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Genetic Variation Among Paper Birch (*Betula Papyrifera* MARSH.) Populations in Germination, Frost Hardiness, Gas Exchange and Growth

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(Received 8th May 2000)

Abstract

Patterns of genetic variation in paper birch (*Betula papyrifera* MARSH.) were evaluated at the population level. A sample of 18 populations from the south interior, central interior and north coast of British Columbia were examined in a number of traits related to germination (germination capacity, germination speed, peak value and germination value), fall and winter frost hardiness, gas exchange (transpiration rate, stomatal conductance, net photosynthesis, instantaneous water use efficiency and mesophyll conductance) and biomass accumulation after the first and the second growing season. Analysis of variance or covariance revealed significant differences among the populations in all studied attributes except for stomatal conductance and height after the second growing season. Proportion of total variance attributed to population effect was up to 92% for germination parameters, 63% for fall frost hardiness, 22% for winter frost hardiness, 63% for biomass after the first growing season, 20% for biomass after the second growing season, and 5% for gas exchange variables. Germination speed and capacity were positively correlated and were higher in populations from colder climates. Central interior populations had the highest level of fall frost hardiness and were the most uniform with respect to that trait. There were large variations in fall frost hardiness within north coastal and south interior populations. Revealed patterns of variations have implications for paper birch genetic resources management and conservation.

Key words: inter- and intra-population variation, quantitative and adaptive attributes, geographic trend.

Introduction

Paper birch (*Betula papyrifera* MARSH.) is a pioneer tree occurring throughout British Columbia except for the outer coastal mainland and northwestern part of Vancouver Island. It can be found over a wide range of environmental conditions but grows best at low and middle elevations in moist and warm locations (SIMARD and VYSE, 1992). It tolerates low winter temperatures as well as high summer temperatures if water supply is sufficient. Paper birch seedlings have high growth potential that is often not fully realized on sites where nutrient and water supply is somewhat limited.

Paper birch is one of the three commercially most important hardwood species in the interior of British Columbia (MASSIE et al., 1994). Its economic importance may increase in the future

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as it is used to produce value-added wood products such as veneer and furniture. Other economic uses include production for biomass and dimension lumber. Paper birch is also an ecologically important component of boreal broadleaf-conifer mixtures where it can have beneficial as well as detrimental effects on conifer species (COMEAU and THOMAS, 1996).

At present, very little is known about genetic variation in paper birch in British Columbia. Since environmental conditions vary extensively within the species natural range, it is reasonable to expect genetic differentiation among paper birch populations in a number of traits. Other species from the genus *Betula* often show significant differences among populations as well as among families within populations. For instance, ERIKSSON and JONSSON (1986) listed examples of genetic differentiation in growth related traits for European silver birch (*B. pendula* ROTH.), European hairy birch (*B. pubescens* EHRH.) and yellow birch (*B. alleghaniensis* BRITTON). In case of paper birch, variation in traits related to growth and gas exchange has already been reported for four populations from climatically diverse locations in British Columbia (WANG et al., 1998a and b). In this paper we report results of a common garden experiment based on 18 paper birch populations that cover much of the natural range of this species in the province. The populations were examined for differences in germination, frost hardiness, growth and biomass allocation and in characteristics related to gas exchange (net photosynthesis, stomatal conductance, mesophyll conductance and water use efficiency). Knowledge of genetic variation at the population level constitutes base-line information needed for the conservation as well as utilization of genetic resources of the species.

Materials and Methods

Plant material

Bulk, open-pollinated seeds of paper birch collected from 18 wild populations in British Columbia (*Table 1*) were used in the study. In spring of 1997, the seeds were sown in styroblocks (PSB313B®) in a commercial nursery on Vancouver Island (latitude 48° 35', longitude 123° 24', elevation 50 m). In fall, 1997, the seedlings were potted into plastic containers (volume 2650 ml). Four hundred and fifty plants (25 per population) were randomly selected in spring of 1998 from the plants potted in the previous year. The selected plants were placed on wooden pallets and secured with wire grid. The plants were fertilized and watered using a drip irrigation system with individual capillaries running to each pot.

Table 1. – British Columbia Forest Service seedlot number and location of sampled paper birch populations.

