

**WATER-USE EFFICIENCY AND PRODUCTIVITY IN NATIVE CANADIAN
POPULATIONS OF *POPULUS TRICHOCARPA* AND *POPULUS BALSAMIFERA***

by

VIRGINIE M. POINTEAU

B.A., The University of Texas at Austin, 1996

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

March 2008

© Virginie M. Pointeau, 2008

ABSTRACT

Afforestation and reforestation programs utilizing available fields for biofuel production, carbon sequestration, and other uses linked to climate change are looking to tree physiologists to identify species and genotypes best-suited to their purposes. The ideal poplar genotype for use in Canadian programs would be drought-resistant, cold-climate adapted, and fast-growing, thus requiring an understanding of links between a variety of physiological traits linked to growth and productivity. This study examined the basis for variations in water-use efficiency within four selected populations of *Populus trichocarpa* and *Populus balsamifera* (2 provenances each). Each species included both a northern and a southern provenance. Correlations between water-use efficiency, nitrogen-use efficiency, $^{13}\text{C}/^{12}\text{C}$ isotope ratio, stomatal conductance, and overall productivity were evaluated. Gas exchange variables measured included net photosynthesis, transpiration rate, stomatal conductance, and intercellular CO_2 content. Water-use efficiency and ^{13}C content across all genotypes were highly correlated. Results suggested that variation in water-use efficiency was primarily related to variation in stomatal conductance across all genotypes. Whereas differences in net photosynthesis in this study were not significant between species, *P. balsamifera* did reveal a higher average stem volume overall. Although variation in stomatal conductance was the major determinant of differences in water-use efficiency, positive correlations were found between ^{13}C isotope abundance and net photosynthesis in both *P. balsamifera* provenances. In this regard, results for the northern *P. balsamifera* provenance are the most consistent across all gas-exchange and growth trait correlations, in terms of meeting expectations for sink-driven water-use efficiency. The findings in this study suggest the possibility of identifying poplar genotypes with an absence of trade-off between water-use efficiency and nitrogen-use efficiency, notably among genotypes from the northern *P. balsamifera* provenance, near Gillam.

TABLE OF CONTENTS

Abstract.....	ii
Table of Contents.....	iii
List of Tables.....	v
List of Figures.....	vi
List of Illustrations.....	vii
List of Abbreviations.....	viii
Acknowledgements.....	ix
Dedication.....	x
CHAPTER I Introduction and Literature Review.....	1
Introduction.....	1
<i>Populus</i> for Afforestation.....	3
Water-Use Efficiency.....	4
Nitrogen-Use Efficiency.....	8
Gibberellins and Productivity.....	9
Considerations & Current Work.....	10
CHAPTER II Objectives.....	12
CHAPTER III Materials & Method.....	13
Materials.....	13
Methods.....	16
Gas Exchange.....	16
Water-Use Efficiency.....	17
Carbon Isotope and Total Nitrogen Composition.....	17
Dry Matter Production.....	18
Stomatal Density.....	19
Statistical Analyses.....	19
CHAPTER IV Results.....	20
Trends Throughout Populations.....	20
Resource-Use Efficiencies.....	25
Provenance-Level.....	26
Genotype Level.....	31

CHAPTER V	Discussion.....	35
	Water-Use Efficiency.....	35
	Nitrogen-Use Efficiency.....	38
	NUE and WUE Trade-off.....	40
	Considerations for Future Work.....	41
CHAPTER VI	Conclusions.....	44
BIBLIOGRAPHY	46
APPENDIX	Analysis of Variance Model.....	57

LIST OF TABLES

Table 1:	Provenance locations, local provenance groupings, and climate data...14
Table 2:	One-way ANOVA for significant species- and genotype-level effects for gas-exchange and growth traits.....21
Table 3:	Pearson correlation among genotypes.....23
Table 4:	Analysis of variance components.....57
Table 5:	Sources and components of variance for the analysis of variance model.....58

LIST OF FIGURES

Figure 1:	Net photosynthesis for all genotypes within provenances.....	22
Figure 2:	$\delta^{13}\text{C}$ foliar correlations with $\delta^{13}\text{C}$ stem, WUE, stomatal conductance, and intercellular CO_2	25
Figure 3:	Net photosynthesis correlations with intercellular CO_2 concentrations for each provenance.....	27
Figure 4:	$\delta^{13}\text{C}$ correlations with net photosynthesis for each provenance	28
Figure 5:	$\delta^{13}\text{C}$ correlations with photosynthetic NUE for each provenance.....	29
Figure 6:	$\delta^{13}\text{C}$ correlations with stem volume for each provenance.....	30
Figure 7:	$\delta^{13}\text{C}$ plotted against photosynthetic NUE for all genotypes in each of the four provenances.....	32
Figure 8:	Fresh total biomass for all genotypes within provenances.....	33

LIST OF ILLUSTRATIONS

Illustration 1: Gas-exchange measurements using the LICOR 6400.....34

LIST OF ABBREVIATIONS

Carbon.....	C
Carnduff provenance.....	CAR
Gillam provenance.....	GIL
Intercellular CO ₂ content (μmol CO ₂ mol air ⁻¹).....	<i>c_i</i>
Leaf amphistomaticity (adaxial D _s /abaxial D _s).....	SR
MacMillan Island provenance.....	MC
Net photosynthesis (μmol CO ₂ m ⁻² s ⁻¹).....	A
Nitrogen.....	N
Nitrogen-use efficiency.....	NUE
Quesnel Lake provenance.....	QL
Root-to-shoot ratio.....	R:S
Specific leaf area (cm ² g ⁻¹).....	SLA
Stable carbon isotope composition (‰).....	δ ¹³ C
Stomatal conductance (mol H ₂ O m ⁻² s ⁻¹).....	<i>g_s</i>
Total stomatal density (per mm ²).....	D _s
Transpiration (mmol H ₂ O m ⁻² s ⁻¹).....	E
Water-use efficiency.....	WUE

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to all who have helped me in this endeavour. First and foremost, my supervisor, Dr. Rob Guy, for his patient and insightful mentorship; Dr. Shawn Mansfield and Dr. Richard Pharis, for serving on my committee and offering their knowledge and perspective; Dr. Andrew Riseman, for serving as my external examiner; my lab assistant, Nadja Johannsen; my labmates, Raju Soolanayakanahally, Dheeraj Chillakuru, Limin Liao, and Shofiul Azam; David Kaplan, manager of the UBC Greenhouse; Dr. Tony Kozak, for sharing his statistics expertise; Norman Hodges, for bringing his computer expertise to the rescue multiple times. And to my community of friends in 3621, 3623 and beyond: thank you.

Plant material was obtained from a provenance trial established by Dr. Cheng Ying in Surrey, BC for *P. trichocarpa*, and from the Shelterbelt Centre, PFRA-AAFC in Indian Head, SK for *P. balsamifera*. Funding was provided by NSERC and BioCap.

To Tanya

&

To my parents, Jack & Françoise Pointeau

INTRODUCTION & LITERATURE REVIEW

Introduction

Upon signing the Kyoto Protocol, Canada adopted the goal of reducing greenhouse gas emissions by 6% below its 1990 levels of 5.96×10^8 tons of CO₂, by the year 2012 (McKenney et al. 2004; Peterson *et al.* 1999). Such reductions could be achieved by either decreasing fossil fuel emissions, or increasing net carbon sequestration through reforestation and afforestation programs, among other means (Cannell 1999). Spatial simulation studies taking both biology and economics into account have estimated that between 12.7% and 32% of agricultural lands in western Canada are available for afforestation (McKenney et al. 2004; Van Kooten et al. 1999). The use of such lands for biofuel production would address both above-mentioned options simultaneously, since CO₂ would essentially be recycled in the processes of planting and subsequently converting trees to fuel (Lemus and Lal 2005; Povellato et al. 2007). The CO₂ released by burning fuel would originally have been removed from the atmosphere by the bioenergy crop, making the use of plant biomass as a source of fuel carbon-neutral (Hall and House 1995). Between 1993 and 2002, approximately 2650 hectares of privately owned land in the Canadian prairie provinces were converted to tree plantations, with white spruce and hybrid poplar making up 93% of the planted area (Hall et al. 2005). Although afforestation alone, even on the global scale, would not be

sufficient to stabilize atmospheric CO₂ levels, let alone lower them, forestry does have both short- (Cannel 1999) and long-term roles to play in contributing to Kyoto Protocol commitments. Ongoing reforestation and afforestation efforts, implying in part the conversion of depleted or marginal agricultural soils to tree plantations, is believed to have multiple ecological benefits beyond Kyoto goals, including protecting the soil from water and wind erosion, capturing nutrients, increasing soil stability through the development of roots at depths, and increasing biodiversity (Laureysens et al. 2005; Mann and Tolbert 2000; Pellis et al. 2004; Sage 1998). Others have found increases in nonnative species, coupled with a reduction in species diversity (Clavijo et al. 2005). Potential benefits to biodiversity, including the return of native plant and animal species, could require the use of native tree species for afforestation purposes (Cowie et al. 2007).

The establishment of tree plantations on lands depleted from years of tillage could increase soil quality by adding substantial levels of carbon to the soils (Grigal and Berguson 1998; Guo and Gifford 2002), although such benefits may take years to materialize; soil C may actually be depleted during the first 6-10 years following afforestation, as the plantation is being established (Paul et al. 2002; 2003). It has been suggested that plantations of hybrid poplar species could potentially increase organic carbon stored in soils to levels twice as high as those of soils covered with either row crops or grasses (Mann and Tolbert 2002), though others argue that, especially in the first 10 years following afforestation, soil C levels may be less beneath tree plantations than under agriculture (Paul et al. 2003). Studies involving a variety of tree species have been highly variable, showing both net increases and decreases in soil carbon levels (Del Galdo et al. 2003; Garten 2002; Tolbert et al. 2002). Establishing forest rotations of 20-

50 years, and including litter in soil C calculations will increase recorded levels of soil C accumulation (Paul et al. 2002). Approximately 150 tonnes of carbon in total could be sequestered per hectare of established tree plantation (Natural Resources Canada 2007); where soil C is concerned, however, current literature is lacking in long-term data spanning more than a few years (Paul et al. 2003).

***Populus* for Afforestation**

Populus tree species, including poplars, cottonwoods and aspens (Taylor 2002) (henceforth referred to as poplars), are among the favored tree species for use in afforestation efforts for numerous reasons: they have a wide natural distribution in the Northern Hemisphere, with 30 recognized species, providing a large pool of genetic variation; they have the ability to reproduce asexually, by sprouting from roots or growing from abscised or broken branches; they are fast-growing trees, capable of reaching 40 m in less than 20 years (Bradshaw et al. 2000; Ceulemans and Deraedt 1999). Poplars can reach reproductive maturity in only four years in a plantation setting (Vanden Broek 2005), and have wide interspecific crossability and high plasticity (Gielen and Ceulemans 2001), thus making them relatively easy to manipulate in ongoing efforts to improve yield on tree plantations. Besides their usefulness in rapid C sequestration and as a source of bioenergy (Hall and House 1995), poplars may also prove to be ideal as model trees for use in studying processes and physiology unique to trees, which *Arabidopsis* cannot provide (Jansson and Douglas 2007; Taylor 2002). The relatively small genome size of *Populus*, together with the multiple poplar genomic maps currently developed (Taylor 2002), render any research done on poplar physiology

potentially useful and applicable to research needs on other tree species. *Populus* represents the first tree species to have its genome entirely sequenced (and the third plant, after *Arabidopsis* and rice) (Tuskan et al. 2004, 2006).

The high productivity of poplars depends on the availability of water, and typically limits their cultivation mostly to floodplains and bottomlands, where water is not a limiting factor (Monclus et al. 2006). In Canada, hybrid poplars are most successful in the relatively milder climates of southern Ontario and southwest British Columbia (Guy et al. 2001). The majority of the 7 million hectares of marginal agricultural lands currently available in Canada for poplar cultivation, however, is located in the prairie provinces, where few hybrid poplar species have proven successful (Guy et al. 2001). In order to take full advantage of available lands, hybrids must be developed that combine cold hardiness with both high productivity and high drought tolerance. The ideal poplar genotype for use in Canadian carbon sequestration programs would be drought-resistant, cold-climate adapted, and fast-growing (Guy et al. 2001), thus requiring a thorough understanding of links between water-use efficiency (WUE), photosynthesis, endogenous gibberellin levels, nitrogen-use efficiency (NUE), and other physiological traits linked to growth and productivity.

Water-Use Efficiency

Water-use efficiency has commonly been defined as the ratio of biomass production to unit water used (Monclus et al. 2006). Given the difficult and labor-intensive nature of measuring total water-use throughout a growing season, WUE is often estimated at the leaf level by measuring the ratio of photosynthesis (A) to transpiration (E). Long-term water-use efficiency can also be estimated through measurements of stable carbon isotope abundance ($\delta^{13}\text{C}$) (Farquhar et al. 1982, 1989). The heavier of the two stable carbon isotopes, ^{13}C , makes up approximately 1.11% of the carbon present in the biosphere, compared to 98.89% for ^{12}C (Griffiths 1993). $\delta^{13}\text{C}$ represents the ratio of ^{13}C to ^{12}C in a plant sample, expressed in per mil (‰) relative to the commonly-used Vienna Pee Dee belemnite standard (Coplen 1994; Condon et al. 2002). $\delta^{13}\text{C}$ in C_3 plants reflects a plant's level of discrimination against the heavier and less abundant ^{13}C during various processes from diffusion of CO_2 from the atmosphere to carboxylation (Farquhar et al. 1982). Plant discrimination against ^{13}C is linked to levels of intercellular CO_2 (c_i), which in turn reflect changes in either stomatal conductance or photosynthetic capacity (Silim et al. 2001). $\delta^{13}\text{C}$ values are negatively correlated with the ratio of internal versus atmospheric CO_2 levels (c_i/c_a). Low c_i caused by high photosynthetic rates will lead to less negative $\delta^{13}\text{C}$ values (Gornall and Guy 2007). More positive $\delta^{13}\text{C}$ values are linked to higher WUE, while more negative $\delta^{13}\text{C}$ values are linked to lower WUE. Thus both $\delta^{13}\text{C}$ values and c_i/c_a serve as indicators of plant WUE.

