With the possible exception of families 10 and 38, most families are likely to have lethal equivalents less than 2 (*Table* 4, Column 4). These numbers may increase when the test period is extended to maturity. However, we consider the above numbers to be fairly small, and conclude that the inbreeding program as outlined in ERIKSSON et al. (1984) has a good chance of progressing without losing lines. Early purging of lethal alleles may not be necessary in *Salix viminalis*. It would be, however, desirable to monitor the changes in lethal equivalents as the generations progress.

Acknowledgements

The technical assistance of URBAN PETTERSSON, HARTMUT WEICHELT, and EWA WINKLER is greatly appreciated. We also thank Drs. GÖSTA ERIKSSON, DAG LINDGREN, and FRANK SORENSEN for critically reviewing the manuscript. This study was partly funded by the Swedish Energy Board and by Swedish Council for Forestry and Agricultural Research.

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Peatland and Upland Black Spruce Populations in Alberta, Canada: Isozyme Variation and Seed Germination Ecology

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(Received 25th September 1991)

Summary

Seeds from three pairs of peatland and upland black spruce (*Picea mariana* (MILL.) B. S. P.) populations in Alberta were used to study variation in isozymes and seed germination. In the isozyme study, 16 enzyme systems coded by 28 putative genetic loci were investigated. In the germination study, seeds were germinated at 15° C, 25° C, or 35° C and 0, —5, —10 or —15 bars. The data were analyzed by ANOVA and SNK-tests. It was found that isozyme variability was similar among the populations, sites and between habitats. Results of F_{st}-values, X^2-tests, and genetic identities, indicated there is little genetic differentiation between upland and peatland habitats. In the germination study, seeds from upland and peatland habitats did not show expected adaptive responses to temperature, moisture and their interaction, but differentiation among the 6 populations was significant. Overall, seeds germinated best at 25° C, and seeds from peatland populations germinated more slowly and poorly at low temperature than did upland populations. Differences in germination among populations were most likely due to maternal effects and/or locally site-specific selection. Results from both studies indicate that there is little ecotypic differentiation between upland and peatland black spruce populations in Alberta.

Key words: *Picea mariana* (MILL.) B. S. P., isozyme electrophoresis, discriminant analysis, genetic variation, temperature, water potential.

Introduction

Peatland and upland habitats are very different in terms of nutrient availability, and soil structure, temperature, moisture, pH, and aeration (BRADBURY and GRACE, 1983),

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and vegetation. These differences in environment might result in variable selection pressures such that plant species which grow in both habitats may evolve edaphic ecotypes.

Black spruce (*Picea mariana* (MILL.) B. S. P.) occurs in a wide range of climatic conditions in North America and grows on both organic and mineral soils (FOWELLS, 1965). A wide range of clinal and ecotypic genetic variation has been reported in black spruce morphology, seed germination, phenology, and seedling performance (DIETRICHSON, 1969; MORGENSTERN, 1969a and b; FOWLER and MULLIN, 1977; SEGARAN, 1977; KHALIL, 1975, 1981; FOWLER and PARK, 1982).

In recent years, allozyme analysis has been used to study population differentiation in black spruce. BOYLE and MORGENSTERN (1987) found no significant population differentiation of allele frequencies in New Brunswick, but YEH et al. (1986) reported regional and altitudinal ecotypic allozyme variation in Newfoundland. Edaphic ecotypic differentiation has been illustrated for other tree species using allozymes (FURNIER and ADAMS, 1986) and morphological traits (JENKINSON, 1977; KRUCKEBERG, 1967; TEICH and HOLST, 1974), but it has not been conclusively demonstrated in black spruce from peatland and upland habitats. In Ontario, peatland and upland populations of black spruce were not differentiated morphologically (FOWLER and MULLIN, 1977; PARKER et al., 1983), but peatland populations were more variable for cone and chemical characters (PARKER et al., 1983) and had higher levels of isozyme variation (O'REILLY et al., 1985). BOYLE et al. (1990) found that one peatland and one upland black spruce population in Ontario were similar in allozyme frequencies, but the trees were more inbred on the upland site. Black spruce is abundant on peatland and upland sites in western Canada, but the genetic structure of populations and the extent of ecotypic variation have not been well studied.

