻¹), specific leaf area (SLA, cm² g⁻¹), leaf (LWR, g g⁻¹) and root (RWR, g g⁻¹) weight ratios, photosynthetic capacity (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), intercellular-to-ambient [CO₂] (C_i/C_a, $\mu\text{mol } \mu\text{mol}^{-1}$), and carbon isotope discrimination ($\delta\delta^{13}\text{C}$, ‰).

	RGR	SLA	LWR	RWR	A	gs	C _i /C _a	$\delta\delta^{13}\text{C}$
Northern.1 vs Southern.1	0.88 (0.0037)	0.42 (0.3060)	0.74 (0.0358)	0.57 (0.1425)	-0.55 (0.1550)	-0.06 (0.8866)	-0.11 (0.7886)	0.33 (0.4303)
Northern.1 vs Northern.2	-0.31 (0.4622)	0.79 (0.0208)	0.35 (0.3936)	0.22 (0.6033)	0.37 (0.3633)	0.02 (0.9710)	0.65 (0.0786)	0.72 (0.0460)
Southern.1 vs Southern.2	-0.19 (0.6473)	0.28 (0.5077)	0.65 (0.0790)	0.63 (0.0933)	0.26 (0.5281)	0.51 (0.1956)	0.35 (0.3954)	0.43 (0.2924)
Northern.2 vs Southern.2	0.34 (0.4140)	0.36 (0.3857)	0.66 (0.0774)	0.12 (0.7713)	0.36 (0.3872)	0.14 (0.7391)	0.28 (0.5050)	0.21 (0.6174)

Table 4.4. Analysis of variance for leaf gas exchange characteristics in first-season full-sib families of black spruce under controlled environments. These traits are: leaf photosynthetic capacity (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), intercellular-to-ambient $[\text{CO}_2]$ (C_i/C_a , $\mu\text{mol } \mu\text{mol}^{-1}$).

		A		g_s		C_i/C_a	
Source of variation	d.f.	M.S.	p	M.S.	p	M.S.	p
Bloc	2	148.96	0.0601	0.4875	0.0275	0.0041	0.5596
Environment (E)	1	109.30	0.0772	0.0007	0.8385	0.0913	0.0524
Main plot Error	2	9.53		0.0138		0.0052	
Family (F)	7	1.71	0.8958	0.0033	0.7863	0.0022	0.2636
E x F	7	8.34	0.0999	0.0068	0.3746	0.0027	0.1772
Sampling error	86	1.79		0.0035		0.0008	
Subplot error	28	4.29		0.0060		0.0017	
		Mean=4.32		Mean=0.25		Mean=0.89	
		C.V.=31%		C.V.=24%		C.V.=3%	

Table 4.5. Analysis of variance for weighted gas exchange characteristics in first-season full-sib families of black spruce under controlled environments. These traits are: gs-weighted assimilation (A_{gs} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and C_i/C_a (C_i/C_{ags} , $\mu\text{mol } \mu\text{mol}^{-1}$), A-weighted stomatal conductance (gs_A , $\text{mol m}^{-2} \text{s}^{-1}$) and C_i/C_a (C_i/C_{aA} , $\mu\text{mol } \mu\text{mol}^{-1}$).

Source of variation	A_{gs}			C_1/C_{ags}			g^SA			C_1/CaA		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p
Bloc	2	43.24	0.1682	2	0.0367	0.0073	2	0.3343	0.0580	2	0.0240	0.0913
Environment (E)	1	136.52	0.0585	1	0.0532	0.0050	1	0.0248	0.3868	1	0.0179	0.1124
Main plot Error	2	8.75		2	0.0003		2	0.0206		2	0.0024	
Family (F)	7	1.93	0.7817	7	0.0010	0.7528	7	0.0044	0.5832	7	0.0015	0.0516
E x F	7	6.77	0.0962	7	0.0039	0.0488	7	0.0029	0.8083	7	0.0008	0.2852
Sampling error	86	1.54		86	0.0008		86	0.0031		86	0.0005	
Subplot error	28	3.44		28	0.0017		28	0.0054		28	0.0007	
Mean=4.16			Mean=0.89			Mean=0.24			Mean=0.89			
C.V.=30%			C.V.=3%			C.V.=23%			C.V.=3%			

Table 4.6. Analysis of variance for leaf carbon isotope discrimination index in first-season full-sib families of black spruce under controlled environments. Expressing the leaf carbon isotope discrimination as the deviation from family (x environment x replicate) mean $\delta^{13}\text{C}$ makes the coefficient of variation to reach very high values since the average of deviations tends toward zero.