Since WUE is defined as the ratio of net photosynthesis (A) to transpiration (E), or A/E , then changes in WUE, and thus changes in $\delta^{13}\text{C}$, must be due to changes in either stomatal conductance or photosynthetic capacity (Silim et al. 2001), assuming an

otherwise constant environment. Trees displaying both high WUE and high productivity, therefore, likely indicate WUE primarily associated with variations in photosynthetic capacity, as opposed to variations in stomatal conductance, which would theoretically lead to a decrease in productivity (Guy and Holowachuk 2001; Sun et al. 1996).

Previous work done on *Populus trichocarpa* Torr. & A. Gray \times *Populus deltoides* Marsh. (Bunn et al. 2004; Rae et al. 2004), *Populus deltoides* Marsh. \times *Populus nigra* L. (Monclus et al. 2005), and *Populus trichocarpa* Torr. & A. Gray (Gornall & Guy 2007) suggested that population-level variation in WUE in these species was not clearly associated with variation in either stomatal conductance or photosynthetic capacity, as evidenced in each study by the lack of any correlation between WUE, or $\delta^{13}\text{C}$ values, and indices of productivity such as photosynthesis, biomass or stem volume. Studies conducted on *Populus deltoides* \times *P. nigra* clones by Marron et al. (2005) and Monclus et al. (2005), and on *Populus trichocarpa* \times *Populus deltoides* by Rae et al. (2004), exploring relationships between various leaf-level traits and overall productivity, found no correlation between stable C isotope discrimination (Δ) and total biomass. Others have found positive correlation between growth and WUE in *Populus* \times *euramericana* Dode (Guinier) clones (Voltas et al. 2006), indicating the possibility for WUE in poplar hybrids driven at least in part by photosynthetic capacity. Likewise, Zhang et al. (2004) found there to be positive correlations between WUE, productivity and $\delta^{13}\text{C}$ in *Populus davidiana* Dode. Bungart and Hützl (2004), in a study using several different poplar species and hybrids, also found positive correlations between WUE (measured as biomass gain per total transpiration) and growth rates. In conifer species, positive correlations between growth traits and $\delta^{13}\text{C}$ values have consistently been found,

suggesting variation in WUE driven primarily by photosynthetic capacity for *Picea glauca* (Moench) Voss (Sun et al. 1996; Silim et al. 2001), *P. sitchensis* (Bong.) Carr. (Silim et al. 2001), *Pinus contorta* Dougl. ex Loud. (Guy & Holowachuk 2001) and in *Picea rubens* Sarg. and *Picea mariana* (Mill.) BSP (Major et al. 2007). Benowicz et al. (2001) found that differences in intrinsic WUE in paper birch (*Betula papyrifera* Marsh.) were due to differences in net photosynthesis, and not stomatal conductance, whereas Wang et al. (1998) attributed higher WUE in paper birch to decreased stomatal conductance (g_s). Ripullone et al. (2004), in a study looking at the effects of nitrogen supply on WUE in *Populus × euroamericana* Dode, also found a positive correlation between WUE and $\delta^{13}\text{C}$. These results, together with the high heritability of both productivity and C isotope discrimination (Δ) in poplar species (Rae et al. 2004; Monclus et al. 2005), suggest the possibility of selecting poplar genotypes with high WUE driven by high photosynthetic rates, and thus at no cost to growth. Alternatively, a lack of correlation between WUE and growth indices could have its advantages in a breeding program, as it would allow for the independent selection of both high WUE and high productivity; normal fluctuations in WUE and g_s , in this case, should not affect growth (Monclus et al. 2006). While correlations between WUE and $\delta^{13}\text{C}$ values are presently well-accepted in the field of plant physiology, positive correlation between $\delta^{13}\text{C}$ and productivity in poplars has not been consistently established.

Guy and Holowachuck (2001) suggest that high sink demand resulting from increased growth leads to an increase in photosynthetic rate, and, subsequently, more positive $\delta^{13}\text{C}$ values and higher WUE. Blum (2005), on the other hand, in a review addressing links between yield potential, drought resistance and WUE, maintains that

WUE in crops results from water-use primarily, rather than from productivity or carbon assimilation. Similarly, in a study addressing wheat and barley cultivars, Condon et al. (2002) suggest that low carbon discrimination resulting from higher photosynthetic capacity could lead to a negative correlation between photosynthesis and biomass in crops. In order to best utilize the potential for rapid growth and high productivity seemingly inherent to poplar species, more studies addressing the links between WUE, $\delta^{13}\text{C}$, net photosynthesis and productivity in a variety of poplars and poplar hybrids are needed.

Nitrogen-Use Efficiency

Positive correlations between nitrogen content and net photosynthesis in C_3 plants are widely recognized (Evans 1989), and have been identified in a variety of gymnosperm (Field et al. 1983; Robinson et al. 2001; Major et al. 2007) and angiosperm species (Sobrado 1991), including *Populus trichocarpa* (Gornall & Guy 2007). Ripullone et al. (2004) found that an increase in both WUE and productivity in *Populus × euroamericana* were caused by effects of N on photosynthetic rates, rather than changes in stomatal conductance or transpiration. Stomatal conductance, and subsequently both c_i/c_a and WUE, is also affected by nitrogen availability.

Photosynthetic nitrogen-use efficiency is defined as the ratio of net photosynthesis to N content per unit leaf area (Larcher 2003). The expected positive linear relationship between photosynthetic capacity and nitrogen content (Larcher 2003) implies a possible inherent trade-off between WUE and NUE, given that any increase in c_i (leading to increased NUE) will lead to more negative $\delta^{13}\text{C}$ values and lower WUE

(Patterson et al. 1997). This is consistent with studies done on various evergreen shrub and tree species (Field et al. 1983), white spruce (Livingston et al. 1999), and paper birch (Wang et al. 1998), although others found an absence of intrinsic trade-off between WUE and NUE for both white and black spruce (Patterson et al. 1997), suggesting the possibility of selecting for both high WUE and NUE separately. It is possible that relatively higher WUE resulting from high photosynthetic capacity could result in higher levels of yield even in nitrogen-deficient environments (Livingston et al. 1999; Pritchard and Guy 2005). In times of drought, however, a tendency in plants to increase stomatal closure will simultaneously increase WUE and decrease NUE, as shown in hybrid willow (Weih et al. 2006). Current literature is lacking in studies addressing correlations, or lack thereof, between WUE and NUE, and the implications of such links on growth and productivity, specifically in poplar species and their hybrids.

Gibberellins and Productivity

Productivity in trees is also thought to have possible links with concentrations of endogenous gibberellins (GAs) (Pharis et al. 1991). In a recent review, Kende and Zeevaart (1997) discuss literature linking gibberellin to stem and cell elongation (Raskin and Kende 1984), and suggesting a GA role in cell division in multiple plant species (Sauter and Kende 1992). A study conducted on maize parental inbreds and their hybrids, by Rood et al. (1988), found that inbreeding depression in maize was partly due to low levels of endogenous GAs, whereas hybrids displaying vigorous shoot growth contained higher GA concentrations. In a study conducted on soy beans and broad beans (Yuan and Xu 2001), the application of exogenous gibberellin was shown to increase

photosynthetic rate, leading to a subsequent increase in photosynthetic carbon fixation, and further demonstrating correlations between gibberellin content and growth. Later studies showed both evidence for (Pharis et al. 1991) and against (Pearce et al. 2004) positive correlations between endogenous GA concentration and superior growth in poplar species. The over-expression of a GA 20-oxidase gene in a study using hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) resulted in increased growth rate, larger leaves and greater biomass (Eriksson et al. 2000), though direct links to secondary growth and wood formation have yet to be entirely established (Israelsson et al. 2003). Although the use of genetic engineering for the improvement of plantation trees may or may not be implemented, further studies exploring links between naturally-occurring GA concentrations and WUE, NUE and overall productivity in poplar species native to Canada would certainly be beneficial for breeding programs seeking to breed native poplar species.

Considerations and Current Work

Abundant studies addressing WUE, $\delta^{13}\text{C}$, net photosynthesis and various growth indices in numerous tree species have substantially increased our understanding of the links and correlations present among these traits; however, while positive correlation between intrinsic WUE and $\delta^{13}\text{C}$ has clearly and consistently been shown in C_3 plants, correlations between WUE indices (including $\delta^{13}\text{C}$) and growth in poplar species have yet to be established. Likewise, current literature is lacking in studies addressing links, or lack thereof, between WUE and NUE in tree species, and especially in poplars. Further work on naturally occurring, inherent gibberellin concentrations in poplar

species, and possible correlations with WUE, growth and productivity, would lend additional insight and guidance to tree breeding programs and hybridization efforts intent on creating the ideal poplar trees for biofuel production, carbon sequestration, or other uses linked to climate change.

This study endeavoured to determine the basis for variations in WUE within selected provenances of *Populus trichocarpa* and *Populus balsamifera*, along with correlations between WUE, NUE, C isotope content ($\delta^{13}\text{C}$), net photosynthesis, stomatal conductance, and overall productivity. It was expected that genotypes displaying positive correlations between $\delta^{13}\text{C}$ values (proxy for long-term WUE) and productivity would also display a high photosynthetic capacity, and that genotypes displaying relatively high WUE as a result of high sink-driven photosynthetic capacity would show a less severe or absent trade-off with NUE.

OBJECTIVES

1. Determine whether variation in WUE within selected provenances of *Populus trichocarpa* growing along the coast of British Columbia, and *P. balsamifera* growing along a north-south transect from Manitoba to Saskatchewan are related more to photosynthetic capacity or to stomatal conductance.
2. Further define possible links between water-use efficiency, $\delta^{13}\text{C}$, nitrogen-use efficiency, stomatal conductance, net photosynthesis and overall productivity.
3. Identify genotypes with relatively high water-use efficiency combined with high productivity.

MATERIALS & METHODS

Materials

Populus trichocarpa Torr. & A. Gray (black cottonwood) and *Populus balsamifera* L. (balsam poplar) clones from selected populations located along two north-south transects in western Canada were used in this study. As the emphasis of this study was on within-population variations in physiological traits, only four provenances in total were selected: two *Populus trichocarpa* provenances from the British Columbia Ministry of Forests collection, and two *Populus balsamifera* provenances, one each from Manitoba and Saskatchewan, from the Natural Resources Canada AgCanBaP collection. One northern provenance and one southern provenance were utilized for each species. From BC, the two selected provenances were Quesnel River (QL), located at 52°65'N 122°26'W, and McMillan Island (MC), located at 49°14'N 121°81'W. From Manitoba, the Gillam provenance was used (GIL), located at 56°27'N 94°41'W, and from Saskatchewan, the Carnduff provenance (CAR), located at 49°14'N 101°56'W. For QL and MC, numbers were increased by grouping three local populations together for each provenance (Table 1). The area encompassing the local groups for each *P. trichocarpa* provenance is comparable to the area defining the Gillam provenance, roughly 40×14 km. Climate data collected for all *P. trichocarpa* genotypes showed very similar elevations, temperature ranges, and precipitation for all groupings within provenances

(Table 1). The specific locations stated above represent the average latitude and longitude for each provenance (Table 1).

	Lat °N	Long °W	Elev (m)	MAT	MWMT	MCMT	MAP	NFFD
QAUS	52.43	122.28	442	6.2	17	-4.1	458	197
QLKE	52.48	122.19	488	6.1	16.7	-4.2	467	197
QFRS	53.04	122.31	472	5.9	16.4	-5.6	667	194
Average	52.65	122.26	467.33	6.07	16.7	-4.63	530.67	196.00
MCMN	49.11	122.35	15	9.6	17.2	2.1	1648	294
HRSO	49.17	121.57	40	9.1	17.3	1	1771	288
HRSP	49.14	121.51	30	9.9	18.1	1.4	1785	298
Average	49.14	121.81	28.33	9.53	17.53	1.50	1735	293.33
GIL 3	56.21	94.36	115					
GIL4	56.20	94.34	123					
GIL5	56.19	94.32	126					
GIL6	56.19	94.30	129					
GIL7	56.22	94.24	104					
GIL10	56.56	94.28	131					
GIL12	56.28	94.38	133					
GIL13	56.28	94.42	137					
GIL14	56.29	94.48	124					
GIL15	56.30	95.01	90					
Average	56.27	94.41	121.20					
Gillam, MB	56.21	94.42	145	-4.2	15.3	-25.8	499.4	128.6
CAR1	49.11	101.50	533					
CAR2	49.13	101.56	545					
CAR3	49.13	101.56	545					
CAR4	49.13	101.56	545					
CAR5	49.14	101.56	543					
CAR7	49.15	101.57	548					
CAR8	49.14	101.58	550					
CAR11	49.16	101.58	553					
CAR14	49.13	101.57	546					
CAR15	49.16	101.57	549					
Average	49.14	101.56	545.70					
Estevan, SK	49.13	102.58	580	3.7	19.5	-14.8	4.33	172.8

Table 1. Provenance locations, local provenance groupings, and climate data. *P. trichocarpa* provenances are QL (QAUS, QLKE and QFRS) and MC (MCMN, HRSO, HRSP); *P. balsamifera* provenances are GIL and CAR. Climate data include mean average temperature (MAT), mean warmest month temperature (MWMT), mean coldest month temperature (MCMT), mean average precipitation (MAP), and number of frost-free days (NFFD). Temperatures are in degrees Celsius; precipitation is in centimeters. Climate data for British Columbia were obtained through ClimateBC (Centre for Forest Conservation Genetics, University of British Columbia); climate data for Saskatchewan and Manitoba were obtained from Environment Canada (Canadian Climate Normals or Averages 1971-2000). For the CAR and GIL provenances, the closest weather stations with all necessary data were located in Estevan, SK (approximately 90 km west of Carnduff) and Gillam, MB, respectively.