Among all the requirements for seed germination, a suitable combination of temperature and moisture may be the most crucial (MAYER and POLJAKOFF-MAYBER, 1982). Since seed germination characteristics are, at least partially, under genetic control (WHITTINGTON, 1973), local populations of a widely distributed species could develop adaptive variability in germination ecology (QUINN and COLOSI, 1977). Provenance differences in seed germination response to moisture or temperature have been explained by the conditions of the site of origin for several species (FRASER, 1971; MOORE and KIDD, 1982; GIBSON and BACHELARD, 1987).

In western Canada, the soil is cooler and wetter in peatlands than in uplands (VAN CLEVE et al., 1981). Therefore, black spruce seeds from these two habitats might have different germination responses to temperature and/or moisture conditions. We hypothesized that seeds from upland trees would germinate better at high temperatures and/or low water potentials than seeds from peatland trees.

Comparative studies of population differentiation using isozyme, morphological and ecophysiological traits can provide valuable information about the effects of evolution on different facets of the genome, the relationships among which are poorly unterstood (LINHART et al., 1989). Only a few such studies have been reported (WHEELER and GURIES, 1982; MERKLE and ADAMS, 1987; FARMER et al., 1988; LINHART et al., 1989; LOOPSTRA and ADAMS 1989). Here, we report some of the results of such a study in which patterns of variation in isozyme variation and seed germination ecology were examined for peatland and upland populations of black spruce. Another paper will present results of a comprehensive examination of seedling morphology and ecophysiology under a variety of environmental conditions.

Table 1. — Locations and stand descriptions of the populations.

	Site	Habitat	Species	Trees/ha	Age	Mean Height (Ht, m)	Mean diameter at 1.5 m Ht (cm)
1.	55 08'N 114 15'W	peatland	black spruce	1875	42	4.15	5.84
			tamarack	2250			
		upland	black spruce	325	81	15.90	20.80
			white spruce	416			
			aspen	240			
2.	55 02'N 114 02'W	peatland	black spruce	4176	41	4.47	5.37
			tamarack	528			
		upland	black spruce	1056	55	12.37	17.90
			white spruce	112			
			tamarack	16			
3.	53 25'N 116 01'W	peatland	black spruce	1648	71	5.8	7.95
			tamarack	2250			
		upland	black spruce	592	91	16.62	21.00
			white spruce	528			
			tamarack	144			

Methods

Three pairs of peatland and upland populations were selected in Alberta (*Table 1*). Sites 1 and 2 were about 25 km apart and 120 km away from site 3. Peatland and upland populations at each site are about 1 km apart. Within each of the 6 populations, 40 black spruce trees were selected for collection of cones. They were dominant or codominant, and at least 40 m apart. Average stand height, diameter at 1.5 m height, and age were estimated from the first 15 trees in each stand (*Table 1*). Trees were aged by counting the rings on discs cut from the stem base. Stand density was estimated by making total counts of all trees (taller than 1 m) in a 25 m x 25 m plot (*Table 1*).

More than 50 one-year-old cones were collected from the upper portion of the crown of each tree in June, 1989. Cones were stored at 3 °C from 1 to 4 weeks and then dried at 50° C for 24 hours. Seeds were extracted by shaking dried cones, then dewinged, cleaned, and stored by seed lot at 3° C.

Isozyme Study

Seeds from the 40 sample trees in each stand were germinated at 25° C for 5 days. Haploid megagametophytes were used for electrophoresis. The extraction buffer contained 0.1M tris, 0.2M sucrose, 2% PEG 8000, 0.6% PVP, 1M EDTA, 0.1% BSA, 0.002M ascorbic acid, and 0.66% mercaptoethanol. The horizontal electrophoresis gels (22 cm x 7 cm x 1.2 cm) contained 12.5% starch. Banding patterns of 16 enzyme systems were studied using three buffer systems. A tris-citrate/lithium-borate buffer system (pH 8.1) (RIDGEWAY et al., 1970) was used to analyze glutamate dehydrogenase (GDH), shikimate dehydrogenase (SKD), colorimetric esterase (CE), leucine-aminopeptidase (LAP), and malic enzyme (ME). This system was run at 300 v and 4 °C for about 2.5 hours. A histidine-tris (pH 7.0) buffer system (CHELIAK and PITEL, 1984) was used to analyze 6-phosphogluconic dehydrogenase (6PG), acid phosphatase (APH), aspartate aminotransferase (AAT), phosphoglucose isomerase (PGI), and glucose-6-phosphate dehydrogenase