Source of variation	d.f.	MS	p
Bloc	2	0.0134	0.6442
Environment (E)	1	0.0258	0.4117
Main plot Error	2	0.0243	
Family (F)	7	0.4839	0.0367
E x F	7	0.2075	0.3945
Sampling error	92	0.1223	
Subplot error	28	0.1899	
Mean=0.0004			
C. V.=10000%			

Table 4.7. Principal components analysis among seed-source climatic variables and first-season seedling traits in full-sib families of black spruce under a simulated northern climate.

Component	1	2	3	4
Eigenvalue	7.21	2.19	1.59	0.59
Percent of total variance	0.60	0.18	0.13	0.06

Variables	Loadings			
Annual soil temperature	<u>-0.36</u>	0.01	<u>-0.16</u>	0.09
Annual climatic moisture index	0.36	<u>-0.01</u>	0.15	<u>-0.26</u>
Growing season vapor pressure deficit	<u>-0.36</u>	0.01	<u>-0.16</u>	0.08
Growing season photoperiod	<u>0.36</u>	<u>-0.01</u>	0.16	<u>-0.16</u>
Relative growth rate	0.27	-0.26	0.19	<u>-0.50</u>
Specific leaf area	-0.31	0.19	-0.12	<u>-0.38</u>
Leaf weight ratio	-0.26	<u>0.47</u>	0.13	-0.10
Root weight ratio	0.27	<u>0.42</u>	-0.19	0.19
Photosynthetic capacity	-0.27	-0.25	<u>0.45</u>	-0.08
Stomatal conductance	<u>-0.09</u>	-0.12	<u>0.72</u>	0.36
Intercellular-to-ambient CO ₂ ratio	0.28	0.30	-0.23	<u>0.42</u>
Carbon isotope discrimination index	0.12	<u>0.58</u>	0.18	<u>0.37</u>

Component interpretation	Length of growing season	Undefined	Moisture	Moisture
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Table 4.8. Principal components analysis among seed-source climatic variables and first-season seedling traits in full-sib families of black spruce under a simulated southern climate.

Component	1	2	3	4
Eigenvalue	5.74	2.47	1.93	1.30
Percent of total variance	0.48	0.21	0.16	0.11

Variables	Loadings			
Annual soil temperature	-0.40	-0.12	-0.03	-0.01
Annual climatic moisture index	<u>0.41</u>	0.01	0.08	-0.03
Growing season vapor pressure deficit	-0.40	-0.13	-0.03	-0.02
Growing season photoperiod	<u>0.41</u>	0.08	<u>0.05</u>	-0.00
Relative growth rate	0.27	-0.00	<u>0.46</u>	-0.22
Specific leaf area	-0.10	<u>0.42</u>	0.37	-0.44
Leaf weight ratio	-0.22	-0.26	<u>0.51</u>	0.11
Root weight ratio	0.19	0.32	<u>-0.51</u>	0.02
Photosynthetic capacity	0.22	<u>-0.52</u>	-0.05	0.09
Stomatal conductance	0.26	-0.15	0.21	<u>0.55</u>
Intercellular-to-ambient CO ₂ ratio	0.01	<u>0.51</u>	0.26	0.37
Carbon isotope discrimination index	-0.23	0.26	0.02	<u>0.54</u>

Component interpretation	Length of growing season	Moisture	Undefined	Undefined
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Figure 4.1. Relative growth rate and primary carbon allocation traits in full-sib families of black spruce under different environments and at two stages within the juvenile period. Values are means $\pm$ S.E.M., n=3. Bars are: open (one season) or dotted (two seasons), simulated northern climate; filled (one season) or hatched (two seasons), simulated southern climate. Families 1, 2 and 3 are of a same southern origin; families 4, 5, 6 are of a same mid-latitudinal origin; and families 7 and 8 are of a same more northerly origin.