The study included three replicates for 10 genotypes from each of the four provenances, for a total of 120 trees. The trees were organized into three blocks, with each block containing one of each clone from each of the four provenances. Trees were grown from cuttings rooted in Rootainers™ (Spencer-Lemaire Industries Limited, Edmonton, AB, Canada), on February 7, 2007. Cuttings were just long enough to contain two buds (approximately 5 cm); the lower bud was buried in the soil for rooting. Between 13 and 15 genotypes were started for each provenance. Roots were allowed to develop over a period of 4 weeks. On March 8, dead or weakly-rooted cuttings were culled from each provenance, reducing the collection to 10 genotypes per provenance. These stecklings were transferred to 4-litre pots and arranged randomly along a single flood bench in the University of British Columbia greenhouse. A random-number generator was used to assign trees to pre-numbered slots along the bench. The trees were allowed to grow for an additional 4½ weeks before gas-exchange measurements began on April 8.

Cuttings were planted in a mixture of 75% peat moss and 25% perlite. During the initial four weeks of growth, the cuttings received only enough water to keep the soil damp, so as not to risk damage to emerging roots. On the flood bench, the trees were initially watered three times a week with a 120 ppm fertigation solution (15-5-15 Cal-Mag, The Scotts Company, Marysville, OH). After two weeks on the flood bench, the trees received 400 ml 20-20-20 Soluble Plant Food (Sunshine®) solution (approx. 31g/100 L water) once a week for the remainder of the experiment. During the entire experiment, the stecklings received 18 hours of light per day, with HPS 400 Watt lights installed over the flood bench to supplement natural light. The trees were 60 days old

when the first gas-exchange measurements were taken.

Methods

Gas Exchange

Gas exchange variables were measured using a LI-6400 portable gas-exchange system (LI-COR Biosciences, Lincoln, NB, USA). The leaf chamber was attached to a tripod to ensure stability, and allow for necessary adjustments to varying tree heights. All measurements were conducted April 8-24, 2007, between 8:00 am and 1:00 pm. Measurements were conducted on one block at a time, with each block taking 3-5 days. A random number generator was used to determine sampling order within each block. In order to ensure constant light at saturation levels, an artificial light source (LI-COR 6400-02B LED Light Source) was used for all measurements. Light level was set at $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to ensure light saturation, estimated at approximately $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Conditions inside the leaf chamber were set as follows: CO_2 concentration at $360 \mu\text{l}\cdot\text{L}^{-1}$; relative humidity at 45%; and block temperature at 20°C . Two recently fully-expanded sun leaves from the upper stem of each tree were measured twice, and combined to provide an average for each tree. Leaves were allowed 10-12 minutes to adjust to chamber conditions prior to recording measurements.

Recorded data for gas-exchange parameters included net photosynthesis (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 content (c_i , $\mu\text{mol CO}_2 \text{ mol air}^{-1}$) (equations derived by von Caemmerer and Farquhar 1981).

Water-Use Efficiency

Instantaneous WUE ($\mu\text{mol CO}_2 \cdot \text{mol}^{-1} \text{H}_2\text{O}$) was calculated as the ratio of net photosynthesis to transpiration, or A/E (Farquhar et al. 1982). The ratio of net photosynthesis to stomatal conductance, or A/g_s , was also calculated, to compare with A/E . Leaf values for $\delta^{13}\text{C}$ were used as a surrogate for long-term WUE (Farquhar et al. 1982, 1989). Stem values for $\delta^{13}\text{C}$ were collected from the base of all third harvest stems (not old wood), both for comparison purposes, and to ensure the value of foliar analyses as accurate representations of whole-canopy trends.

Carbon Isotope and Total Nitrogen Composition

Leaf samples were collected from each tree immediately after gas-exchange measurements were completed. Samples were kept in storage at -20°C until they could be oven-dried; samples were dried at 70°C to constant mass. A stainless-steel ball mill was used to grind dried leaf samples into a fine powder; 1-2 mg sub-samples were analyzed for $\delta^{13}\text{C}$ and total C and N content at the Stable Isotope Facility at the University of California at Davis. Following leaf sample combustion to CO_2 and N_2 at 1000°C in an on-line elemental analyzer (PDZEuropa ANCA-GSL), stable isotope ratios of C and N were measured by continuous flow isotope ratio mass spectrometry (20-20 Mass Spectrometer, Sercon, Crewe, UK). $\delta^{13}\text{C}$ values were calculated based on a working standard mixture of ammonium sulfate and sucrose with $\delta^{13}\text{C}$ V-PDB -23.83 .

$\delta^{13}\text{C}$ reflects the ratio of the two stable carbon isotopes ^{13}C and ^{12}C within a plant, relative to an arbitrary standard. The C isotope composition of the samples was calculated using the following equation:

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

Where R_{standard} and R_{sample} refer to the ratios of $^{13}\text{C}/^{12}\text{C}$ of the standard and the sample, respectively. The current standard, Vienna-PDB, was originally based on a fossil belemnite from the Peedee Formation (PDB) (Coplen TB 1994). Net photosynthesis (A : $\mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$) and leaf N density ($\mu\text{mol N cm}^{-2}$) were used to calculate photosynthetic nitrogen-use efficiency (NUE: $\mu\text{mol CO}_2 \mu\text{mol N}^{-1} \text{ s}^{-1}$).

Dry Matter Production

Destructive sampling of trees for measurement of dry matter production occurred throughout the course of the experiment, as gas-exchange measurements were completed on each tree. Stem basal diameter, height and fresh mass, and root fresh mass were measured for each clone. Stem volume was estimated from height and basal diameter, using the formula for a cone ($\frac{1}{3}\pi r^2 h$), and used as a measure of total above-ground growth. Total biomass (g) was estimated by combining fresh mass measurements for stem and roots. Given the need to conserve fresh frozen material for further work, dry biomass could not be recorded. Leaf area, fresh and dry mass were recorded for all leaves used for gas-exchange measurements. Specific Leaf Area (SLA: $\text{cm}^2 \text{ g}^{-1}$) was calculated based on leaf area and dry mass.

Stomatal Density

Leaf stomatal impressions were taken for each leaf used in gas-exchange. These impressions were made using clear fingernail polish, which, once dry on the leaf, was peeled off and applied to a slide (as per Gornall and Guy 2007). Stomatal counts for each leaf were done on three separate areas along the impression, then averaged, for both abaxial (lower) and adaxial (upper) sides of the leaf, for all genotypes. Leaf amphistomaticity (SR) was calculated as the ratio of adaxial to abaxial stomatal densities. Adaxial and abaxial counts were added together to yield total stomatal density for each genotype (D_s).

Statistical Analyses

Statistical analyses were conducted in SigmaPlot 9.0 and SAS 9.1. Variables were analyzed by analysis of variance (ANOVA) using a two-factor factorial in a randomized complete block design (see Appendix for complete ANOVA model); the general linear models (GLM) procedure was used to address unequal numbers of observations within factors. Genotypes were nested within species and latitude. The Kolmogorov-Smirnov test for normality and Bartlett's test for homogeneity of variances were conducted on the residuals. Significance levels were tested at $\alpha = 0.05$ for all data. Genotype means (average of three values for each genotype) were used in regression analysis; Pearson correlation coefficients (r) were calculated using all data. Linear regressions were conducted to describe relationships between physiological traits (WUE, $\delta^{13}\text{C}$, NUE, g_s , c_i , A , and stem volume).

RESULTS

Trends throughout populations

Results from one-way analyses of variance (ANOVA) for all trees revealed significant differences both between species and within provenances for most physiological traits (Table 2). There were no significant differences between latitudes for any tested variable, except SLA, which is greater for northern provenances, with means of $233.13 \text{ cm}^2\text{g}^{-1}$ and $215.52 \text{ cm}^2\text{g}^{-1}$ for north and south, respectively ($p=0.0217$). ANOVA revealed no differences in net photosynthesis (A) between species or latitudes (Table 2 and Fig. 1). Significant differences in A were found within provenances ($p=0.0037$), however, with values ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) ranging as follows for each provenance: Gillam, 10.11-16.26; Carnduff, 12.78-15.56; Quesnel Lake, 11.98-16.23; MacMillan Island, 11.29-15.21. A showed no correlation across all genotypes with any of the gas-exchange traits, including stomatal conductance (g_s), intercellular CO_2 (c_i), and transpiration (E); or with water-use efficiency indices including A/E , A/g_s and $\delta^{13}\text{C}$ (Table 3, A/g_s not shown). A was positively correlated with both leaf N density ($p=0.0080$) and photosynthetic NUE ($p=0.0353$) (Table 3). A was also positively correlated with stem volume ($p=0.0156$) and total biomass ($p=0.0002$), and negatively correlated with SLA ($p=0.0055$) (Table 3). Correlations did not exist, however, between these growth parameters and any of the three WUE indices mentioned above (Table 3).

	A	<i>g_s</i>	Stem Volume	Leaf Area	SLA	WUE (A/E)	$\delta^{13}\text{C}$	R : S	NUE
Species	df: 1 SS: 0.5481 MS: 0.5481 F: 0.10 <i>p</i> : 0.7593	df: 1 SS: 1.3676 MS: 1.3676 F: 114.74 <i>p</i> : <0.0001	df: 1 SS: 1.4349 MS: 1.4349 F: 8.38 <i>p</i> : 0.0049	df: 1 SS: 15640.2625 MS: 15640.263 F: 88.98 <i>p</i> : <0.0001	df: 1 SS: 18900.3000 MS: 18900.300 F: 28.39 <i>p</i> : <0.0001	df: 1 SS: 12.4415 MS: 12.4415 F: 85.69 <i>p</i> : <0.0001	df: 1 SS: 25.5774 MS: 25.5774 F: 95.99 <i>p</i> : <0.0001	df: 1 SS: 0.7384 MS: 0.7384 F: 13.30 <i>p</i> : 0.0008	df: 1 SS: 1.4348E-9 MS: 1.434E-9 F: 6.92 <i>p</i> : 0.069
Gen (Sp*lat)	df: 36 SS: 207.0155 MS: 5.7504 F: 2.08 <i>p</i> : 0.0037	df: 36 SS: 1.0323 MS: 0.0287 F: 2.41 <i>p</i> : 0.0007	df: 36 SS: 25.8261 MS: 0.7174 F: 4.19 <i>p</i> : <0.0001	df: 36 SS: 48095.1018 MS: 1335.9751 F: 7.60 <i>p</i> : <0.0001	df: 36 SS: 58182.1632 MS: 1616.1712 F: 2.43 <i>p</i> : 0.0006	df: 36 SS: 17.8693 MS: 0.4964 F: 3.42 <i>p</i> : <0.0001	df: 36 SS: 32.1817 MS: 0.8939 F: 3.36 <i>p</i> : <0.0001	df: 36 SS: 1.9994 MS: 0.0555 F: 1.68 <i>p</i> : 0.0287	df: 36 SS: 1.4674E-8 MS: 4.076E-10 F: 1.97 <i>p</i> : 0.0072

Table 2. One-way ANOVA for significant species- and genotype-level effects for the following gas-exchange and growth traits: net photosynthesis (A), stomatal conductance (g_s), stem volume, leaf area, specific leaf area, water-use efficiency (WUE), stable carbon isotope content ($\delta^{13}\text{C}$), root-to-shoot ratio (R:S), and nitrogen-use efficiency (NUE).

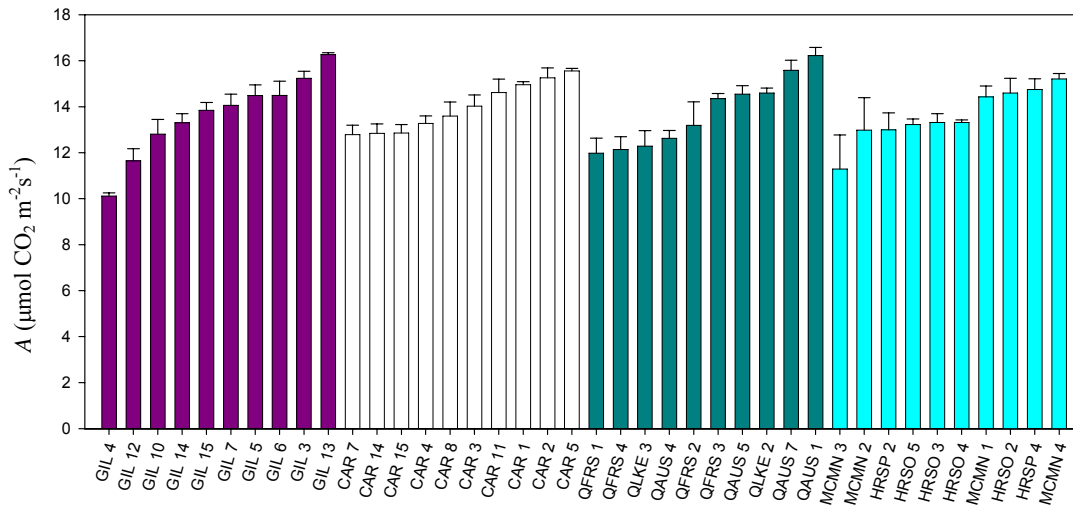


Figure 1. Net photosynthesis (A) for all genotypes within provenances. Gillam (GIL) and Carnduff (CAR) represent the northern and southern *Populus balsamifera* provenances, respectively. Quesnel Lake (QFRS, QLKE, QAUS) and MacMillan Island (MCMN, HRSP, HRSO) represent the northern and southern *Populus trichocarpa* provenances, respectively.