Seedlot	Location	Latitude	Longitude	Elevation (m)
Ep 42401	Sardine Creek	52° 47′	122° 14′	760
Ep 42402	Bush Creek	50° 49′	119° 45′	1250
Ep 42403	Lee Creek	50° 46′	119° 32′	600
Ep 42414	Skeena River	54° 30′	128° 34′	70
Ep 42415	Little Oliver Creek	54° 42′	128° 16′	270
Ep 42416	Burdick Creek	55° 11′	127° 47′	480
Ep 42417	Juniper Creek	55° 08′	127° 43′	350
Ep 42418	St. Mary River	49° 38′	116° 03′	990
Ep 42419	Wilson Creek	50° 04′	117° 23′	915
Ep 42420	Porcupine Creek	49° 15′	117° 10′	840
Ep 42421	Frost Lake	53° 47′	122° 38′	975
Ep 42423	Mars Creek	51° 22′	118° 18′	990
Ep 42424	Cuisson Lake	52° 31′	122° 24′	762
Ep 42425	Raft Creek	52° 31′	121° 31′	830
Ep 42426	Eaglet Lake	54° 06′	122° 21′	685
Ep 42427	Tabor Lake	53° 55′	122° 22′	915
Ep 42428	Amanita Lake	54° 08′	121° 47′	760
Ep 42429	Barnes Creek	50° 34′	118° 50′	850

Germination

Seed germination tests were conducted prior to sowing in spring, 1997. Four replications of at least 100 seeds from each of the 18 populations were used in the standard germination tests. The unstratified seeds were placed on top of moist filter paper and kept at temperatures between 20 °C to 30 °C for 21 days. After seven days, germinants were counted every day and assessed according to ISTA (International Seed Testing Association, 1985) rules. The following variables were determined: germination capacity (GC), germination speed (R_{50} and R'_{50}), peak value (PV) and germination value (GV). Among these parameters, GC is the percentage of total germinated seeds, R_{50} is the number of days it takes to germinate 50 % of the total seeds (CHING, 1959) and R'_{50} is the number of days it takes to germinate 50 % of the viable (and non-dormant) seeds (THOMSON and EL-KASSABY, 1993). In order to estimate PV, the accumulated number of germinants was divided daily by the number of corresponding days. The maximum value obtained is called PV and it represents the mean daily germination of the most vigorous seeds (CZABATOR, 1962). Multiplying PV by mean daily germination gives GV, which represents germination speed and/or germination completeness (CZABATOR, 1962).

Frost hardiness

Frost hardiness of paper birch stems was measured in October, November and December, 1997. Six seedlings per population were randomly selected for each trial. Frost hardiness was determined by the measurement of electrolyte leakage from tissues exposed to sub-freezing temperatures (GLERUM, 1985). The following sets of test temperatures were used: –6 °C, –12 °C, –18 °C on 14 October, –12 °C, –18 °C, –24 °C on 11 November and –18 °C, –32 °C, –44 °C on 15 December. Stem segments (5 mm long) placed in plastic vials with 0.3 ml of deionized water were frozen at a rate of 4 °C per hour in a programmable freezer and kept at each test temperature for 1 hour. The frozen samples were thawed for 2 hours at 4 °C before adding 3.3 ml of deionized water. The electrical conductivity of the test solution was measured after the samples were held for approximately 18 hours at room temperature. Following the conductivity measurements the tissues were killed in a water bath (90 °C for 1 hour) and left to equilibrate for 18 hours before the second conductivity measurements. Frost injury indices (I_t = index at temperature t) were calculated following GLERUM (1985). Higher values of I_t indicate lower frost hardiness. Based on data from tests conducted at –18 °C in October, November and December, the approximate date by which I_{-18} for each population approached 50 % was obtained.

One additional test was performed in December to evaluate the maximum possible heritability of cold resistance using the multiple measurement method (FALCONER, 1989). Three measurements per plant, on the lower, middle and upper stem sections, were taken on 18 plants exposed to –32 °C.

Biomass and gas exchange

Height (H1), stem diameter at the root-shoot transition zone (DIAM1), shoot dry weight (SDW) and root dry weight (RDW) were measured at the end of the first growing season on 30 seedlings per population. Ratio of RDW to SDW was also calculated (RDW/SDW). Height (H2) and stem diameter at the root-shoot transition zone (DIAM2) were measured after the second growing season on 25 plants per population and their stem volume (SVOL) was calculated assuming conical form of the stem.