(G6P). Finally, a continuous tris-citrate buffer system (0.028 M citric acid, 0.13M tris, pH 7.0) was used to analyze malate dehydrogenase (MDH), fluorescent esterase (FLE), isocitrate dehydrogenase (IDH), adenylate kinase (AK), phosphoglucomutase (PGM), and aconitase (ACO). The last two systems were run at 200 v and 4 °C for about 3 hours. Staining techniques and procedures were similar to those of YEH and O'MALLEY (1980). Additional staining solutions were: AK — 100 ml Tris (Trizma base, 0.2M, pH 8.0), 2 ml 1% NADP, 1 ml 10% MgCl, 2 ml 1% PMS, 2 ml 1% MTT; GGP — 100 units of G-6PGH, 100 mg glucose, 100 mg ADP, 7 mg hexokinase; APH — 300 mg Na-alpha-napthyl acid phosphate, 150 mg Fast garnet GBC salt, 90 ml Na acetate (0.2M, pH 5.0), 2 ml 10% MgCl; FLE — 10 mg 4-methylumbelliferyl acetate, 24 ml Na acetate (0.2M, pH 5.0); LAP 100 ml Tris/Maleic buffer (Tris 0.2M and Maleic acid 0.2M, pH 5.3), 2 ml 10% MgCl, 100 mg L-leucyl-beta-napthylamide, 140 mg Fast black K Salt; and, SKD — 80 ml Tris (0.2M, pH 8.0), 0.60 g agar, 2 ml 1% MTT, 2 ml 1% PMS, 2 ml 1% NAP, and 4 mg shikimic acid.

The inheritance and linkage of the allozymes for the loci studied here have been determined (YEH et al., in preparation[2]). To infer parental genotypes, 8 haploid megagametophytes per parent tree were assayed individually. Megagametophytes from trees that were homozygous at most loci were run on each gel as standards. When interpreting the electrophoretic banding patterns, multiple loci for a given enzyme system were designated as 1 for the locus coding the most anodal zone, and 2 for the next, etc. The most common allele at each locus was designated as 0, with alleles coding faster moving bands, as 1, 2, etc., and slower, as —1, —2, etc.

Data analysis was done using univariate methods. Heterogeneity X^2-tests (WORKMAN and NISWANDER, 1970) were employed to detect differences in allele frequencies among the 6 populations, 3 sites, populations within habitats, and between habitats. BIOSYS-I (SWOFFORD and SELANDER, 1981) was used to calculate the mean number of alleles per locus based on all the loci, percentage of polymorphic loci (frequency of most common allele $\leq$ 0.99), F-statistics (WRIGHT, 1965), genetic identity (NEI, 1978), and average expected heterozygosity based on HARDY-WEINBERG expectation.

Seed Germination Test

A preliminary test showed that almost no germination occurred at 10 °C or 40 °C. On this basis, 15 °C, 25 °C, and 35 °C, representing a range of temperatures under which seed germination could be expected to occur in nature, were chosen for the test. Seeds were germinated in the dark in 3 germinators, each set for one of the 3 constant temperatures resulting in a simple split-plot experimental design with temperatures as whole plots. Within each germinator, seeds from each population were subjected to 4 water potentials (0 MPa, —0.5 MPa, —1.0 MPa, and —1.5 MPa) to give a factorial set of 12 treatments. Polyethylene glycol (PEG) 6000 was added to distilled water to provide the appropriate water potentials at each temperature. The concentrations of PEG were calculated by extrapolation of the equation given by MICHEL and KAUFMANN (1973), and the water potentials produced were checked with a Wescor Dew Point Microvoltmeter HR-33T. The solutions contained 0.025% Thiram to prevent fungus infection.

35 trees per population had enough seeds for the planned experiments. Three seeds per tree from the 35 trees in each population were bulked to form each replicate. Four replicates of each population were exposed to each of the 12 treatments. The 105 seeds of each replicate were sown on filter paper (#3, Whatman) in a petri dish (diameter 9 cm). 5 ml of the solution with appropriate water potential was added to each petri dish, and the petri dish was wrapped with Saran Wrap to prevent water evaporation. A total of 96 petri dishes (6 populations x 4 water potentials x 4 replicates) were placed in each germinator at random.

Germinants (radicale breaking seed coat) were counted and removed once a day for 30 days. Then, a cutting test was done to obtain the total number of filled seeds for each replicate. Total germination percent (G% = (number of germinants/number of filled seeds) x 100) per replicate and the number of days to reach 50% of the G% (R50) were calculated for each replicate. Analysis of variance (ANOVA) was conducted using SPSS-PC (NORUSIS, 1988). Significant differences among means were further examined by the SNK-test. The significance level used in all statistical tests in this study was $p \leq 0.05$.