Stomatal conductance (g_s) varied between species ($p < 0.0001$) as well as within provenances ($p = 0.0007$), but not between latitudes; *P. balsamifera* showed higher g_s than *P. trichocarpa*, with means of $0.693 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ versus $0.477 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$, respectively (Table 2). Stomatal conductance values within provenances ranged from $0.51\text{-}0.88$ and $0.60\text{-}0.79 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ for Gillam and Carnduff (*P. balsamifera*), and from $0.32\text{-}0.68$ and $0.34\text{-}0.68 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ for Quesnel Lake and MacMillan Island (*P. trichocarpa*), respectively. Stomatal conductance was highly positively correlated with c_i ($p < 0.0001$) and E ($p < 0.0001$), and negatively correlated with all WUE indices (A/E : $p < 0.0001$; $\delta^{13}\text{C}$: $p < 0.0001$; A/g_s : $p < 0.0001$) (Table 3). Stomatal conductance was also positively correlated with D_s ($p < 0.0001$) and negatively correlated with SR ($p = 0.0013$) (Table 3). SR, in turn, was negatively correlated, and D_s positively correlated, with all gas-exchange parameters (c_i , g_s , and E) (Table 3). Reverse trends were found for

	A	g_s	c_i	E	WUEi	Stem Vol	R:S	Leaf Dry	Leaf Area	SLA	Adaxial	Abaxial	SR	D_s	$\delta^{13}C$	N	NUE	Biomass
A		0.1253 0.4409	-0.263 0.1008	0.1965 0.2242	0.2571 0.1093	0.3800 0.0156	0.0502 0.7584	0.4007 0.0104	0.2295 0.1542	-0.431 0.0055	0.0861 0.5975	-0.003 0.9851	0.0884 0.5877	0.0235 0.8856	0.1880 0.2455	0.4135 0.0080	0.3338 0.0353	0.5631 0.0002
g_s			0.8726 <.0001	0.9742 <.0001	-0.858 <.0001	0.2026 0.2099	-0.244 0.1294	-0.109 0.5030	-0.252 0.1166	-0.307 0.0538	-0.408 0.0090	0.6759 <.0001	-0.491 0.0013	0.6296 <.0001	-0.811 <.0001	0.1723 0.2878	-0.094 0.5623	0.2276 0.1579
c_i				0.8543 <.0001	-0.972 <.0001	0.0199 0.9030	-0.278 0.0819	-0.259 0.1062	-0.336 0.0341	-0.140 0.3876	-0.486 0.0015	0.6041 <.0001	-0.520 0.0005	0.5247 0.0005	-0.864 <.0001	-0.030 0.8564	-0.158 0.3315	-0.053 0.7452
E					-0.872 <.0001	0.2290 0.1552	-0.238 0.1394	-0.099 0.5418	-0.245 0.1274	-0.312 0.0503	-0.392 0.0124	0.6372 <.0001	-0.456 0.0031	0.5912 <.0001	-0.767 <.0001	0.1444 0.3742	0.0070 0.9660	0.2442 0.1289
WUEi						-0.015 0.928	0.2568 0.1097	-0.272 0.089	0.3288 0.0383	0.0903 0.5795	0.4201 0.007	-0.576 0.0001	0.462 0.0027	-0.514 0.0007	0.8252 <.0001	0.0810 0.6193	0.099 0.5421	0.048 0.7693
Stem Vol							-0.196 0.2255	0.5724 0.0001	0.4199 0.0070	-0.335 0.0344	-0.061 0.7103	0.2537 0.1143	-0.097 0.5501	0.2651 0.6594	0.0719 0.0007	0.5144 0.0007	-0.170 0.2934	0.8679 <.0001
R:S								0.0799 0.6241	0.1011 0.5350	0.0634 0.6975	0.2952 0.0645	-0.321 0.0434	0.3032 0.0572	-0.267 0.0955	0.2070 0.1999	-0.012 0.9428	-0.001 0.9964	-0.034 0.8353
Leaf Dry									0.8852 <.0001	-0.305 0.0558	0.2213 0.1700	-0.190 0.2409	0.2256 0.1617	-0.143 0.3775	0.3687 0.0192	0.2782 0.0822	0.0426 0.7943	0.6958 <.0001
Leaf Area										0.1549 0.3400	0.3620 0.0217	-0.428 0.0058	0.4197 0.0070	-0.367 0.020	0.4534 0.0033	-0.097 0.5496	0.2803 0.0798	0.5324 0.0004
SLA											0.2894 0.0701	-0.462 0.0027	0.3853 0.0141	-0.427 0.0059	0.1598 0.3247	-0.751 <.0001	0.4425 0.0042	-0.390 0.0129
Adaxial												-0.503 0.0009	0.9395 <.0001	-0.251 0.1190	0.3203 0.0439	-0.129 0.4278	0.1969 0.2233	-0.010 0.9496
Abaxial													-0.687 <.0001	0.9629 <.0001	-0.529 0.0005	0.3823 0.0149	-0.470 0.0022	0.1316 0.4183
SR														-0.476 0.0019	0.3867 0.0137	-0.208 0.1986	0.3196 0.0444	-0.043 0.7932
D_s															-0.492 0.0013	0.3879 0.0134	-0.465 0.0025	0.1441 0.3749
$\delta^{13}C$																-0.020 0.9043	0.1640 0.3120	0.1271 0.4347
N																	-0.649 <.0001	0.4893 0.0014
NUE																		-0.084 0.6045

Table 3. Pearson correlations: r-values on top, with p-values below. Highlighted cells indicate $p < 0.0001$; bold indicates $p < 0.01$; net photosynthesis (A), stomatal conductance (g_s), intercellular CO₂ (c_i), transpiration (E), water-use efficiency (WUE), stem volume, root-to-shoot ratio (R:S), leaf dry weight (g), leaf area (cm²), specific leaf area (SLA: cm²g⁻¹), adaxial and abaxial stomatal densities, leaf amphistomaticity (SR), stomatal density (D_s), stable C isotope abundance ($\delta^{13}C$), nitrogen content, nitrogen-use efficiency (NUE) and total biomass (g).

correlations with WUE and $\delta^{13}\text{C}$, with negative correlations between D_s and WUE indices, and positive correlations between SR and WUE indices (Table 3). No correlations were found between either SR or D_s and A, stem volume, root-to-shoot ratio (R:S) or biomass (Table 3). Abaxial D_s is higher in *P. balsamifera* than in *P. trichocarpa* ($p < 0.0001$), with means of 174 mm^{-2} and 102 mm^{-2} , respectively; no differences were found within provenances. Interestingly, adaxial and abaxial stomatal densities were negatively correlated ($p = 0.0009$), indicating a constant total density of stomata despite differences in leaf amphistomaticity. Pearson correlations revealed a positive significant correlation between SR and SLA ($p = 0.0141$, Table 3), indicating thinner leaves (SLA is defined here as cm^2g^{-1}) with increasing leaf amphistomaticity.

Stem volume was higher in *P. balsamifera* with a mean of 5.38 cm^3 compared with 4.31 cm^3 for *P. trichocarpa* ($p = 0.0049$), and differed within provenances ($p < 0.0001$), with values ranging from $3.56\text{-}12.2 \text{ cm}^3$ and $3.17\text{-}7.82 \text{ cm}^3$ for Gillam and Carnduff, respectively; and from $1.36\text{-}9.06 \text{ cm}^3$ and $2.20\text{-}7.39 \text{ cm}^3$ for Quesnel Lake and MacMillan Island, respectively (Table 2). Leaf area was substantially larger in *P. trichocarpa* provenances with a mean of 103 cm^2 compared to 80.6 cm^2 for *P. balsamifera* ($p < 0.0001$), and differed within provenances ($p < 0.0001$). SLA was higher in *P. trichocarpa*, with a mean of $236.88 \text{ cm}^2\text{g}^{-1}$, compared with $211.78 \text{ cm}^2\text{g}^{-1}$ for *P. balsamifera* ($p < 0.0001$). SLA also differed within provenances ($p = 0.0006$) (Table 2), with the following ranges in value for each provenance: Gillam, $160\text{-}274 \text{ cm}^2\text{g}^{-1}$; Carnduff, $193\text{-}214 \text{ cm}^2\text{g}^{-1}$; Quesnel Lake, $194\text{-}293 \text{ cm}^2\text{g}^{-1}$; and MacMillan Island, $163\text{-}248 \text{ cm}^2\text{g}^{-1}$.

Resource-Use Efficiencies

Stem values for $\delta^{13}\text{C}$ were highly positively correlated with foliar values ($p < 0.0001$). Given that stem values better integrate $\delta^{13}\text{C}$ through time and space, and thus provide a much more accurate read for whole-plant WUE, the correlation between foliar and stem values lent added confidence to my reliance on foliar $\delta^{13}\text{C}$ to represent the entire tree as a surrogate for long-term water-use efficiency (Fig. 2A). Additionally, the close comparison of these two traits indicated a consistent environment in the greenhouse throughout the duration of the study, where all gas-exchange measurements were taken.

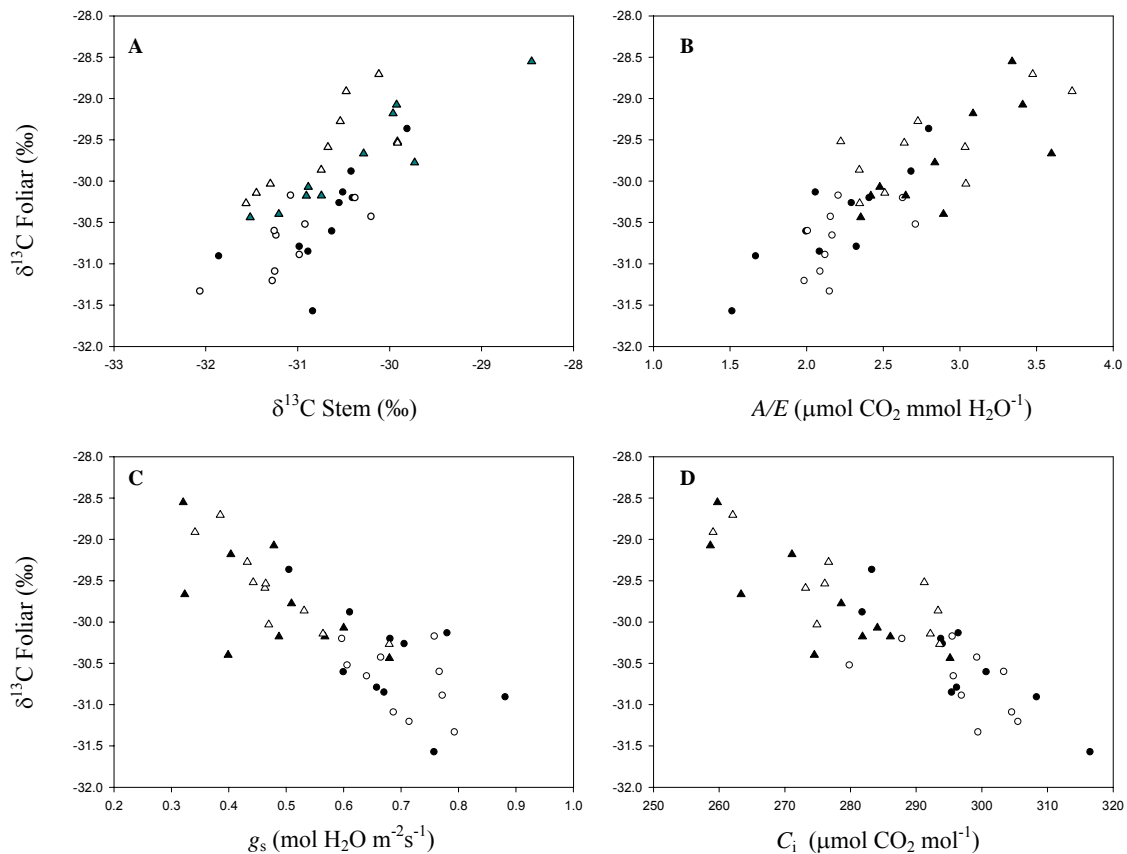


Figure 2. $\delta^{13}\text{C}$ foliar correlations with (A) $\delta^{13}\text{C}$ stem ($r=0.7333$, $p<0.0001$); (B) intrinsic water-use efficiency (A/E) ($r=0.8252$, $p<0.0001$); (C) stomatal conductance (g_s) ($r=-0.811$, $p<0.0001$); and (D) intercellular CO_2 (c_i) ($r=-0.864$, $p<0.0001$). Circles represent the *P. balsamifera* provenances (Gillam: closed, Carnduff: open) and triangles represent the *P. trichocarpa* provenances (Quesnel Lake: closed, MacMillan Island: open).

Both A/E and $\delta^{13}\text{C}$ (foliar unless otherwise noted) were tested with ANOVAs as indices of water-use efficiency. ANOVA revealed differences between species ($p < 0.0001$ for both) as well as within provenances ($p < 0.0001$ for both) (Table 2). A/E was higher and $\delta^{13}\text{C}$ less negative among the *P. trichocarpa* provenances; the mean A/E for *P. trichocarpa* was $2.86 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$, compared with $2.20 \text{ CO}_2 \text{ mmol H}_2\text{O}^{-1}$ for *P. balsamifera*, while the mean $\delta^{13}\text{C}$ for *P. trichocarpa* genotypes was -29.67‰ , compared with -30.60‰ for *P. balsamifera*. $\delta^{13}\text{C}$ was positively correlated with A/E ($p < 0.0001$), as expected (Fig. 2B). $\delta^{13}\text{C}$ was negatively correlated with both stomatal conductance (g_s) ($p < 0.0001$) and intercellular CO_2 (c_i) ($p < 0.0001$) (Table 3 and Figure 2C & D, respectively), as well as with E ($p < 0.0001$) (Table 3).

ANOVAs revealed differences for NUE within ($p = 0.0072$) but not between provenances (Table 2). Across all provenances, NUE showed no correlation with $\delta^{13}\text{C}$ or A/E , but was correlated with A ($p = 0.0353$) and SLA ($p = 0.0042$) (Table 3).