Gas exchange rates were measured on 21 plants per population between 20 and 25 July, 1998. Plants measured on the same day constituted one block for statistical analysis to

account for day-to-day variations in gas exchange rates due to variable weather conditions. Exchange rates of carbon dioxide and water vapour were determined using an open gas exchange system (LCA4, The Analytical Development Company, England) with a Parkinson broadleaf chamber (PLC3, The Analytical Development Company, England). The carbon dioxide concentration and the relative humidity of air entering the leaf cuvette was controlled at 320 µL/L and 45%, respectively. The temperature was recorded and used as a covariate in statistical analyses. An artificial light source (35 W Eye Dichro-Cool halogen lamp, Iwasaki Electric Co., Japan) delivering 1200 µmol/m²/s of photon flux density was used to illuminate the leaf chamber. Measurements were taken on sunny days with photosynthetically active radiation exceeding 1000 µmol/m²/s of photon flux density to ensure that the measured leaves were induced to high light. Gas exchange rates of each plant were measured twice on a fully expanded sun leaf and the average of the two measurements was used in the statistical analyses. Based on carbon dioxide and water vapour exchange rates the following parameters were obtained as in von CAEMMERER and FARQUHAR (1981): transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), net photosynthesis (A, µmol CO_2 m^{-2} s^{-1}) and intercellular CO_2 concentration (Ci). Photosynthetic instantaneous water use efficiency (A/E) was calculated as the ratio of A to E. Mesophyll conductance (gm, mol CO_2 m^{-2} s^{-1}) was determined by dividing A by Ci (LUDLOW and JARVIS, 1971).

Statistical analyses

All variables were subject to analysis of variance (ANOVA) or covariance (ANCOVA) with significance level of $P < 0.05$. The following transformations were used to meet the assumptions of normal distribution and homoscedasticity: $(H1)^{0.5}$, $(SDW)^{0.5}$, $(RDW)^{0.5}$, log(RDW/SDW), $(A)^2$, $(gs)^{0.5}$, log(A/E) and $1/(gm)^{0.5}$. A completely randomized design was used to evaluate parameters related to germination, frost hardiness and biomass. The data were analyzed by one-way ANOVA according to the general linear model:

$$Y_{ij} = \mu + \tau_j + \varepsilon_{(i)j}$$

where µ = common mean, τ_j = population effect (j = 1,...,18); $\varepsilon_{(i)j}$ = error term (i = 1,...,n where n is sample size per population).

A randomized complete block design was used to evaluate parameters related to gas exchange. The data were subjected to two-way ANCOVA according to the general linear model:

$$Y_{ijl} = \mu + \tau_j + \beta_i + \varepsilon_{ij} + \omega_{(ij)l} + \text{covariate}$$

where µ = common mean, τ_j = population effect (j = 1,...,18); β_i = block (test date) effect (i = 1,...,4); ε_{ij} = population x block interaction, and $\omega_{(ij)l}$ = sampling error (l = 1,....,p where p is sample size per population per block). Only significant ($P < 0.05$) covariates were included in the final models: leaf temperature for A, gs and gm, and water vapour pressure deficit for A/E.

In order to relate the population variations in germination attributes and frost hardiness to the climatic conditions of the seed collection sites, the following climatic variables were derived from climatic models based on latitude, longitude and elevation (REHFELDT et al., 1999): mean temperature of the coldest month (MTCM), mean temperature of the warmest month (MTWM), mean summer precipitation (MSP) and frost free period (FFP). The models were developed for British Columbia and adjacent regions of Yukon, Alberta and the United States between 48° 30' and 62° north. Geographic variations were examined using canonical correlation and canonical redundancy analyses for germination data and linear regression for frost hardiness data. Canonical correlations were performed between germination variables (R'_{50}, GC, GV, PV) and climatic variables (MTCM, MTWM, MSP and FFP) using the CANCORR procedure of SAS (SAS, 1988).

Results

Germination

Statistically significant ($P < 0.05$) differences among the populations were found for all germination attributes (*Table 2*).