Results

Allozyme Frequencies and Variability

The 16 enzymes were coded by a total of 28 electrophoretically demonstrable loci. Of these, 20 loci were polymorphic with a total of 51 alleles. The 8 monomorphic loci were Aat-3, Aph-1, G6p-1, Me-1, Pgi-2, Pgi-3, Pgm-1, and 6pg-1. Estimated allele frequencies for the 6 populations, are given in *table* 2. The mean number of alleles per locus, percentages of polymorphic loci, and mean observed and expected heterozygosities were similar among the 6 populations, and between the 2 habitats (*Table 3*).

Table 2. — Allele frequencies of the polymorphic loci for the 6 populations (the least frequent allele at each locus is not shown).

			Peatland			Upland		
			Site					
Locus	# alleles per locus	Allele	1	2	3	1	2	3
Aat-1	2	1	.975	1.000	.975	.975	.975	.925
Aat-2	2	0	1.000	1.000	.988	.988	.988	.950
Aco-1	4	0	.613	.762	.625	.625	.525	.650
		1	.213	.125	.287	.275	.250	.213
		2	.162	.112	.087	.100	.225	.138
Ak-1	2	0	1.000	1.000	.988	1.000	1.000	1.000
Ak-2	2	0	.813	.800	.775	.788	.813	.788
Aph-2	2	0	.988	.988	1.000	1.000	1.000	1.000
Ce-1	2	0	1.000	1.000	1.000	1.000	1.000	.988
Fle-1	2	0	.925	.938	.887	.962	.950	.850
Gdh-1	2	0	.725	.738	.750	.800	.675	.700
Idh-1	2	0	1.000	1.000	.988	1.000	1.000	1.000
Idh-2	3	0	.450	.438	.538	.300	.438	.550
		1	.550	.550	.463	.700	.563	.438
Lap-1	2	1	.538	.550	.538	.550	.512	.500
Mdh-1	4	0	.962	.950	.950	.962	.962	.938
		-1	.025	.038	.038	.025	.025	.063
		2	.013	.000	.013	.013	.013	.000
Mdh-2	3	0	.975	.988	.913	.962	.950	.913
		1	025	.013	.075	.038	.050	.075
Mdh-3	3	0	.863	.800	.875	.863	.788	.887
		1	.112	.125	.063	.087	.150	.050
Me-2	4	0	.887	.850	.850	.887	.813	.875
		1	.112	.112	.125	.100	.162	.112
		2	.000	.025	.025	.013	.025	.013
Pgi-1	2	0	.988	1.000	1.000	1.000	.988	1.000
Pgm-2	3	0	.325	.275	.350	.325	.425	.363
		1	.663	.712	.637	.663	.575	.637
		2	.013	.013	.013	.013	.000	.000
Skd-1	4	0	.637	.650	.625	.675	.688	.712
		-1	.175	.188	.200	.188	.125	.150
		1	.138	.162	.150	.125	.175	.125
6pg-2	3	1	.663	.712	.675	.625	.663	.738
		2	.313	.250	.275	.313	.287	.213

[2]) YEH, F. C., EL-KASSABY, Y. A. and KHALIL, M. A. K.: The genetics and linkage of isozymes in black spruce (*Picea mariana*).

Table 3. — Genetic variability at 28 loci (standard errors).

Data Source		Mean number of alleles per locus[1]	Percentage of loci polymorphic[2]	Mean heterozygosity observed	Mean heterozygosity expected[3]
Peatland	1	1.9 (0.2)	57.1	0.152 (0.039)	0.163 (0.040)
	2	1.8 (0.2)	50.0	0.148 (0.037)	0.156 (0.038)
	3	1.9 (0.2)	60.7	0.161 (0.039)	0.173 (0.039)
Upland	1	1.8 (0.2)	53.6	0.158 (0.040)	0.155 (0.038)
	2	1.8 (0.2)	57.1	0.160 (0.040)	0.174 (0.041)
	3	1.9 (0.2)	57.1	0.162 (0.039)	0.174 (0.038)
Mean		1.9	55.9	0.157	0.166
Habitat Peatland		2.1 (0.2)	60.9	0.154 (0.037)	0.164 (0.039)
Upland		2.0 (0.2)	67.7	0.160 (0.039)	0.169 (0.039)
Site	1	1.9 (0.2)	60.7	0.155 (0.039)	0.159 (0.039)
	2	2.0 (0.2)	60.7	0.154 (0.038)	0.166 (0.040)
	3	2.0 (0.2)	64.3	0.161 (0.038)	0.173 (0.038)

[1]) Calculated on the basis of all loci.
[2]) Frequency of the most common allele $\leq$ 0.99.
[3]) Unbiased estimate (Nei 1978) based on HARDY-WEINBERG expectation.