Provenance-level

Further correlation analyses carried out at the provenance level revealed some notable differences between provenances. Whereas A and c_i showed no correlation across all provenances combined (Table 3), both *P. balsamifera* provenances, Gillam ($p = 0.004$) and Carnduff ($p = 0.004$), revealed a negative correlation between A and c_i (Fig. 3). These same provenances also showed a positive correlation between A and $\delta^{13}\text{C}$, with a p-value of 0.017 for Gillam, and 0.028 for Carnduff (Fig. 4), whereas no correlation existed across provenances combined (Table 3). $\delta^{13}\text{C}$ and NUE were negatively correlated for the Quesnel Lake provenance ($p = 0.004$) and positively correlated for

Carnduff ($p=0.042$) (Fig. 5). $\delta^{13}\text{C}$ and stem volume were positively correlated for the MacMillan Island provenance ($p=0.011$) (Fig. 6).

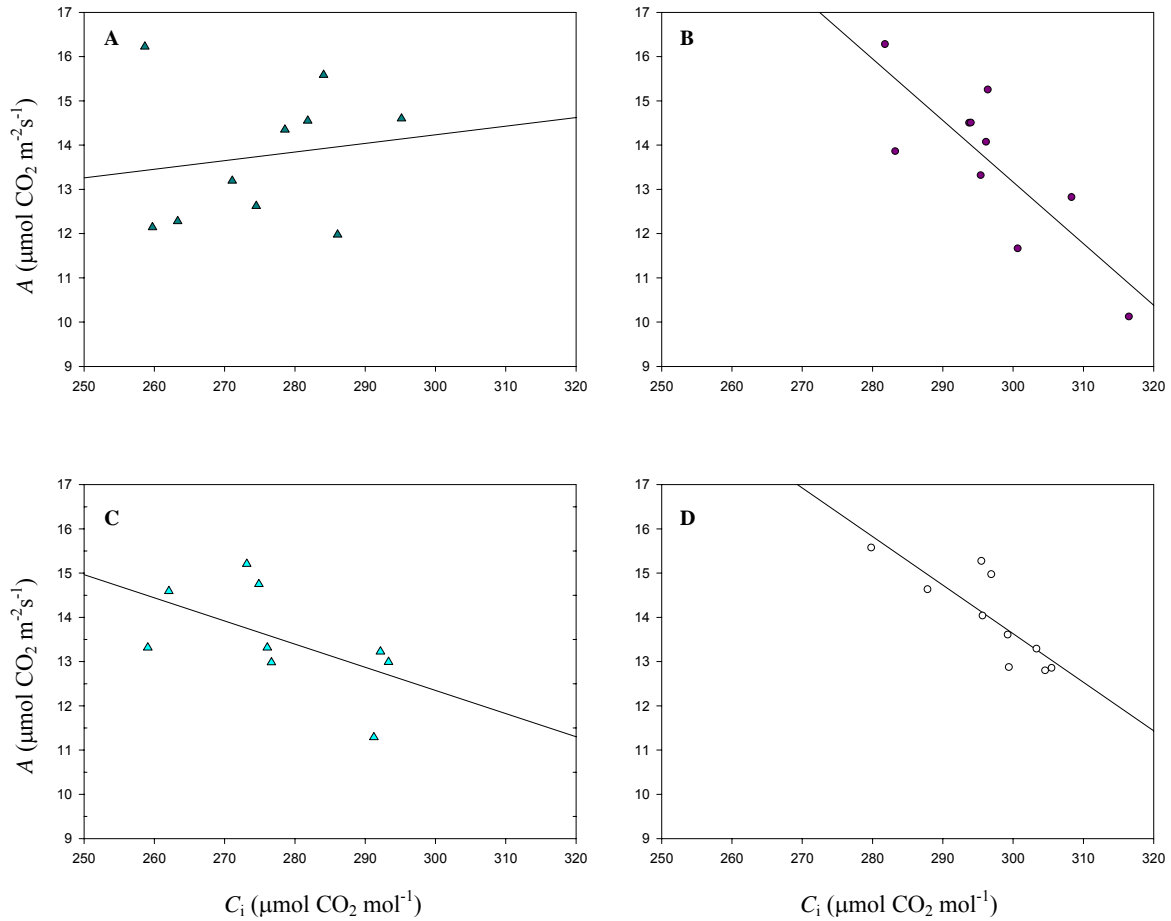


Figure 3. Net photosynthesis (A) correlations with intercellular CO_2 concentrations (c_i) for each provenance. The *P. trichocarpa* provenances are Quesnel Lake (**A**: $r=0.156$, $p=0.668$) and MacMillan Island (**C**: $r=-0.395$, $p=0.258$); the *Populus balsamifera* provenances are Gillam (**B**: $r=-0.813$, $p=0.004$) and Carnduff (**D**: $r=-0.819$, $p=0.004$).

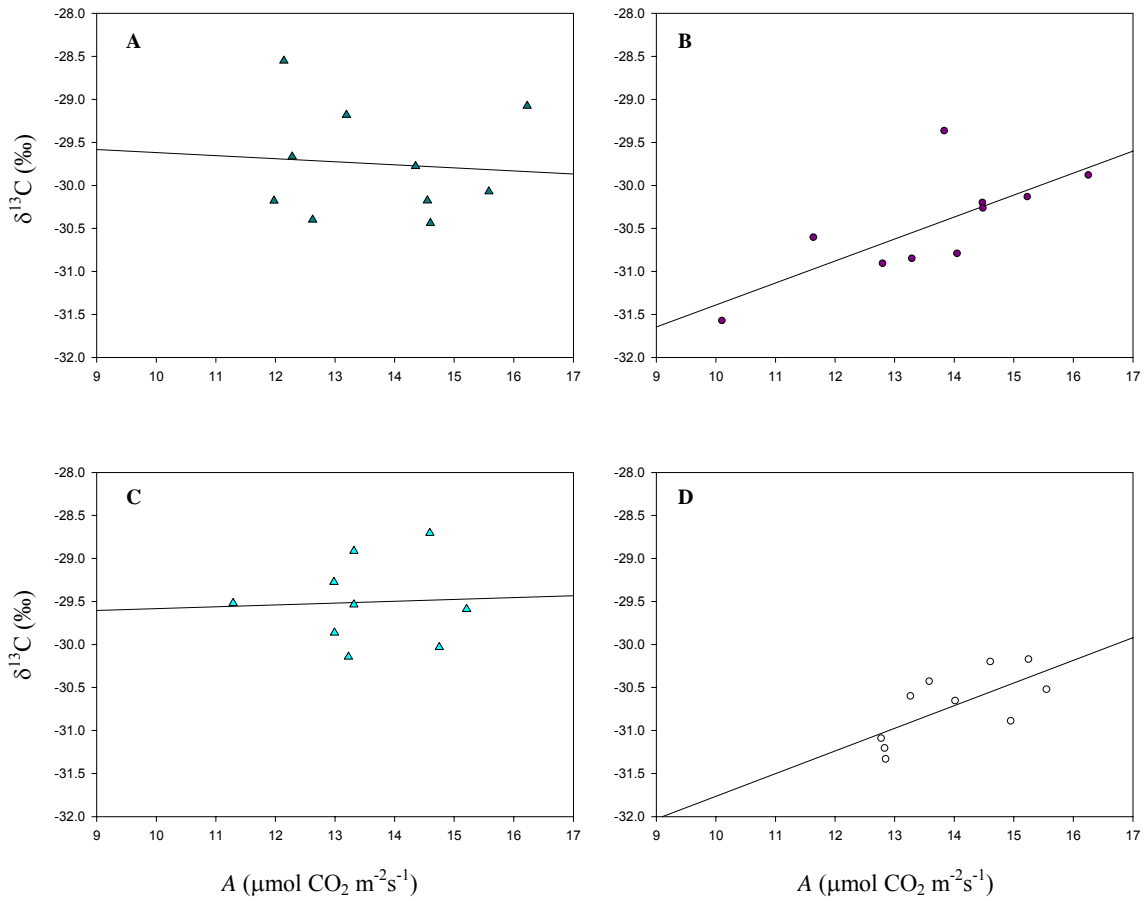


Figure 4. $\delta^{13}C$ correlations with net photosynthesis (A) for each provenance. The *P. trichocarpa* provenances are Quesnel Lake (**A**: $r = -0.086$, $p = 0.813$) and MacMillan Island (**C**: $r = -0.072$, $p = 0.843$); the *Populus balsamifera* provenances are Gillam (**B**: $r = 0.730$, $p = 0.017$) and Carnduff (**D**: $r = 0.687$, $p = 0.028$).

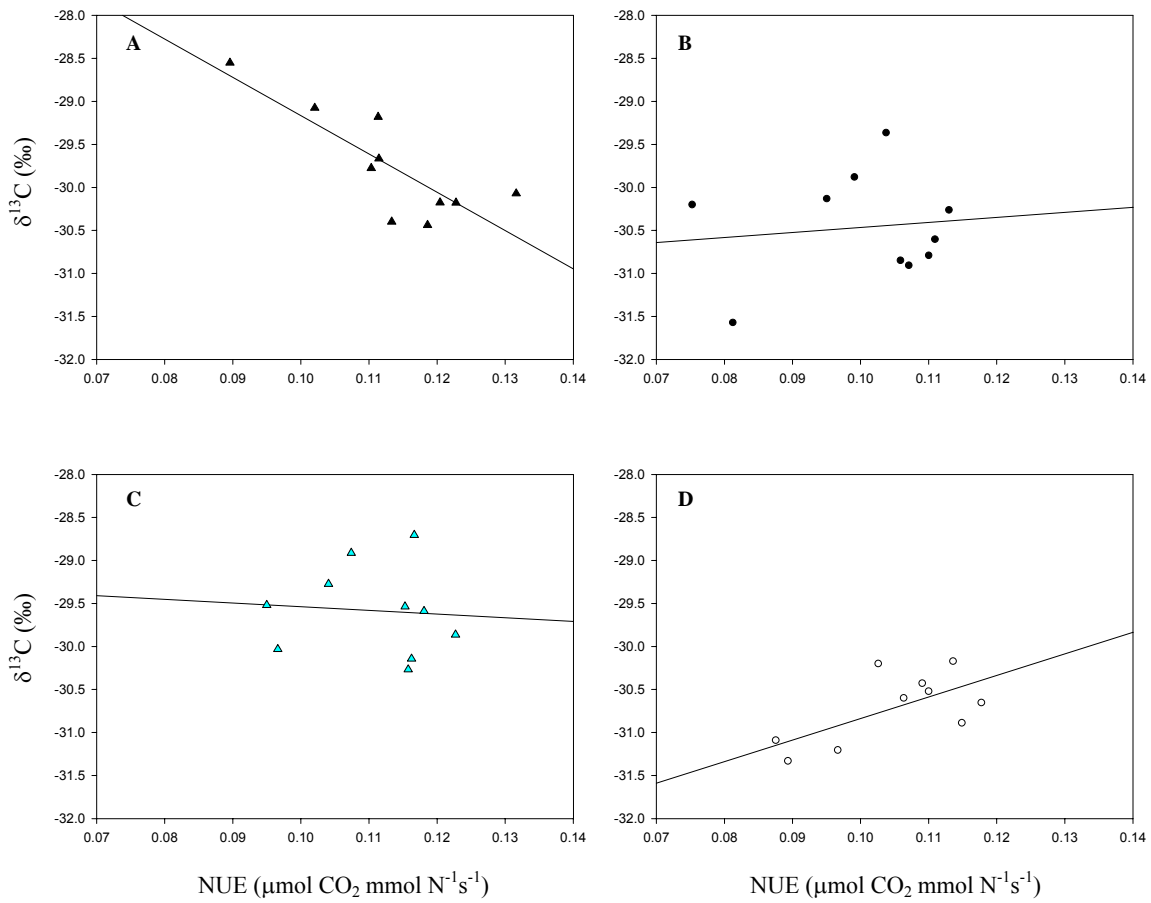


Figure 5. $\delta^{13}\text{C}$ correlations with photosynthetic NUE for each provenance. The *P. trichocarpa* provenances are Quesnel Lake (**A**: $r=-0.818$, $p=0.004$) and MacMillan Island (**C**: $r=-0.079$, $p=0.829$); the *Populus balsamifera* provenances are Gillam (**B**: $r=0.220$, $p=0.541$) and Carnduff (**D**: $r=0.650$, $p=0.042$).