Table 2. – Analysis of variance for attributes related to germination (A), gas exchange (B)[1]), frost hardiness (C)[2]) and biomass after the first growing season (D) and biomass after the second growing season (E).

	Source of Variation	Degrees of Freedom	Components of Variance (%)					Expected Mean Squares
A			R'_{50}	GC	PV	GV		
	Population (P)	17	91.82*	89.73*	87.33*	90.91*		$\sigma^2_E + 4\sigma^2_P$
	Error (E)	54	8.18	10.27	12.67	9.09		σ^2_E
B			gs	A	gm	WUE		
	Population (P)	17	0.23	1.49*	4.56*	5.09*		$\sigma^2_E + 20.4\sigma^2_P + 5.1\sigma^2_{P•B}$
	Block (B)	3	24.62*	6.86*	3.21*	1.04		$\sigma^2_E + 72.1\sigma^2_B + 4.0\sigma^2_{P•B}$
	P•B	51	1.45	4.91	3.79	1.90		$\sigma^2_E + 5.2\sigma^2_{P•B}$
	Error (E)	305	73.70	86.73	88.44	91.98		σ^2_E
C			L_5oct	L_{18}oct	L_{18}nov	L_{24}nov	L_{18}dec	
	Population (P)	17	48.96*	28.94*	59.87*	62.47*	21.62*	$\sigma^2_E + 6\sigma^2_P$
	Error (E)	90	51.04	71.06	40.13	37.53	78.38	σ^2_E
D			H1	DIAM1	SDW	RDW	RDW/SDW	
	Population (P)	17	62.77*	29.58*	43.23*	13.09*	53.19*	$\sigma^2_E + 30\sigma^2_P$
	Error (E)	522	37.23	70.42	56.77	86.91	46.81	σ^2_E
E			H2	DIAM2	SVOL			
	Population (P)	17	0.83	19.85*	13.61*			$\sigma^2_E + 24.8\sigma^2_P$
	Error (E)	430	99.17	80.15	86.39			σ^2_E

[1]) Covariates are not included

[2]) Sample results of October (oct), November (nov) and December (dec) frost hardiness tests are presented including results for temperatures that produced the largest differences among the populations at each test date.

*) Significant at $P \leq 0.05$

Table 3. – Overall mean, standard deviation (SD) and mean population maximum and minimum for traits related to germination and gas exchange (A), biomass and frost hardiness (B)[1]).

A

Trait (unit)	R'_{50} (days)	GC (%)	PV (#seedlings per day)	GV	gs (mol H_2O/ m^2s)	A (µmol CO_2/ m^2s)	gm (mol CO_2/ m^2s)	WUE (µmol CO_2/ mmol H_2O)
Mean	7.07	76.35	7.76	29.10	0.195	15.71	0.207	4.92
SD	0.17	3.86	0.69	3.06	0.062	3.02	0.227	0.62
Max	8.07	94.38	10.98	48.80	0.230	17.44	0.380	5.28
Min	6.09	50.39	4.53	11.10	0.182	14.19	0.133	4.57

B

Trait (unit)	H1 (cm)	DIAM1 (mm)	SDW (g)	RDW (g)	RDW/ SDW	H2 (cm)	DIAM2 (mm)	SVOL (cm^3)	L_5oct (%)	L_{24}nov (%)	L_{18}dec (%)
Mean	19.18	3.27	0.45	0.54	1.38	182.98	18.13	159.63	41.73	45.00	12.82
SD	4.09	0.34	0.14	0.18	0.44	20.61	1.56	35.26	15.58	13.48	3.87
Max	29.19	3.71	0.72	0.67	2.26	190.56	19.46	184.97	73.36	75.64	20.48
Min	12.12	2.93	0.26	0.36	0.62	173.00	16.88	136.02	13.55	23.42	9.82

[1]) Sample results of October (oct), November (nov) and December (dec) frost hardiness tests are presented.

The differences among the populations were large, particularly in germination completeness. For example, GC ranged from 50.4% to 90.4% and GV ranged from 11.1 to 48.8 (*Table 3*). Since two populations had GC around 50%, the speed of germination was represented only as R'_{50}. All germination attributes were interrelated: GC, GV and PV were highly correlated with each other (r values > 0.92) as well as with R'_{50} (r = –0.68, –0.79, –0.86, respectively). The correlations indicate that populations with higher GC germinated faster and that GV is a good index of paper birch germination completeness and speed.