Overall, there was very little differentiation for allozyme frequencies among the 6 populations, 3 sites, populations within habitats, or between habitats based on X^2-tests, F_{st} values, and genetic identities (*Table 4*). The X^2-test showed significant differentiation among the 6 populations only at one locus, and among populations of the upland habitat at two loci (*Table 4*). These represent 3.5% and 7.0% respectively of the total number of loci detected. Hence, this is close to the 5% occurrence of significant differences which is expected by chance when using $p \leq 0.05$. The data indicated greater differentiation among populations within habitat types (F_{st} = 0.005 and 0.012) than between habitat types (F_{st} = 0.001). Variation among upland populations (F_{st} = 0.012) was twice that among peatland populations (F_{st} = 0.005), although these differences may not be significant.

Table 4. — Results of analysis of allozyme variability, A: hierarchical heterogeneity X^2-tests (df in brackets), B: F_{st}-values, and C: mean genetic identities[a]).

Locus	Among all populations	Between habitats	Among populations within peatlands	Among populations within uplands
Aat-1	8.02 (5)	2.65 (1)	2.03 (2)	3.34 (2)
Aat-2	8.67 (5)	3.62 (1)	2.09 (2)	2.96 (2)
Aco-1	18.30 (15)	3.64 (3)	8.64 (6)	6.06 (6)
Ak-1	3.13 (5)	1.04 (1)	2.09 (2)	0.00 (2)
Ak-2	0.56 (5)	0.00 (1)	0.35 (2)	0.20 (2)
Aph-1	3.14 (5)	2.09 (1)	1.05 (2)	0.00 (2)
Ce-1	3.13 (5)	1.04 (1)	0.00 (2)	2.09 (2)
Fle-1	9.74 (5)	0.03 (1)	1.43 (2)	8.28 (2)*
Gdh-1	3.74 (5)	0.10 (1)	0.13 (2)	3.51 (2)
Idh-2	3.13 (5)	1.04 (1)	2.09 (2)	0.00 (2)
Idh-3	17.66 (5)*	1.03 (1)	3.86 (2)	12.77 (2)*
Lap-1	0.68 (5)	0.21 (1)	0.03 (2)	0.44 (2)
Mdh-1	7.61 (5)	1.09 (3)	3.39 (6)	3.12 (6)
Mdh-2	10.94 (10)	0.76 (2)	6.94 (4)	3.24 (4)
Mdh-3	9.02 (10)	0.06 (2)	4.02 (4)	4.93 (4)
Me-1	7.49 (15)	1.11 (3)	4.24 (6)	2.13 (6)
Pgi-1	4.17 (5)	0.00 (1)	2.09 (2)	2.09 (2)
Pgm-2	7.24 (10)	2.45 (2)	1.08 (4)	3.70 (4)
Skd-1	8.67 (15)	2.28 (3)	4.33 (6)	2.07 (6)
6pg-1	4.70 (10)	0.77 (2)	1.39 (4)	2.54 (4)
Total	139.00 (160)	25.00 (32)	51.00 (64)	63.00 (64)
F_{st}	0.010	0.001	0.005	0.012
I.	0.9998	1.0000	1.0000	0.9989

[a]) Frequencies of all the alleles of the polymorphic loci were used in the analyses.
*) Chi-square value is significant at 5% level.

Table 5. — ANOVA results for total germination percent (G%), and R50 (number of days to 50% of the G%).