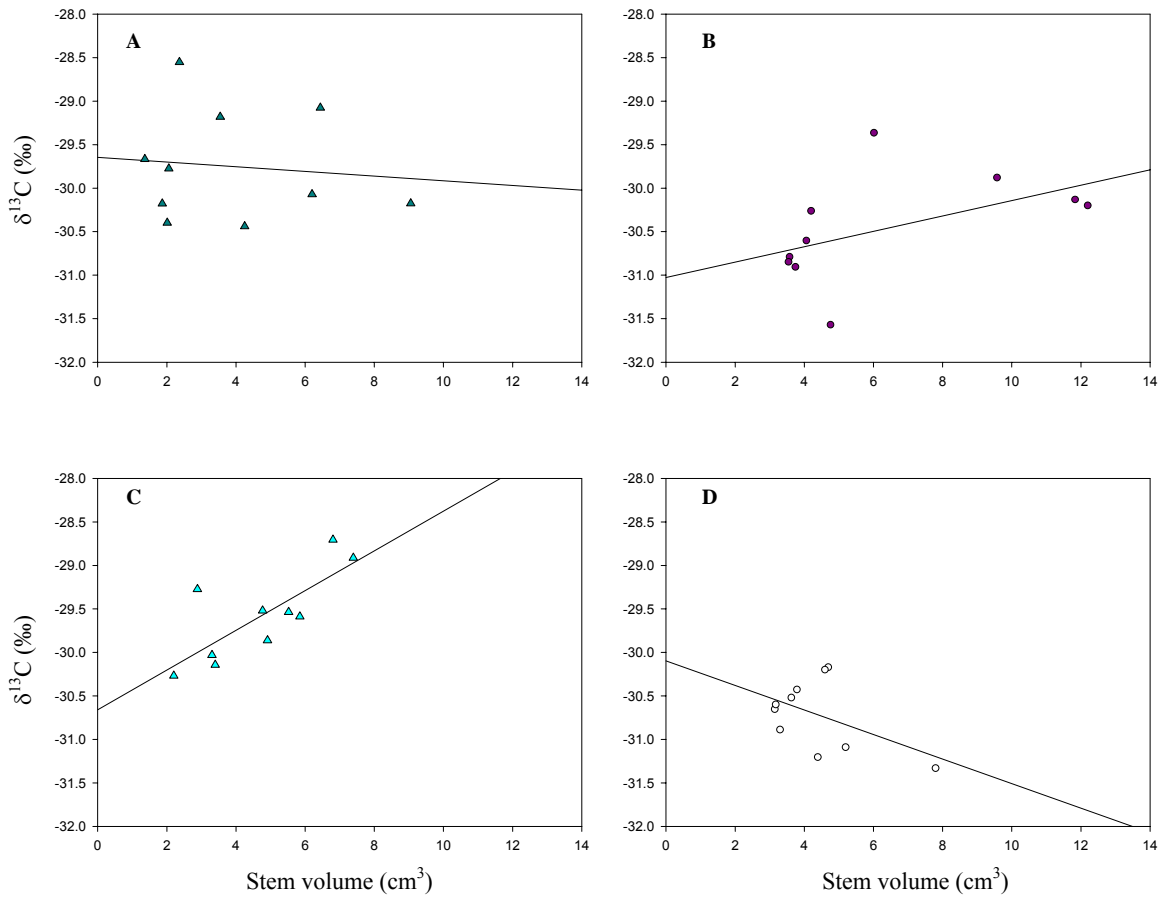


Figure 6. $\delta^{13}\text{C}$ correlations with stem volume for each provenance. The *P. trichocarpa* provenances are Quesnel Lake (**A**: $r=-0.117$, $p=0.748$) and MacMillan Island (**C**: $r=0.761$, $p=0.011$); the *Populus balsamifera* provenances are Gillam (**B**: $r=0.496$, $p=0.145$) and Carnduff (**D**: $r=-0.485$, $p=0.156$).

Figure 2A revealed possible population differences in the leaf to stem gradient in $\delta^{13}\text{C}$. A subsequent two-way analysis of variance was carried out on the differences between leaf and stem values for each provenance. The ANOVA revealed significant differences between the southern *P. trichocarpa* provenance (MCMN) and the southern *P. balsamifera* provenance (CAR), with a p-value <0.05 based on Bonferroni's adjustment (original p-value was 0.002; Bonferroni's adjustment assigned significance equal to 0.05 for $\alpha \leq 0.0083$). Means for leaf to stem differences in $\delta^{13}\text{C}$ each provenance were as follows: Quesnel Lake, -0.816‰; MacMillan Island, -1.58‰; Gillam, -1.01‰; and Carnduff, -0.636‰.

Genotype-level

Figure 7 shows the scatter plot of $\delta^{13}\text{C}$ and NUE divided into quadrants, with approximately 10 genotypes per quadrant. The plot was divided in this manner to facilitate the identification of genotypes most- and least-likely to have sink-driven WUE, in the upper-right and lower-left quadrants, respectively. Of the ten genotypes located in the upper right quadrant, five revealed high levels of net photosynthesis within their respective provenance: from MacMillan Island, MCMN4 and HRSO2; from Quesnel Lake, QAUS7 and QFRS3; and from Gillam, GIL3 (Fig. 7 and Fig. 1). Of these five, four have high *A* coupled with high biomass, relative to their respective provenance: HRSO2, MCMN4, GIL3, and QAUS7 (Fig. 8).

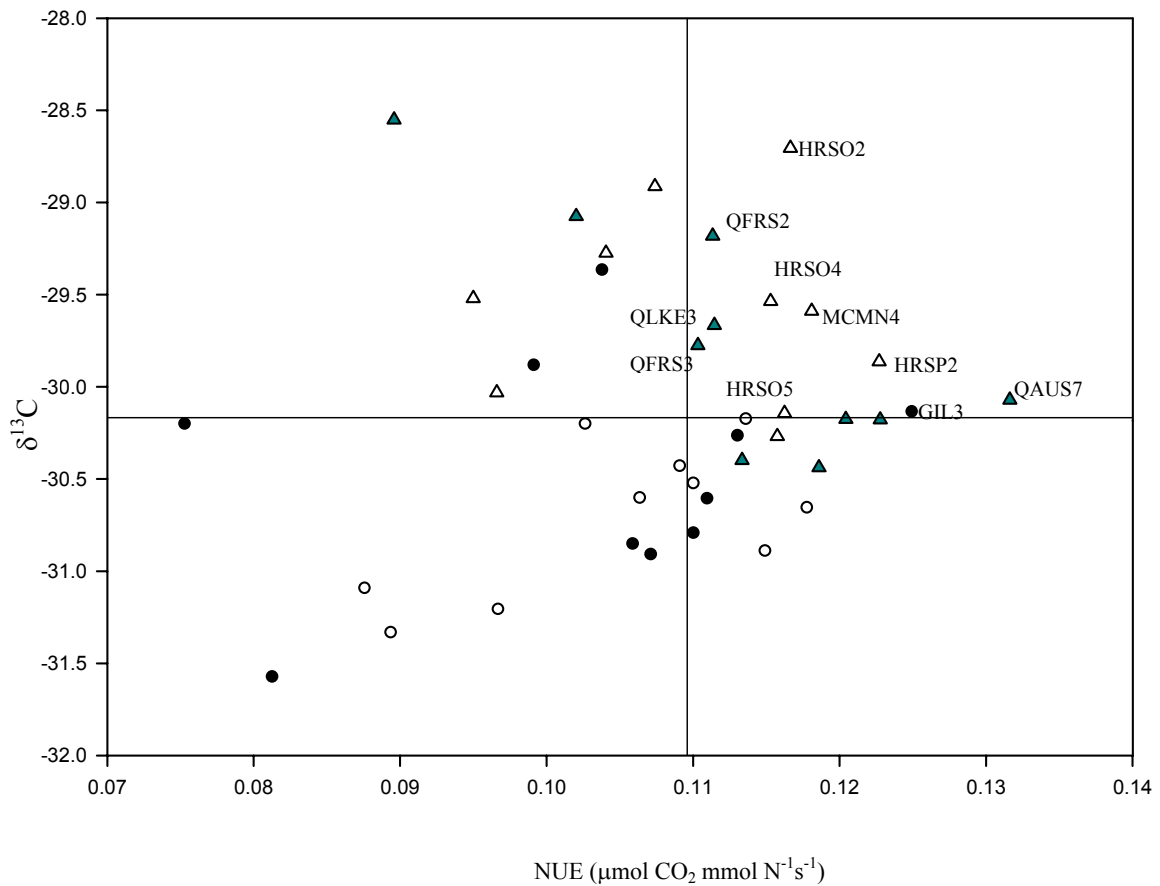


Figure 7. $\delta^{13}\text{C}$ plotted against photosynthetic NUE for all genotypes in each of the four provenances. Plot is divided into four quadrants, with each containing as close to 10 genotypes as possible. Genotypes are labeled in the upper right quadrant, indicating both high $\delta^{13}\text{C}$ and high NUE. Level of net photosynthesis for each labeled genotype can be compared to other genotypes within its provenance (see Fig. 1). The same can be done for biomass (see Fig. 8). Circles represent the *P. balsamifera* provenances (Gillam: closed, Carnduff: open) and triangles represent the *P. trichocarpa* provenances (Quesnel Lake: closed, MacMillan Island: open).

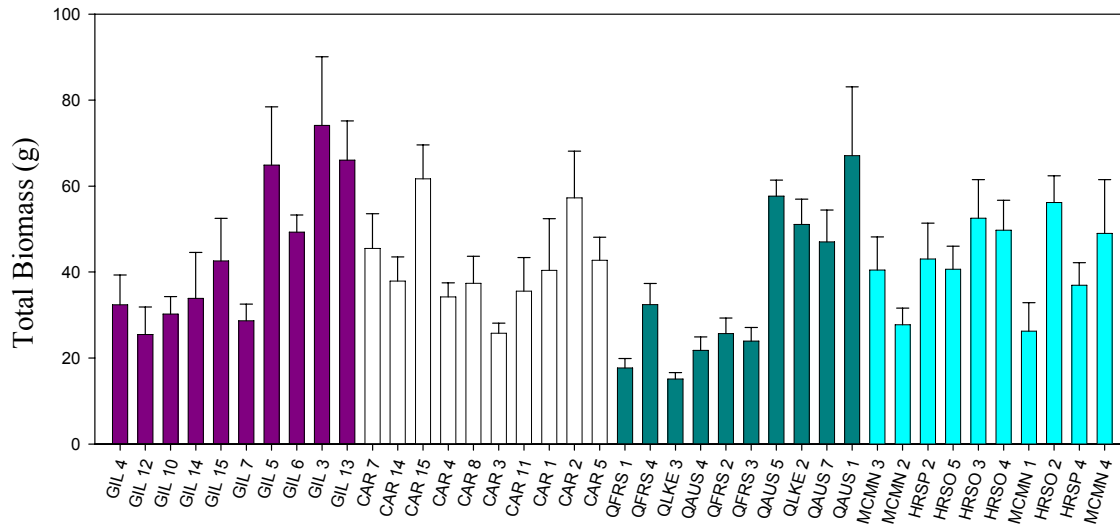


Figure 8. Fresh total biomass (g) for all genotypes within provenances. Gillam (GIL) and Carnduff (CAR) represent the northern and southern *Populus balsamifera* provenances, respectively. Quesnel Lake (QFRS, QLKE, QAUS) and MacMillan Island (MCMN, HRSP, HRSO) represent the northern and southern *P. trichocarpa* provenances, respectively. Genotypes within each provenance are sorted according to increasing net photosynthesis. For comparison with net photosynthesis measurements for each genotype, see Figure 1.



Illustration 1. Gas-exchange measurements using the LICOR 6400.

DISCUSSION

Water-Use Efficiency

The two stable carbon isotopes, ^{13}C and ^{12}C , respectively make up 1.11% and 98.89% of the atmospheric carbon pool (Griffiths 1993), giving the atmosphere a more negative isotopic composition relative to the standard Vienna-PDB (Coplen 1994, 1995). Carbon isotopic composition of C_3 plants is more negative than that of the atmosphere due to discrimination against the heavier of the two isotopes during diffusion through the stomata and at the sites of carboxylation, thus giving C_3 plants isotopic compositions ranging from -30 to -22‰ (Farquhar 1982). These values fluctuate with the level of internal CO_2 partial pressure (c_i), becoming more negative, and thus less rich in ^{13}C , as c_i increases. Given the direct link between $\delta^{13}\text{C}$ values and c_i , and between c_i and WUE, where WUE is defined as the ratio of C assimilation to transpiration (A/E), C isotope composition is commonly used as a surrogate index for plant water-use efficiency (Farquhar et al. 1982, 1989; Griffiths 1993). WUE and $\delta^{13}\text{C}$ across all genotypes were highly correlated in my results (Fig. 2B); as an additional precaution, $\delta^{13}\text{C}$ foliar values were compared with $\delta^{13}\text{C}$ stem values, to ensure accuracy. High correlation (Fig. 2A) here as well lent further confidence to my use of $\delta^{13}\text{C}$ foliar values throughout this paper as a measure of WUE.

High WUE results either from decreased stomatal conductance accompanied by decreased growth, or from increased photosynthetic capacity followed by increased

productivity (Gornall & Guy 2007; Condon et al. 2002). Highly significant correlation between $\delta^{13}\text{C}$ and stomatal conductance across populations ($p < 0.0001$, Table 3) suggests variations in WUE caused primarily by variations in stomatal conductance. This is consistent with previous work done on *Populus trichocarpa* Torr. & A. Gray \times *Populus deltoides* (Rae et al. 2004), *Populus deltoides* \times *Populus nigra* (Monclus et al. 2005), and *Populus trichocarpa* (Gornall & Guy 2007). However, for WUE to be primarily linked to variations in stomatal conductance, I would expect to find complementary negative correlations between $\delta^{13}\text{C}$ and net photosynthesis, stem volume and/or biomass; no such correlations were found (Table 3), suggesting a lack of definitive association between WUE and variations in either stomatal conductance or photosynthetic capacity across all populations in this study. Others have found positive correlation between growth and WUE in poplar species (Voltas et al. 2006), suggesting a clear link between WUE and photosynthetic capacity. In conifer species, positive correlations between growth traits and $\delta^{13}\text{C}$ values have consistently been found, suggesting WUE determined primarily by variations in photosynthetic capacity for these trees (Sun et al. 1996; Guy & Holowachuk 2001; Silim et al. 2001; Major et al. 2007). Benowicz et al. (2001) found that differences in intrinsic WUE in paper birch were due to variations in net photosynthesis, and not stomatal conductance, whereas Wang et al. (1998) attributed higher WUE in paper birch (*Betula papyrifera*) to decreased g_s . Whereas Gornall & Guy (2007) found links between gas-exchange traits and growth in *P. trichocarpa*, the absence of correlation between net photosynthesis and g_s or c_i in this present study further implies complete independence of growth from WUE across species and provenances (Table 3). The same results were found for studies done on *Populus deltoides* \times *Populus nigra* clones (Marron et al. 2005;

Monclus et al. 2005). As suggested by Monclus et al. (2006), this could have its advantages in a breeding program, as it would allow for the independent selection of both high WUE and high productivity; normal fluctuations in WUE and g_s , in this case, should not affect growth.