Canonical correlations indicate that all germination attributes were related to the climate of seed collection sites. Out of four pairs of canonical variables, the first one was statistically significant ($P < 0.05$) and it explained 49% of the variation in germination attributes. The first canonical variable of the climatic variables (CLIM1) was strongly and negatively correlated with MTCM (*Table 4*). Among germination attributes, GC, PV and GV loaded highly ($P < 0.01$) on CLIM1 (*Table 4*) and all three increased with decreasing temperatures of seed collection sites. Speed of germination was also significantly higher ($P<0.05$) in populations from locations characterized by lower winter temperatures (*Table 4*).

Table 4. – (A) correlations[1]) between climatic variables and their first canonical variable (CLIM1); (B) correlations between germination variables and their first canonical variable (GERM1); (C) correlations between germination variables and CLIM1.

A climatic variables	CLIM1	B germination variables	GERM1	C germination variables	CLIM1
MTCM	– 0.65	R'_{50}	–0.58	R'_{50}	–0.47
MTWM	– 0.25	GC	0.94	GC	0.76
MSP	– 0.06	GV	0.96	GV	0.78
FFP	– 0.09	PV	0.91	PV	0.74

[1]) Critical values of the correlation coefficient are 0.456 at P = 0.05 and 0.575 at P = 0.01.

Frost hardiness

Statistically significant differences ($P < 0.05$) among the paper birch populations were found in depth of frost hardiness and time of frost hardiness induction. Populations differed significantly in all three tests at every test temperature except for the last test in December at –44 °C. As much as 62.5% of the total variance in frost injury index (I_t) was due to population (*Table 2*). The largest differences among the populations in I_{-18} were found in the middle of November, i.e. at the time when the seedlings were rapidly becoming frost hardy (*Figure 1*). The least frost hardy population (# 42419) reached a LT_{50} of –18 °C 25 days later than the most frost hardy populations (# 42424 and # 42426). Based on the amount of the total variance attributed to the population effect, the differences among the populations in I_t were much smaller in December than were found in fall during frost hardiness development (*Table 2*). In December all populations would survive the lowest temperatures that could occur in their natural locations since none of the populations had I_t higher than 38% after exposure to –44 °C.

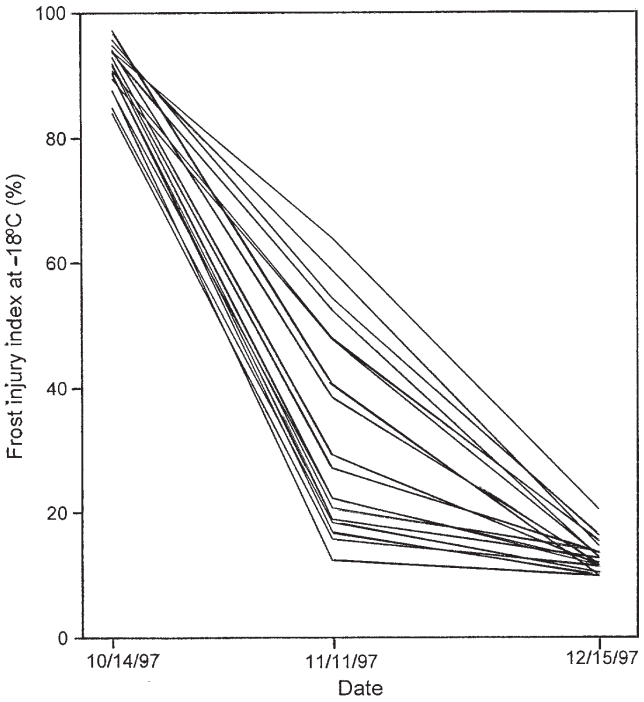

Figure 1. – Frost injury index of 18 paper birch populations at –18°C versus test date.

Geographic variations in paper birch fall frost hardiness development (evaluated by the date each population reached a hardiness level of I_{-18} = 50%) show that northern interior populations were very different from all other populations (*Figure 2*). Northern interior populations were the most frost hardy

and formed the most uniform group in terms frost resistance. Northern coastal populations and southern interior populations were frost hardy to similar levels. However, there were large differences among the populations within each group. Good correlation was found between MTCM and I_t for tests in October and November (r = 0.75 and 0.65, respectively).