Figure 4.2. Biomass and secondary carbon allocation traits in full-sib families of black spruce under different environments and at two stages within the juvenile period. Values are means $\pm$ S.E.M., n=3. Bars are: open (one season) or dotted (two seasons), simulated northern climate; filled (one season) or hatched (two seasons), simulated southern climate. Families 1, 2 and 3 are of a same southern origin; families 4, 5, 6 are of a same mid-latitudinal origin; and families 7 and 8 are of a same more northerly origin.

Figure 4.3. Leaf gas exchange characteristics in full-sib families of black spruce under different environments and at two stages within the juvenile period.

Values are means $\pm$ S.E.M., n=3. Bars are: open (one season) or dotted (two seasons), simulated northern climate; filled (one season) or hatched (two seasons), simulated southern climate. Families 1, 2 and 3 are of a same southern origin; families 4, 5, 6 are of a same mid-latitudinal origin; and families 7 and 8 are of a same more northerly origin.

Figure 4.4. Sensitivity of stomatal conductance to photosynthetic capacity (a) and relationships between leaf %N and photosynthetic capacity (b) for full-sib families of black spruce under different environments and at different stages within the juvenile period. Values are means, $n=3$. Symbols are: open circle (one season) or triangle (two seasons), simulated northern climate; closed circle (one season) or triangle (two seasons), simulated southern climate.

Figure 4.5. Source of variation in carbon isotope discrimination (a, b), its variation among (c) and relationship with leaf photosynthetic gas exchange (d) in first-season full-sib families of black spruce under different environments. Leaf carbon isotope discrimination was expressed as the deviation from the Family (x Environment x Replicate) mean $\delta^{13}\text{C}$. Values are means $\pm$ S.E.M., n=3. Bars are: open, simulated northern climate; filled simulated southern climate. In (c, d), symbols are: open circle, simulated northern climate; closed circle, simulated southern climate. Families 1, 2 and 3 are of a same southern origin; families 4, 5, 6 are of a same mid-latitudinal origin; and families 7 and 8 are of a same more northerly origin.

Figure 4.6. Source of variation in carbon isotope discrimination (a, b), its variation among (c) and relationship with leaf photosynthetic gas exchange (d) in two-season old full-sib families of black spruce under different environments. Leaf carbon isotope discrimination was expressed as the deviation from the Family ($\times$ Environment $\times$ Replicate) mean $\delta^{13}\text{C}$. Values are means $\pm$ S.E.M., n=3. Bars are: dotted, simulated northern climate; hatched simulated southern climate. In (c, d), symbols are: open triangle, simulated northern climate; closed triangle, simulated southern climate. Families 1, 2 and 3 are of a same southern origin; families 4, 5, 6 are of a same mid-latitudinal origin; and families 7 and 8 are of a same more northerly origin.

Figure 4.7. Leaf carbon isotope discrimination in relation to growth and primary carbon allocation traits in full-sib families of black spruce under different environments and at different stages within the juvenile period.

Leaf carbon isotope discrimination was expressed as the deviation from the Family ($\times$ Environment $\times$ Replicate) mean $\delta^{13}\text{C}$. Values are means, $n=3$.

Symbols are: open circle (one season, thin dotted line) or triangle (two seasons, thick dotted line), simulated northern climate; closed circle (one season, thin solid line) or triangle (two seasons, thick solid line), simulated southern climate.

Figure 4.8. Environmental and temporal responses to seed-source mean annual temperature (at 10 cm depth) for growth rate and primary carbon allocation traits in full-sib families of black spruce. Values are means, $n=3$. Symbols are: open circle (one season, thin dotted line) or triangle (two seasons, thick dotted line), simulated northern climate; closed circle (one season, thin solid line) or triangle (two seasons, thick solid line), simulated southern climate.