Significant correlations between A and both biomass and stem volume across all genotypes in this study suggest that more productive genotypes could potentially be identified based on leaf photosynthesis, and independent of WUE. Measures of leaf photosynthesis using gas-exchange instruments have recently been shown to be reliable in predicting relative growth rate in a wide variety of tree species (Kruger & Volin 2006), although others have found no link between leaf photosynthesis or net assimilation rate and biomass accumulation (Warren & Adams 2005). Whereas differences in A in this study are not significant between species, *P. balsamifera* revealed a higher average stem volume overall (5.38 cm³ vs. 4.31 cm³ in *P. trichocarpa*). This may simply be due to differences in resource allocation, given the lack of difference in total fresh biomass (not shown), and the greater R:S in *P. trichocarpa* (Table 2). Greater growth in *P. balsamifera* could also be linked to a shorter growing season (fewer frost-free days: average of 151 for *P. balsamifera* vs. 245 for *P. trichocarpa*; see Table 1), as suggested by previous work done on *Populus* species (Gornall & Guy 2007).

Several other trends were revealed at the species and genotype levels. Positive correlations were found between $\delta^{13}\text{C}$ and A in both *P. balsamifera* provenances (Fig. 4), together with negative correlations between A and c_i (Fig. 3). No correlations were found between these traits in either of the *P. trichocarpa* provenances. These results present potentially more complex relationships between WUE and growth for *P.*

balsamifera, suggesting that high WUE may result from a combination of lower g_s and increased photosynthetic capacity for the Gillam and Carnduff provenances. The non-significant yet negative trend between $\delta^{13}\text{C}$ values and stem volume for the Carnduff provenance (Fig. 6) leaves this possibility unresolved for that provenance specifically. For Gillam, although correlation between $\delta^{13}\text{C}$ values and stem volume is not significant, it is positive (Fig. 6), thus making this provenance the most consistent across all gas-exchange and growth trait correlations, in terms of meeting expectations for sink-driven WUE. The southern *P. trichocarpa* provenance, MacMillan Island, may also have partially sink-driven WUE, given the positive correlation between C isotope composition and stem volume ($p=0.011$, Fig. 6) found for this provenance, however trends across gas-exchange and growth traits are not consistent and remain somewhat inconclusive.

Nitrogen-Use Efficiency

Positive correlations between nitrogen content and net photosynthesis in C_3 plants are widely recognized (Evans 1989), and have been identified in a variety of gymnosperm (Field et al 1983; Robinson et al. 2001; Major et al. 2007) and angiosperm species (Sobrado 1991), including *Populus trichocarpa* (Gornall & Guy 2007). A and leaf N density were positively correlated in this study across all genotypes ($p=0.008$, Table 3), although at the species level, only *P. trichocarpa* revealed a positive $A \times N$ correlation ($p=0.013$, not shown), while *P. balsamifera* showed a positive yet non-significant correlation ($p=0.152$, not shown). Photosynthetic nitrogen-use efficiency is defined as the ratio of net photosynthesis to N content per unit leaf area (Larcher 2003). The expected positive linear relationship between photosynthetic capacity and nitrogen

content (Larcher 2003) implies a possible inherent trade-off between WUE and NUE, given that any increase in c_i (leading to increased NUE) will lead to more negative $\delta^{13}\text{C}$ values and lower WUE (Patterson et al. 1997). This is consistent with studies done on various evergreen species (Field et al. 1983), white spruce (Livingston et al. 1999), and paper birch (Wang et al. 1998), although others found an absence of intrinsic trade-off between WUE and NUE for both white and black spruce (Patterson et al. 1997). In times of drought especially, a tendency in plants to increase stomatal closure will simultaneously increase WUE and decrease NUE, as shown in hybrid willow (Weih et al. 2006). My results revealed no correlation, and thus no obvious trade-off, between $\delta^{13}\text{C}$ and photosynthetic NUE across all genotypes. At the species level, however, *P. trichocarpa* revealed a negative correlation between these two traits ($p=0.016$, not shown), thus indicating a probable trade-off in resource use. This trend is consistent with the positive correlation between A and N here as well. The *P. balsamifera* provenances, Gillam and Carnduff, were not significantly correlated, though revealed a slightly positive r-value of 0.16, coupled with no apparent correlation between A and N, as mentioned previously. ANOVA revealed no significant differences in NUE at the species level between *P. balsamifera* and *P. trichocarpa* (Table 2), but higher WUE for *P. trichocarpa*, suggesting that these opposing trends separating the two species are likely due to differences in WUE. Having tested only two provenances for each species, however, makes it impossible to draw definitive conclusions here. Further examination of $\delta^{13}\text{C} \times \text{NUE}$ correlations among the four tested provenances revealed a significant negative correlation for the Quesnel provenance ($p=0.004$, *P. trichocarpa*), and a positive correlation for Carnduff ($p=0.042$, *P. balsamifera*: Fig. 5).

NUE and WUE Trade-off

The presence of this trade-off, or lack thereof, opens up another avenue by which to better manage the goals of a potential hybridization program. High productivity in poplars is commonly associated with high water availability (Monclus et al. 2006). It is possible that apparent trade-offs in resource-use efficiencies among certain species are linked to water availability, and that trees grown under ideal watering schedules would be more likely to show little or no trade-off between WUE and NUE, as suggested by Weih et al. (2006) in an experiment using different watering regimes conducted on hybrid willow. This may be the case for *P. balsamifera* and *P. trichocarpa*, where WUE appears more closely associated with variations in stomatal conductance than with variations in photosynthetic capacity. However, unless WUE were primarily sink-driven (see Voltas et al. 2006, discussed earlier), growth would likely be compromised as a result of drought and, subsequently, prolonged reduced stomatal conductance (Monclus et al. 2006; Zhang et al. 2004). Current literature is lacking in studies addressing correlations, or lack thereof, between WUE and NUE, and the implications of such links on growth and productivity, specifically in poplar species and their hybrids. Further work would need to be done with *P. balsamifera* and *P. trichocarpa* to determine the effects of drought on both WUE and NUE.

Considerations for future work

This study admittedly encompasses only a small percentage of available plant material for the species and provenances presented herein. More extensive work including a greater number of provenances would be very useful in more accurately determining trends within species. On a provenance level, a more thorough study of Gillam genotypes would be especially interesting, given the trends revealed within that particular provenance. Studies addressing the link, or lack thereof, between WUE and NUE in tree species, and especially in poplars, are currently lacking in the literature. The findings in this study suggest the possibility of identifying poplar genotypes with an absence of trade-off between WUE and NUE, notably among genotypes from the northern *P. balsamifera* provenance, near Gillam. It would be interesting to survey a wider selection of *P. balsamifera* provenances, in an attempt to determine whether the trends seemingly present in the GIL provenance are consistently present across *P. balsamifera* populations.

The experimental design for this work was meant to explore differences within provenances primarily, and was in fact successful to that end, revealing large differences for a variety of traits. However, definitive statements at both provenance and species levels can hardly be made here. Comparisons of northern versus southern provenances, for example, would likely reveal more accurate results given a clearer separation between northern and southern provenances, and also given more similar latitudes for each end. For this study, QL was defined as the northern provenance for *P. trichocarpa*, despite being located quite a bit further south than Gillam, Manitoba, the location of the northern provenance for *P. balsamifera* (Table 1). It would be worthwhile to acquire plant

material from a *P. trichocarpa* provenance located at latitudes as similar to those of the GIL trees as possible; such material was not available for this study. This would allow further inquiry into previous findings that populations of poplar species with shorter growing seasons assimilate biomass at a faster rate (Gornall & Guy 2007), and would allow comparisons to be made between the two species in question here.

Follow-up work with these poplar species should include a more in-depth analysis of the underlying causes of leaf to stem differences in $\delta^{13}\text{C}$. Such work might reveal differences in C allocation at leaf and stem levels, as well as possibly identify provenances with photosynthetic bark, among other explanations. Further studies focused on leaf amphistomaticity for both species would also likely prove interesting, and hopefully shed some light on links between leaf morphology, stomatal density and gas-exchange parameters. Further analysis may show some links between transfer conductance processes (see Warren 2006) and positive correlations between WUE and SR.

The inclusion of gibberellin (GA) content analyses in further studies would likely be quite telling as well. All harvested stems from this study were freeze-dried and set aside for such analysis, to be carried out as soon as possible. Unfortunately, results could not be included in this paper due to time constraints. Growth and productivity have been linked with concentrations of endogenous GAs in various plant species (Kende and Zeevaart 1997; Rood et al. 1988) including poplars (Pharis et al. 1991; Eriksson et al. 2000). A better understanding of the role of GAs in poplar growth and productivity, and their potential link to other physiological traits, would lend additional insight and guidance to tree breeding programs and hybridization efforts intent on creating the ideal

poplar trees for biofuel production, carbon sequestration, or other uses linked to climate change.

CONCLUSION

The original driving motivation behind this study was to explore and ultimately identify ideal candidates for future hybridization of *P. balsamifera* and *P. trichocarpa* genotypes, in the hopes of developing a hybrid poplar that would thrive in continental Canada. In order to begin identifying which genotypes would fit the goals of such a breeding program, including frost and drought tolerance, rapid growth and high productivity, I took into account WUE, NUE and productivity first and foremost. Individual genotypes displaying both high WUE and high NUE, together with high productivity, would likely have at least partially sink-driven WUE, and thus would be less likely to compromise growth in times of moderate water stress. A scatter plot showing the relationship between WUE and NUE across all genotypes combined has the upper right quadrant occupied mostly by genotypes from the Quesnel Lake and MacMillan Island provenances (Fig. 7), with Quesnel Lake displaying a likely trade-off between WUE and NUE (Fig. 5A). However, genotypes located in the upper right quadrant are the most likely individuals among the four studied provenances to have both high WUE and high NUE, especially if they also display high productivity. Of the ten individual genotypes most likely to combine high WUE with high NUE (Fig. 7), five also displayed high photosynthesis relative to their provenance: HRSO2, MCMN4, QFRS3, GIL3, and QAUS7 (Fig. 1). Of these five, four have high *A* coupled with high biomass, relative to their respective provenance: HRSO2, MCMN4, GIL3, and QAUS7

(Fig. 8), making these trees excellent candidates for further study. However, while the *P. trichocarpa* provenances have more genotypes in this category, if the goal is to create crosses between *P. trichocarpa* and *P. balsamifera* genotypes, then genotypes in the upper right quadrant of each provenance's $\delta^{13}\text{C} \times \text{NUE}$ scatter plot should be considered (see Fig. 5), based on their productivity and net photosynthesis levels relative to other genotypes within the same provenance. Gillam genotypes appear more promising than Carnduff as *P. balsamifera* candidates for hybridization, given provenance-level correlations most consistent with what would be expected for sink-driven WUE.

Canada, the United States and the Russian Federation currently claim the largest naturally occurring stands of poplars and willows (Ball et al. 2005). Natural poplar stands occupy an estimated 28.3 million hectares in Canada alone (Ball et al. 2005; Volney et al. 2005), with *Populus balsamifera* (balsam poplar), *Populus trichocarpa* (black cottonwood), and *Populus tremuloides* (trembling aspen) being the most widespread and naturally successful among the native species of the country (Farrar 1995). Given that the majority of agricultural lands available for afforestation programs in Canada are located in the prairie provinces, Canadian hybridization efforts must take frost- and drought-hardiness into account, together with rapid growth and productivity. The most appropriate target species for such programs would very likely be among the three most naturally prevalent in Canada.

BIBLIOGRAPHY

- Ball, J., Carle, J., and Del Lungo, A. 2005. Contribution of poplars and willows to sustainable forestry and rural development. *Unasylva* **221**: 3-9.
- Benowicz, A., Guy, R., Carlson, M.R., and El-Kassaby, Y.A. 2001. Genetic variation among paper birch (*Betula papyrifera* MARSH.) populations in germination, frost hardiness, gas exchange and growth. *Silvae Genetica* **50**: 7-13.
- Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential –are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* **56**: 1159-1168.
- Bradshaw, H.D., Jr., Ceulemans, R., Davis, J., and Stettler, R. 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. *Journal of Plant Growth Regulation* **19**: 306-313.
- Bungart, R. and Hüttl, R.F. 2004. Growth dynamics and biomass accumulation of 8-year-old hybrid poplar clones in a short-rotation plantation on a clayey-sandy mining substrate with respect to plant nutrition and water budget. *European Journal of Forest Research* **123**: 105-115.
- Bunn, S.M., Rae, A.M., Herbert, C.S., and Taylor, G. 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. *Forestry* **77**: 307-323.

- Caemmerer, von S. and Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376-387.
- Cannel, M.G.R. 1999. Growing trees to sequester carbon in the UK: answers to some common questions. *Forestry* **72**: 237-247.
- Ceulemans, R. and Deraedt W. 1999. Production physiology and growth potential of poplars under short-rotation forestry culture. *Forest Ecology and Management* **121**: 9-23.
- Clavijo, M. del Pilar, Nordenstahl, M., Gundel, P.E., Jobbagy, E.G. 2005. Poplar afforestation effects on grassland structure and composition in the flooding pampas. *Rangeland Ecology Management* **58**: 474-479.
- Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D. 2002. Improving intrinsic water-use efficiency and crop yield. *Crop Science* **42**: 122-131.
- Coplen, T.B. 1994. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. *Pure & Applied Chemistry* **66**: 273-276.
- Coplen, T.B. 1995. Discontinuance of SNOW and PDB. *Nature* **375**: 285.
- Cowie, A., Schneider, U.A., Montanarella, L. 2007. Potential synergies between existing multilateral environmental agreements in the implementation of land use, land-use change and forestry activities. *Environmental Science & Policy* **10**: 335-352.
- Del Galdo, I., Six, J., Peressotti, A., Cotrufo, M.F. 2003. Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. *Global Change Biology* **9**: 1204-1213.