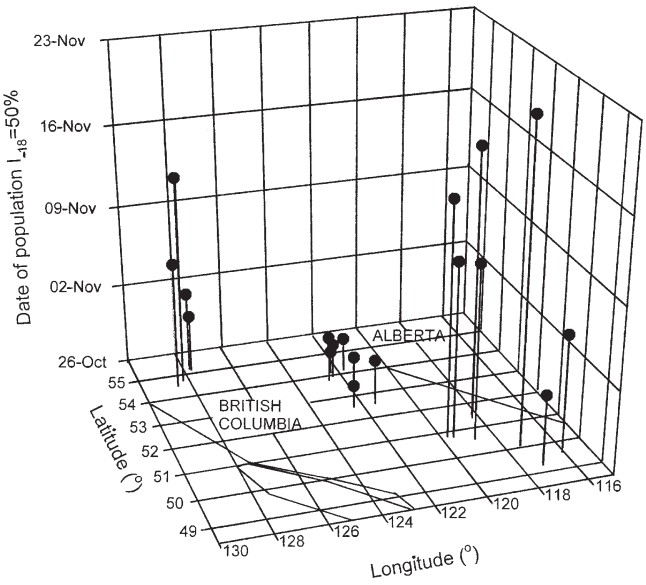

Figure 2. – Fall frost hardiness development of paper birch populations in British Columbia evaluated by date each population became frost hardy to –18°C (mean population I_{-18} = 50%).

Maximum possible heritability of paper birch winter frost hardiness was estimated at 0.67 using the multiple measurements method. Upper and lower portions of paper birch stems were frost hardy to the same levels in December.

Biomass and gas exchange

Large differences in productivity and biomass allocation pattern (RDW/SDW) were found among the paper birch populations after the first growing season (*Table 2*). As much as 63% for H1 and 53% for RDW/SDW of the total variance was due to population effect. After the second growing season no significant differences were found for height but the populations differed significantly in DIAM2 and SVOL (*Table 2*).

Significant differences ($P < 0.05$) among the populations were found for A, gm and A/E even though the between population variance was small for these variables (Table 2). No significant block and population interactions were found indicating that the populations responded in the same way to the day-to-day variations in climatic conditions. Mean population A was high and ranged from 14.76 to 17.55 µmol CO_2 $m^{-2}s^{-1}$ (*Table 3*). Photosynthetic rate was correlated strongly with gm (0.75) and with A/E (0.77). Higher A/E was due to higher A rather than ability to conserve water since no significant differences were found among the populations in gs (*Table 2*).

Discussion

Differences among the populations in germination parameters have been found for a number of species, e.g. European silver birch (REYES et al., 1997), Pacific silver fir (*Abies amabilis* DOUGL.[FORBES]) (DAVIDSON et al., 1996), western hemlock (*Tsuga heterophylla* [RAF.] SARG.) (CAMPBELL and RITLAND, 1982) and red alder (*Alnus rubra* BONG.) (RADWAN and DEBELL, 1981). Variable success is reported in relating the variations to geography. No geographic trend was found for GC in Pacific silver fir populations (DAVIDSON et al., 1996) but speed of germination based on GV was higher in southern populations. In contrast, CAMPBELL and RITLAND (1982) and MORGENSTERN (1969) found, respectively, that northern populations of western hemlock and black spruce (*Picea mariana* [MILL.] B.S.P.) tend to germinate faster. Different geographic trends in germination parameters evaluated at the population level are not surprising considering the number of environmental and species-specific factors involved. Our results show that germination parameters of paper birch were strongly related to the temperature regime of seed collection sites. Germination speed and completeness were higher in populations from colder regions and were not related to moisture regime. Water regime may not be very different between the sites when the seeds germinate since there should be enough soil moisture left from melted snow and since paper birch seldom grows on very wet sites (FOWELLS, 1965).