Figure 4.9. Environmental and temporal responses to seed-source annual climatic moisture index for leaf physiological characteristics in full-sib families of black spruce. Leaf carbon isotope discrimination was expressed as the deviation from the Family ($\times$ Environment $\times$ Replicate) mean $\delta^{13}\text{C}$. The moisture index was determined as the balance between precipitation and potential evapotranspiration. Values are means, $n=3$. Symbols are: open circle (one season, thin dotted line) or triangle (two seasons, thick dotted line), simulated northern climate; closed circle (one season, thin solid line) or triangle (two seasons, thick solid line), simulated southern climate.

CHAPTER FIVE

SYNTHESIS AND CONCLUSIONS

Patterns of geographic differentiation

Populations of black spruce exhibit a range-wide differentiation in juvenile growth, carbon allocation and leaf physiological characteristics. These traits have likely evolved in tight correlation with natural gradients in photoperiod, temperature and water availability (Chapter three), which appear strong enough to differentiate populations of black spruce even at smaller scales (Chapter four). If indeed the juvenile traits of black spruce are responding to seed-source climatic constraints, the irregularity of temperature and water availability within the distribution range may leave room for ecotypic variation. Accordingly, ecotypes within clines is highly likely the pattern of geographic differentiation in black spruce as regards the mean relative growth rate, carbon allocation and leaf physiological characteristics.

Whole-plant carbon allocation and leaf physiology

Water conservation requires matching the leaf-level compromise between carbon acquisition and water loss with that at the whole-plant level between carbon allocation to roots for the exploration of soil water and carbon allocation to shoots for increased growth. In black spruce, because of the two compromises have likely evolved in response to different climatic factors, their co-occurrence is environment-dependent.

Growth and carbon allocation traits respond linearly to seed-source temperature (or other correlated factor such as photoperiod) according to a consistent pattern where low-temperature populations/families allocate more carbon to roots relative to shoots and grow slower. Leaf physiological characteristics, on the other hand, because of their sensitivity to the combined effects of seed-source moisture and cultivation temperature, exhibit highly plastic responses. In general, populations/families of black spruce are more conservative, at the leaf level, in environments that least reflect their origin. At the range-wide scale, 57°N appears to be a cut-point differentiating northern from southern populations (Chapter three). However, sampling from within the southern populations also shows a similar latitudinal differentiation among families (Chapter four). Accordingly, when water is not limiting, only under warmer conditions should populations/families that allocate a higher proportion of carbon to roots relative to shoots also be expected to express a lower stomatal conductance in relation to leaf photosynthetic capacity. These populations/families will therefore exhibit a lower carbon isotope discrimination (Δ).

When grown under warmer, well-watered environments, black spruce allocates a higher proportion of carbon to roots relative to shoots which, in combination with an increased leaf photosynthetic capacity and reduced stomatal conductance, results in a strong coupling between leaf-level water conservation and whole-plant carbon allocation. This leads to reduced

growth for low- Δ populations and is stronger in northern populations/families. Over time, populations/families of black spruce also shift from an early preferential allocation of carbon to shoots to greater investment into root tissues (probably a photoperiod-mediated response), resulting in slower growth. A correlated temporal change in leaf physiological characteristics follows, always resulting in strong water conservation under warmer conditions and in populations/families of northern origin.

The high plasticity of carbon allocation traits in time and leaf physiological characteristics across environments, leads to inconsistent rankings for populations/families. Nevertheless the data suggest the possibility of selecting black spruce families, by use of Δ , for water conservation, under warmer well-watered conditions, but only at the cost of reduced growth.

Water-use efficiency and growth

In black spruce, differentiation in carbon isotope discrimination -thus in water-use efficiency- among diverse populations results primarily from variation in stomatal conductance when water is not limiting. While this leads to a negative relationship between water-use efficiency and plant growth under warmer cultivation conditions, a positive relationship is observed under cooler cultivation conditions owing to a much greater sensitivity of stomatal conductance to photosynthetic capacity. However, still under non water-limiting conditions, there are families within populations

that not only conserve water via increased photosynthetic capacity but also allocate much lower proportions of carbon to roots relative to shoots in comparison with the bulk populations (for the same latitudinal range). Consequently, these may grow faster so long as the relative change in CO₂ assimilation offsets that in whole-plant carbon allocation. This is highly likely early in the development but seems to alter afterwards owing to intrinsic differences in carbon allocation patterns. Therefore, even for those families of black spruce, long-term water conservation comes at the cost of reduced growth.