Source	G%[1] df	G% P-value	R50 df	R50 P-value[2]	R50 df	R50 P-value[3]
T (temperature)	2 (split-factor, not tested)					
W (water potential)	1	0.000	1	0.000		
S (site)	2	0.722	2	0.004	2	0.000
H (habitat)	1	0.037	1	0.000	1	0.000
T x W	2	0.000				
T x S	4	0.625	2	0.458		
T x H	2	0.000	1	0.375		
W x S	2	0.312	2	0.418		
W x H	1	0.512	1	0.798		
S x H	2	0.768	2	0.000	2	0.000
T x W x S	4	0.393				
T x W x H	2	0.291				
T x S x H	4	0.065	2	0.853		
W x S x H	2	0.466	2	0.041		
T x W x S x H	4	0.003				

[1]) G% by S, H, T (15 °C, 25 °C, 35 °C), W (0, —0.5 MPa), and their interactions.
[2]) R50 by S, H, W (0 and —0.5 MPa), and their interactions at temperature 25 °C.
[3]) R50 by S, H, T (25 °C, 35 °C) and their interactions at water potential 0MPa.

Seed Germination

At water potentials lower than —0.5 MPa, no germination occurred. At 15 °C and 0 MPa or —0.5 MPa, or 35 °C and —0.5 MPa, only a few seeds germinated sporadically; thus, R50 was not calculated for these treatments. Therefore, the data were analyzed by 3 ANOVA's, G% at 3 temperatures by 2 water potentials (0 MPa and —0.5 MPa), R50 at 0 MPa by temperatures (25 °C and 35 °C); and R50 at 25 °C by 2 water potentials (0 and —0.5 MPa). These ANOVA results are summarized in *table 5*.

The 4-way interaction (*site x habitat x temperature x water potential*) was significant for G% (*Table 5*), so its means were further analyzed by SNK-test (*Table 6*). At

Table 6. — Means of total germination percent by water potential by temperature by site by habitat.

SITE	HABITAT	WATER POTENTIAL (MPa) 0 TEMPERATURE 15	0 TEMPERATURE 25	0 TEMPERATURE 35	-0.5 TEMPERATURE (°C) 15	-0.5 TEMPERATURE (°C) 25	-0.5 TEMPERATURE (°C) 35
1	Peatland	11.02a	91.94a	91.38a	7.11a	66.05a	6.62a[1]
	Upland	23.43b	91.86a	85.99a	12.54a	58.05a	18.83b
2	Peatland	13.95ab	92.97a	86.62a	4.46a	67.38a	17.21b
	Upland	21.11b	93.35a	83.81a	10.64a	65.88a	16.67b
3	Peatland	13.50ab	91.91a	87.49a	5.49a	61.56a	18.60b
	Upland	23.69b	92.08a	87.44a	11.20a	65.79a	6.37a
Mean	Peatland	12.85	92.27	88.50	5.69	65.00	14.14
	upland	22.74	92.43	85.75	11.46	63.24	13.96
Overall mean		17.78	92.35	87.12	8.57	64.12	14.05

[1]) In each column means marked by different letters are different at 5% significance level by SNK-test.

Table 7. — Means of R50 (number of days to 50% of the total germination percent) by Water Potential by Site by Habitat at temperature 25 °C.

	WATER POTENTIAL (MPa)					
	0			-0.5		
	SITE			SITE		
HABITAT	1	2	3	1	2	3
Peatland	3.56a	3.71a	4.37b	6.24cd	6.66c	7.40e[1]
Upland	3.22a	3.32a	3.41a	6.65c	5.78d	6.02cd

[1]) Means marked by different letters are different at 5% significance level by SNK-test.

Table 8. — Means of R 50 (number of days to 50% of the total germination percent) by Site by Habitat (averaged for 25 °C and 35 °C) at 0 MPa.

	SITE		
HABITAT	1	2	3
Peatland	3.50b[1]	3.69c	4.28d
Upland	3.15a	3.25a	3.26a

[1]) Means marked by different letters are different at 5% significance level by SNK-test.

0 MPa, G% was much lower at 15 °C than at 25 °C or 35 °C. At —0.5 MPa, seeds germinated much better at 25 °C than at 15 °C or 35 °C. On average, G% decreased from 65.75% at 0 MPa to 28.91% at —0.5 MPa. At 15 °C (0 MPa or —0.5 MPa), seeds from upland populations had a higher G% than those from peatland although the difference was only significant for site 1 at 0 MPa. At —0.5 MPa and 35 °C, site 1 showed a higher G% in the upland population compared to the peatland. However, the opposite trend was seen between habitats at site 3.