Geographic variations in germination parameters found in this study correspond to the results of a study by BEVINGTON (1986) who found that northern seed sources of paper birch germinated faster and had higher germination percentages than southern ones based on unchilled seeds germinated at 18 °C. In that study, geographic differences in paper birch germination were based on seeds collected from single trees from various locations in northern and southern regions of the US and Canada rather than on seeds collected from populations. BEVINGTON (1986) suggested that differences in germination parameters may have been partially caused by the thinner and more translucent pericarp found in seeds of northern origin. A thinner pericarp would present less mechanical resistance to radicle growth and would transmit more light and therefore could influence the speed of germination. Higher germination speed may be advantageous for populations with a shorter growing period. In our study, the largest difference in R'_{50} between two populations was only two days. However, the standard germination tests for paper birch are conducted in temperatures (20 °C to 30 °C) that are likely much higher than germination temperatures in natural conditions. It is possible that the population would show greater differences at lower test temperatures. BEVINGTON (1986) found larger differences between northern and southern plants when the tests were conducted at lower temperatures (14 °C to 18 °C).

The likely cause of genetic differences in GC among paper birch populations is differential dormancy level. BEVINGTON (1986) found that the southern and northern seed sources differed in dormancy levels: northern plants responded to shorter chilling treatment than southern ones. Also, GC increased in both groups after chilling. In our experiment, GC values of northern interior populations were very high (up to 94%) suggesting that they did not need stratification. However, sources from regions characterized by fluctuating fall temperatures (e.g. coastal regions or interior wet belt) may require some pre-chilling treatment. A chilling requirement should prevent seed germination in fall when the temperatures in mild areas may be high enough to favour germination. Large differences in GC observed in this study suggest that stratification may be needed for some seed sources. ELLIOTT and TAYLOR (1981) found that while, in general, seeds of red alder populations did not require stratification, there were exceptions.

Differences among the populations in germination parameters have important practical implications for stock production in terms of its genetic diversity and production success as well as for gene conservation efforts. There can be a substantial loss

of genetic diversity during commercial seedling production that favours seedlings from faster germinating seed sources (EL-KASSABY et al., 1992).

Corresponding to the large differentiation among the populations in traits related to germination, there were large differences among the populations with respect to frost hardiness development. Strong genetic control of frost hardiness development in paper birch is indicated by the large portion of the total variance that was attributed to the population effect in October and November tests. Bigger differences in fall frost hardiness than in winter frost hardiness are often observed (SAKAI and LARCHER, 1987). It is unlikely that the differences in December frost hardiness among paper birch populations would be much larger had lower temperatures been selected. The plants were already damaged (on average 29%) after exposure to –44 °C in the December test and no significant differences were found at that temperature. Since temperatures do not get much lower than –44 °C in any of the sampled locations, all tested populations would survive winter temperatures. It implies that if significant frost damage occurs in paper birch, it is likely due to late frost hardiness development or too early dehardening in spring. Similarly, DEANS and HARVEY (1996) found the largest differences among sessile oak (*Quercus petraea* [MATT.] LIEBL.) populations in frost hardiness in fall and spring and no significant differences in December and January.

The geographic pattern of fall frost hardiness development is important for paper birch management and conservation. As a group, populations from the central interior of the province were the most frost hardy in fall and were the most uniform in frost hardiness compared to the populations from the south interior and the north coast. The within region variations correspond to climatic diversity. The central interior plateau is the least diverse with respect to temperature and moisture regime. On the other hand, the south interior of the province is characterized by complex topography, existence of dry and wet belts and a range of temperature extremes. Within this region, the least frost hardy population of all tested populations (# 42419) is a close neighbour of one of the most frost hardy populations (# 42420). Similarly, northern coastal populations, which are located near the transition zone between the coastal and interior climates showed large differentiation in frost hardiness: two populations closer to the coast were clearly less frost hardy in fall than the two populations located further inland. The difference in frost hardiness development between the most and the least frost hardy coastal population was 13 days even though the distance between them was only 45 km.

There was a distinct latitudinal pattern of variation in interior paper birch frost hardiness since central interior plants were more frost hardy than their south interior counterparts. However, it is clear that the complicated overall pattern of variations could not be well represented by latitude, longitude and elevation. Derived climatic variables were more successful in that respect since approximately 56% and 42% of the variations in frost injury index could be explained by MTCM for tests in October and November, respectively. Climatic data for late summer and fall would likely help to further refine the relationship between the climate and the level of paper birch frost hardiness.