Interestingly though, black spruce has also evolved a strategy that enables families to switch, as water becomes more limiting (edaphic or atmospheric, or other correlated factors), from the stomata-based efficiency in water use to that primarily driven by variation in mesophyll biochemical capacity for CO₂ fixation. Because this flexibility in response may result in a positive relationship between water-use efficiency and growth, it could help the species cope with unpredictable environments. This perhaps helps explain the versatility of black spruce along gradients of water availability within its distribution range. Further knowledge of the threshold moisture level (or correlated factors) below which black spruce populations shift from primary limitation to photosynthetic gas exchange caused by stomatal conductance to primary limitation caused by mesophyll biochemical capacity for CO₂ fixation, may prove useful in matching families with specific environments.

Potential response to climate change

Assessed from its juvenile traits, the potential response of black spruce to a warmer future climate will largely depend on the degree of aridity. Under high water availability, long-term water conservation will come at the cost of reduced growth but at a lesser extent for the currently selected full-sib families. Yet, unless the temporal shift in carbon allocation occurs sporadically within the juvenile period, greater allocation of carbon to roots under warmer well-watered environments may have important ecological implications as regards seedling establishment. As more thermophilous plant species shift northwards in response to global warming, greater investment of carbon in roots relative to shoots by juvenile black spruce may foster the need for more intensive vegetation management practices.

Under drier conditions, in contrast, the ability to shift to assimilation-based leaf-level water conservation may help maintain a positive relationship between water-use efficiency and growth. However, the much lower proportional allocation of carbon to roots by some families may significantly reduce their competitiveness for soil resources. Selecting and propagating fast-growing families of northern origin may constitute a silvicultural compromise that could alleviate the adverse effects of warmer and drier conditions.

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APPENDIX

Appendix 1

Table A1. Geographic coordinates and seed-source climatic variables for bulk populations of black spruce used in controlled and field experiments. Entries are: Lat., latitude; Long., longitude; Alt., altitude; D_{ls} , seasonal daylight; T_{aa} , annual air temperature; T_{sa} , annual 10-cm depth soil temperature; VPD_s , seasonal vapour pressure deficit; P_{ra} , annual precipitation; PET_a , annual potential evapotranspiration; CMI_a , annual climatic moisture index.

Population number	Province of origin	Lat. (N)	Long. (W)	Alt. (m)	D_{ls} (h)	T_{aa} (°C)	T_{sa} (°C)	VPD_s (kPa)	P_{ra} (cm)	PET_a (cm)	CMI_a (cm)
1	QC	46°08'	70°38'	229	14.37	4.0	6.8	0.35	108	48	59
2	ON	46°55'	79°42'	305	14.44	2.7	6.4	0.43	82	47	35
3	ON	48°40'	90°11'	460	14.61	2.3	6.0	0.42	71	46	25
4	QC	49°55'	74°20'	518	14.73	-0.1	4.7	0.43	88	40	48
5	ON	50°03'	94°52'	335	14.75	2.1	6.3	0.55	62	43	19
6	QC	50°20'	66°20'	244	14.77	1.1	4.6	0.32	113	28	85
7	MB	52°23'	101°07'	396	15.01	0.9	5.7	0.57	49	46	2
8	SK	54°43'	107°49'	457	15.30	-0.8	4.4	0.45	49	37	12
9	SK	56°03'	108°42'	427	15.49	-0.4	4.7	0.43	46	36	10
10	BC	56°36'	121°23'	854	15.58	1.3	5.2	0.54	49	38	11
11	MB	57°38'	96°45'	274	15.74	-4.9	1.8	0.38	43	24	18
12	BC	58°55'	125°45'	915	15.97	-1.4	4.2	0.53	45	41	4
13	YT	64°08'	139°25'	762	17.26	-5.1	1.6	0.48	31	35	-4

Appendix 2

Table A2. Geographic coordinates and seed-source climatic variables for full-sib families of black spruce used in this study. Entries are: Lat., latitude; Long., longitude; Alt., altitude; D_{ls} , seasonal daylength; T_{aa} , annual air temperature; T_{sa} , annual 10-cm depth soil temperature; VPD_s , seasonal vapour pressure deficit; P_{ra} , annual precipitation; PET_a , annual potential evapotranspiration; CMI_a , annual climatic moisture index.