- Eriksson, M.E., Israelsson, M., Olsson, O., and Moritz, T. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nature Biotechnology* **18**: 784-788.
- Evans, J. 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* **78**: 9-19.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503-537.
- Farquhar, G.D., O'Leary, M.H., Berry, J.A. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121-137.
- Farrar, J.L. 1995. *Trees in Canada*. Fitzhenry & Whiteside Limited, Markham, Ontario:
- Field, C., Merino, J., and Mooney, H.A. 1983. Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia* **60**: 384-389.
- Garten, C.T. Jr. 2002. Soil carbon storage beneath recently established tree plantations in Tennessee and South Carolina, USA. *Biomass and Bioenergy* **23**: 93-102.
- Gielen, B. and Ceulemans, R. 2001. The likely impact of rising atmospheric CO₂ on natural and managed *Populus*: a literature review. *Environmental Pollution* **115**: 335-358.
- Gornall, J.L. and Guy, R.D. 2007. Geographic variation in ecophysiological traits of black cottonwood (*Populus trichocarpa*). *Canadian Journal of Botany* **85**: 1202-1213.

- Griffiths, H. 1993. Carbon isotope discrimination. *In* Photosynthesis and Production in a Changing Environment: a field and laboratory manual. *Edited by* Hall, D.O., Scrulock, J.M.O., Bolhar-Nordenkamp, H.R., Leegood, R.C., and Long, S.P. Chapman & Hall, London. Pp. 181-192.
- Grigal, D.F. and Berguson, W.E. 1998. Soil carbon changes associated with short-rotation systems. *Biomass and Bioenergy* **14**: 371-377.
- Guo, L.B. and Gifford, R.M. 2002. Soil Carbon sequestration and land-use changes: a meta-analysis. *Global Change Biology* **8**: 345-360.
- Guy, R.D. and Holowachuk, D.L. 2001. Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. Ex Loud.: relationship to environment, climate of origin, and growth potential. *Canadian Journal of Botany* **79**: 274-283.
- Guy, R.D., Pharis, R.P., Aitken, S.N., Zhang, R., and Fung, L. Finding the best trees for the job: realizing the full potential of afforestation in Canada. Proceedings of Canadian Nuclear Society Climate Change 2: Canadian Technology Development, Toronto, 3-5 October 2001.
- Hall, D.O. and House, J.I. 1995. Biomass: a modern and environmentally acceptable fuel. *Solar Energy Materials and Solar Cells* **38**: 521-542.
- Hall, R.J., Joss, B.N., Sidders, D.M., and Keddy, T.J. 2005. The FAACS backcast: Afforestation activity in the Prairie Provinces (1990-2002). *The Forestry Chronicle* **80**: 727-735.
- Israelsson, M., Eriksson, M.E., Hertzberg, M., Aspeborg, H., Nilsson, P., and Moritz, T. 2003. Changes in gene expression in the wood-forming tissue of transgenic hybrid aspen with increased secondary growth. *Plant Molecular Biology* **52**: 893-903.

- Jansson, S. and Douglas, C.J. 2007. *Populus*: A model system for plant biology. Annual Review of Plant Biology **58**: 435-458.
- Kende, H. and Zeevaart, J.A.D. 1997. The five “classical” plant hormones. The Plant Cell **9**: 1197-1210.
- Kruger, E.L. and Volin, J.C. 2006. Reexamining the empirical relation between plant growth and leaf photosynthesis. Functional Plant Biology **33**: 421-429.
- Larcher, W. 2003. Physiological Plant Ecology, Fourth edition. Springer, New York.
- Laureysens, I., Pellis, A., Willems, J., and Ceulemans, R. 2005. Growth and production of a short rotation coppice culture of poplar. III. Second rotation results. Biomass and Bioenergy **29**: 10-21.
- Lemus, R. and Lal, R. 2005. Bioenergy crops and carbon sequestration. Critical Reviews in Plant Sciences **24**: 1-21.
- Livingston, N.J., Guy, R.D., Sun, Z.J., and Ethier, G.J. 1999. The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant, Cell and Environment **22**: 281-289.
- Major, J.E., Mosseler, A., Barsi, D.C., and Campbell, M. 2007. Comparative nutrient economy, stable isotopes, and related adaptive traits in *Picea rubens*, *Picea mariana*, and their hybrids. Trees **21**: 677-692.
- Mann, L. and Tolbert, V. 2000. Soil sustainability in renewable biomass plantings. Ambio **29**: 492-498.
- Marron, N., Villar, M., Dreyer, E., Delay, D., Boudouresque, E., Petit, J.M., Delmotte, F.M., Guehl, J.M., and Brignolas, F. 2005. Diversity of leaf traits related to

- productivity in 31 *Populus deltooides* × *Populus nigra* clones. *Tree Physiology* **25**: 425-435.
- McKenney, D.W., Yemshanov, D., Fox, G., Ramlal, E. 2004. Cost estimates for carbon sequestration from fast growing poplar plantations in Canada. *Forest Policy and Economics* **6**: 345-358.
- Monclus, R., Dreyer, E., Delmotte, F.M., Villar, M., Delay, D., Boudouresque, E., Petit, J.M., Marron, N., Bréchet, C., and Brignolas, F. 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltooides* × *P. nigra* clones. *New Phytologist* **167**: 53-62.
- Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.M., Barbarous, C., Le Thiec, D., Bréchet, C., and Brignolas, F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltooides* × *Populus nigra*. *New Phytologist* **169**: 765-777.
- Natural Resources Canada. Afforestation Facts of the Prairie Provinces [online]. Available from <http://www.poplar.ca/pdf/afforfacte.pdf> [accessed 3 December 2007].
- Patterson, T.B., Guy, R.D., and Dang, Q.L. 1997. Whole-plant nitrogen- and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. *Oecologia* **110**: 160-168.
- Paul, K.I., Polglase, P.J., Nyakuengama, J.G., Khanna, P.K. 2002. Change in soil carbon following afforestation. *Forest Ecology and Management* **168**: 241-257.
- Paul, K.I., Polglase, P.J., Richards, G.P. 2003. Predicted change in soil carbon following afforestation or reforestation, and analysis of controlling factors by

- linking a C accounting model (CAMFor) to models of forest growth (3PG), litter decomposition (GENDEC) and soil C turnover (RothC). *Forest Ecology and Management* **177**: 485-501.
- Pearce, D.W., Rood, S.B., and Wu, R. 2004. Phytohormones and shoot growth in a three-generation hybrid poplar family. *Tree Physiology* **24**: 217-224.
- Pellis, A., Laureysens, I., and Ceulemans, R. 2004. Growth and production of a short rotation coppice culture of poplar I. Clonal differences in leaf characteristics in relation to biomass production. *Biomass and Bioenergy* **27**: 9-19.
- Peterson, E.B., Bonnor, G.M., Robinson, G.C., and Peterson, N.M. 1999. Carbon sequestration aspects of an afforestation program in Canada's prairie provinces. Submitted to the Joint Forest Sector Table/Sinks Table, National Climate Change Process. Nawitka Renewable Resources Consultants Ltd. Victoria, British Columbia.
- Pharis, R.P., Yeh, F.C., and Dancik, B.P. 1991. Superior growth potential in trees – what is its basis, and can it be tested for at an early age. *Canadian Journal for Forest Research* **21**: 368-374.
- Povellato, A., Bosello, F., and Giupponi, C. 2007. Cost-effectiveness of greenhouse gases mitigation measures in the European agro-forestry sector: a literature survey. *Environmental Science & Policy* **10**: 474-490.
- Pritchard, E.S. and Guy, R.D. 2005. Nitrogen isotope discrimination in white spruce fed with low concentrations of ammonium and nitrate. *Trees* **19**: 89-98.

- Rae, A.M., Robinson, K.M., Street, N.R., and Taylor, G. 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. *Canadian Journal of Forest Research* **34**: 1488-1498.
- Raskin, I. and Kende, H. 1984. Role of Gibberellin in the growth response of submerged deep water rice. *Plant Physiology* **76**: 947-950.
- Ripullone, F., Lauteri, M., Grassi, G., Amato, M., Borghetti, M. 2004. Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus × euroamericana*; a comparison of three approaches to determine water-use efficiency. *Tree Physiology* **24**: 671-679.
- Robinson, D.E., Wagner, R.G., Bell, F.W., and Swanton, C.J. 2001. Photosynthesis, nitrogen-use efficiency, and water-use efficiency of jack pine seedlings in competition with four boreal forest plant species. *Canadian Journal of Forest Research* **31**: 2014-2025.
- Rood, S.B., Buzzell, R.I., Mander, L.N., Pearce, D., and Pharis, R.P. 1988. Gibberellins: a phytohormonal basis for heterosis in maize. *Science* **241**: 1216-1218.
- Sage, R.B. 1998. Short rotation coppice for energy: toward ecological guidelines. *Biomass and Bioenergy* **15**: 39-47.
- Sauter, M. and Kende, H. 1992. Gibberellin-induced growth and regulation of the cell division cycle in deepwater rice. *Planta* **188**: 362-368.
- Silim, S.N., Guy, R.D., Patterson, T.B., and Livingston, N.J. 2001. Plasticity in water-use efficiency in *Picea sitchensis*, *P. glauca* and their natural hybrids. *Oecologia* **128**: 317-325.

- Sobrado, M.A. 1991. Cost-benefit relationships in deciduous and evergreen leaves of tropical dry forest species. *Functional Ecology* **5**: 608-616.
- Sun, Z.J., Livingston, N.J., Guy, R.D., and Ethier, G.J. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant, Cell and Environment* **19**: 887-894.
- Taylor, G. 2002. *Populus*: Arabidopsis for forestry. Do we need a model tree? *Annals of Botany* **90**: 681-689.
- Tolbert, V.R., Todd, D.E. Jr, Mann, L.K., Jawdy, C.M., Mays, D.A., Malik, R., Bandaranayake, W., Houston, A., Tyler, D., Pettry, D.E. 2002. Changes in soil quality and below-ground carbon storage with conversion of traditional agricultural crop lands to bioenergy crop production. *Environmental Pollution* **116**: S97-S106.
- Tuskan, G.A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., and Bhalerao, R.R. et al. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596-1604.
- Tuskan, G.A., Gunter, L.E., Yang, Z.M.K., Yin, T.M., Sewell, M.M., and DiFazio, S.P. 2004. Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. *Canadian Journal of Forest Research* **34**: 85-93.
- Vanden Broek, A., Villar, M., Van Bockstaele, E., and Van Slycken, J. 2005. Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences for native poplar populations. *Annals of Forest Science* **62**: 601-613.

- Van Kooten, G.C., Krcmar-Nozic, E., Stennes, B., and Van Gorkom, R. 1999. Economics of fossil fuel substitution and wood product sinks when trees are planted to sequester carbon on agricultural lands in western Canada. *Canadian Journal of Forest Research* **29**: 1669-1678.
- Volney, W.J.A., Alfaro, R.I., Bothwell, P., Hogg, E.H., Hopkin, A., Laflamme, G., Hurley, J.E., Warren, G., Metsaranta, J., and Mallett, K.I. 2005. A framework for poplar plantation risk assessments. *Unasylva* **221**: 20-25.
- Voltas, J., Serrano, L., Hernandez, M., and Peman, J. 2006. Carbon isotope discrimination, gas exchange and stem growth of four Euramerican hybrid poplars under different watering regimes. *New Forests* **31**: 435-451.
- Wang, J.R., Hawkins, C.D.B., and Letchford, T. 1998. Photosynthesis, water and nitrogen use efficiencies of four paper birch (*Betula papyrifera*) populations grown under different soil moisture and nutrient regimes. *Forest Ecology and Management* **112**: 233-244.
- Warren, C. 2006. Estimating the internal conductance to CO₂ movement. *Functional Plant Biology* **33**: 431-442.
- Warren, C.R. and Adams, M.A. 2005. What determines interspecific variation in relative growth rate of *Eucalyptus* seedlings? *Oecologia* **144**: 373-381.
- Weih, M., Rönnerberg-Wästljung, A.C., and Glynn, C. 2006. Genetic basis of phenotypic correlations among growth traits in hybrid willow (*Salix dasyclados* × *S. viminalis*) grown under two water regimes. *New Phytologist* **170**: 467-477.

Yuan, L. and Xu, D.Q. 2001. Stimulation effect of gibberellic acid short-term treatment on leaf photosynthesis related to the increase in Rubisco content in broad bean and soybean. *Photosynthesis Research* **68**: 39-47.

Zhang, X., Zang, R., and Li, C. 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Sciences* **166**: 791-797.

APPENDIX

Analysis of Variance Model

Variables were analyzed by analysis of variance (ANOVA) using a two-factor factorial in a randomized complete block design (Table 4, Table 5). Two treatments were identified –Species and Latitude- with genotypes randomly nested within both, while the 3 Time periods were identified as blocks. All these were as defined in the following linear model equation:

$$Y_{ijkl} = \mu + T_i + S_j + L_k + S \times L_{jk} + G/(S \times L)_{l(jk)} + E_{i(jk)l}$$

Where $i=1 \dots n$, $j=1 \dots m$, $k=1 \dots p$, $l=1 \dots r$ ($n=3$, $m=2$, $k=2$, $l=10$).

Source	DF	F-test
Time (T) = Block	2	MS_T/MS_{E2}
Species (S)	1	MS_S/MS_{E1}
Latitude (L)	1	MS_L/MS_{E1}
$S \times L$	1	$MS_{S \times L}/MS_{E1}$
Genotype (G)/(S \times L) = E1	36	$MS_{G/(S \times L)}/MS_{E2}$
Experimental Error = E2	78	No test
Total	$120 - 1 = 119$	

Table 4. Analysis of variance components, including sources, degrees of freedom (DF) and F-tests.

Source	Components of Variance
T_j	$\sigma_E^2 + mpr\sigma_T^2$
S_j	$\sigma_E^2 + n\sigma_G^2 + npr\sigma_S^2$
L_k	$\sigma_E^2 + n\sigma_G^2 + nmr\sigma_L^2$
$S \times L_{jk}$	$\sigma_E^2 + n\sigma_G^2 + nr\sigma_{S \times L}^2$
$G/(S \times L)_{l(jk)}$	$\sigma_E^2 + n\sigma_G^2$
$E_{ijkl(jk)}$	σ_E^2

Table 5. Sources and components of variance for the analysis of variance model.