In contrast to germination and frost hardiness, most of the variation in gas exchange variables was found within populations. Even though populations differed significantly in A, gm and A/E, only a small portion of variance in these variables was attributed to the population effect. However, the estimation of the population variance in gas exchange variables is likely underestimated due to the nature of the measurements. Gas exchange rates change instantly with changing environmental conditions thus inflating the error variance.

No significant differences were found among the populations in gs in test conditions of sunny weather and warm temperatures. In these circumstances, all populations maintained high gs (0.18 to 0.23 mol $H_2O/m^2/s$). Significant differences found for A/E were due to differentiation in A rather than the ability to control transpiration by changing gs. In contrast to our results, WANG et al. (1998b) found significant differences in gs and A/E among four paper birch populations from climatically diverse locations and attributed the differences in A/E to differentiation in gs. However, it is not surprising that the results of both studies were different since the scope of the studied populations and environmental conditions of both tests were very different: four populations grown in a greenhouse in the central interior of British Columbia versus 18 populations grown outdoors on the south coast of the province. The differences between the studies may reflect significant population and environment interaction in conditions of optimum moisture and nutrient supply.

Differences among the populations in A were in part due to different carboxylation efficiency since A and gm were highly correlated (r = 0.75). Mesophyll conductance approximates carboxylation efficiency assuming that other components of gm, an excitation resistance and CO_2 diffusive resistance in the liquid phase (LUDLOW and JARVIS, 1971) were not significant. The assumption is reasonable since the excitation resistance is important at low light only (LUDLOW and JARVIS, 1971) and liquid phase diffusion usually has only a small impact on A (FARQUHAR and SHARKEY, 1982).

Significant geographic trends in paper birch biomass and gas exchange variables have already been reported (BENOWICZ et al., 2000). Populations from regions characterized by colder climates and shorter growing season had higher photosynthetic rates implying that an inverse relationship exists within the species between leaf longevity and photosynthetic capacity. On the other hand, plant size after the first growing season was positively correlated with temperature regime and the length of the growing season in the seed collection sites. It should be noted that reported here smaller differences among the populations in biomass attributes after the second growing season compared to the first growing season were probably environmental in nature. Plants were grown in plastic pots and were quite large in the second year. Larger plants would used up their resources faster than smaller plants and therefore their full growth potential might not be realized. Significant differences among three paper birch populations in height of mature trees have been reported by VIHERA-AARNIO and VELLING (1999).

Revealed patterns of variation have significant implications for paper birch genetic resources management and conservation. Winter temperature should not be an important factor affecting paper birch survival; however, close attention should be paid to fall temperature regimes of prospective plantation sites. Central interior populations should be selected if superior fall frost hardiness is required. Seed transfer from the coast and south interior into the central and north interior may result in significant frost damage. Selection in the climatic transitional zones may produce populations with good growth potential and high frost hardiness. For example, two north coastal populations from the most interior location among the coastal populations (# 42416 and # 42417) and one south interior population closest to the Rocky Mountains (# 42418) had high SDW and developed early frost hardiness. Special care should be exercised with respect to seed transfer within the

south interior of British Columbia where transfer should be guided by local microclimatic conditions. The south interior region also presents the biggest challenge in terms of paper birch gene conservation because of the large differentiation among the populations within this region and difficulties in relating the variation to the macroclimatic variables as evidenced by the variation in frost hardiness.

Acknowledgements

This study was supported by Forest Renewal British Columbia Grant number HQ96060-RE made to Y.A.E.

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Comparison of Multivariate and Univariate Methods for the Estimation of Type B Genetic Correlations

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(Received 14th June 2000)

Summary

Univariate and multivariate statistical methods for type B genetic correlation estimation are numerically compared based on computer simulated half-sib forest genetic experimental data. Multivariate methods have demonstrated desirable properties in effectively handling unbalanced data associated with heterogeneous variances across environments and yielded empirically unbiased estimates of type B genetic correlations for various data structures. Constraining estimates of type B genetic correlations within theoretical parameter space when using multivariate methods helps improve estimation accuracy and narrow the confidence interval. While some univariate methods can also produce unbiased estimates of type B genetic correlations for unbalanced data with heterogeneous variances, multivariate methods tend to estimate type B genetic correla-

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