Family number	Province of origin	Lat. (N)	Long. (W)	Alt. (m)	D_{ls} (h)	T_{aa} (°C)	T_{sa} (°C)	VPD_s (kPa)	P_{ra} (cm)	PET_a (cm)	CMI_a (cm)
1	ON	42°45'	80°20'	213	14.09	7.9	9.5	0.58	89	60	29
2	ON	42°45'	80°20'	213	14.09	7.9	9.5	0.58	89	60	35
3	ON	42°45'	80°20'	213	14.09	7.9	9.5	0.58	89	60	25
4	ON	45°57'	77°24'	190	14.35	4.3	7.3	0.46	82	51	32
5	ON	45°57'	77°24'	190	14.35	4.3	7.3	0.46	82	51	32
6	ON	45°57'	77°24'	190	14.35	4.3	7.3	0.46	82	51	32
7	ON	51°08'	80°52'	11	14.86	-1.1	4.1	0.29	73	31	42
8	ON	51°08'	80°52'	11	14.86	-1.1	4.1	0.29	73	31	42

Figure A1. Diurnal influence of air temperature on leaf and 6 cm-depth soil temperatures, and correlated diurnal variation in photosynthetic photon flux density and saturation vapour pressure deficit under simulated northern and southern environments. Symbols are: open, northern climate
 (Tl=-1.95+1.13Ta, R²=1.0 and Ts=1.93+0.85Ta, R²=0.89); closed, southern
 climate (Tl=-3.03+1.14Ta, R²=1.0 and Ts=2.20+0.88Ta, R²=0.93).

Figure A2. Diurnal influence of air temperature on leaf and 12 cm-depth soil temperatures, and correlated diurnal variation in photosynthetic photon flux density and saturation vapour pressure deficit under southern field conditions. Linear regression relationships are: $T_l=1.22+0.99T_a$, $R^2=0.91$ and $T_s=2.07+0.93T_a$, $R^2=0.92$.

Appendix 4

Table A3. Analysis of variance (repeated measurements) of recorded environmental conditions during the three successive climate simulations. Entries are: [CO₂], ambient carbon dioxide concentration; PPFD, photosynthetic photon flux density; air temperature; relative humidity; and, saturation vapor pressure deficit.

Source of variation	[CO ₂]			PPFD			Temperature			Relative humidity			Saturation vpd		
	d.f.	M.S.	p	d.f.	M.S.	p	d.f.	M.S.	p	M.S.	p	M.S.	p	M.S.	p
Environment (E)	1	2131	0.0005	1	282	0.9074	1	6436	0.0001	22	0.1536	7	0.0001		
Error	269	171		272	20790		270	28		11		<1			
Time (T)	2	21360	0.0001	2	601	0.0891	2	<1	0.3212	2	0.0180	<1	0.0006		
T x E ¹	2	4183	0.0001	2	503	0.1276	2	<1	0.4634	1	0.1632	<1	0.2079		
Error	538	156		544	233		540	<1		1		<1			

Note: 1 - Only on the daily [CO₂] was there a significant T x E interaction, indicating some inconsistency in the seasonal trend among replicates and between climates. However, this has less likely impacted on the overall seasonal average (Table 2.1). In general, the mean daily [CO₂] varied between -0.1% and 5.4% around the target value of 350 ppm. This deviation averaged 2.3% and 3.1% for the northern and southern climates, respectively. This variation is within the precision of ±3% for the controlling system.

Appendix 5

Figure A3. Air temperature and precipitation as measured at the field plot and Ottawa Airport in comparison with the respective 1951-80 climate normals for Ottawa. Bars are: hatched, field plot; dotted, Ottawa Airport; open, 1951-80 climate normal for Ottawa.

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