In ANOVA testing effects of habitat, water potential (0, —0.5 MPa), and site on R50 at 25 °C, the 3-way interaction was significant (*Table 5*), thus the means were further analyzed by SNK-test (*Table 7*). Generally, seeds from upland populations germinated faster than those from peatland ones. The difference was significant for site 2 at —0.5 MPa and site 3 at 0 MPa or —0.5 MPa. Within peatland populations, site 3 had significantly slower germination than site 1 or 2.

In ANOVA testing effects of site, habitat, and temperature (25 °C, 35 °C) on R50 at 0 MPa, the 2-way interaction, habitat x site, was significant (*Table 5*). Upland populations had similar R50's which were significantly smaller than those for peatland populations (*Table 8*). The 3 peatland populations had significantly different R50's. The largest difference between habitats occurred at site 3, where the peatland population showed much slower germination.

Discussion

Allozyme Variation

Environmental differences between upland and peatland habitats may be expected to lead to development of edaphic ecotypes in black spruce (O'Reilly et al., 1985). However, we found little genetic differentiation at the isozyme level between the 2 habitats. This agrees with other isozyme and morphology studies on peatland and upland black spruce in eastern Canada (Fowler and Mullin, 1977; Parker et al., 1983; Boyle et al., 1990).

We found that the majority of genetic variability was intrapopulational, with a very small proportion among populations (F_{st} = 0.01) or sites (F_{st} = 0.005). As in other conifers, large population size and continuous geographical distribution, longevity of stands, pollen and seed dipersal by wind, high outcrossing, and lack of effective barriers to gene flow among populations of black spruce (Guries and Ledig, 1981; Yeh, 1981; Cheliak et al., 1988) likely have contributed to lack of significant genetic variability among populations, and between habitats.

Seed Germination Ecology

We hypothesized that seeds from peatland and upland populations would differ in their germination response as a result of adaptive evolution. Thus, they would germinate better in conditions similar to those of their native habitats. To support this hypothesis, interactions between habitat (H) and temperature (T), water potential (W), or T x W should be significant with seeds from uplands germinating better at higher temperature and lower water potential. Although we found a significant H x T effect on G%, this was due to the better germination by upland populations at low temperature with no consistent difference between habitats at —0.5 MPa and 35 °C (*Table 6*). H x T had no significant effect on R50. H x W had no significant effects on G%, and its effects on R50 varied among the sites (*Table 7*). Further, T x W did not affect G% between habitats, but did show site-dependent effects (*Table 6*). Thus, when significant, the interactions to test the hypothesis did not conform to expectations. However, they often had significant site-dependent effects which might reflect the influence of local site-specific selection.

Several explanations are possible for the lack of concordance of the germination results with expectation. First, selection is expected to be severe during germination and seedling establishment when temperature and moisture are most critical. However, successful germination and establishment may also be determined by the availability of 'safe sites' (Harper, 1977), which may vary little betwen habitats with respect to selection pressure. Secondly, if germination occurs during the wet period in early spring, the differences between the two habitats, with respect to moisture conditions, may be less than we originally expected. Thirdly, even if upland black spruce germinates better in lower moisture and higher temperature conditions than peatland black spruce, drought or heat resistant genotypes could be selected for in peatlands during early seedling growth as the upper peat surfaces may be very dry and warm later in the year (Denyer and Riley, 1964; R. Rothwell, unpublished). Finally, since black spruce is primarily outcrossing, if selection is not strong, extensive gene exchange between adjacent populations may overcome the effects of selection so that little or no ecotypic variation is evident (Ford, 1964).

Seed germination behaviour is influenced by genotype but also reflects conditioning effects of climate and soil of the maternal environment (Rowe, 1964) including site fertility (Youngberg, 1952). We found seeds from upland trees germinated more at 15 °C than peatland seeds (*Table 6*) and also, generally, germinated faster (*Table 7, 8*). Habeck (1958) reported higher germination for upland white-cedar than for lowland. In black spruce, Morgenstern (1969a) found germination speed and percent decreased with increasing soil moisture regime of the site of seed origin. The lower nutrient availability of peatland may be reflected in low seed vigor and poor germination of seed from peatland trees. Therefore, the germination differences between habitats might mainly reflect

the influence of the maternal environment rather than genetic effects.

Conclusions

Both allozyme and germination studies showed little genetic differentiation between upland and peatland habitats. There were some significant habitat effects on germination although not as hypothesized, and often they were site-dependent. In general, seeds from upland trees germinated better than those from peatland. These differences might reflect selection and/or maternal effects. Either way, they have significant implications in reforestation and will be examined further in greenhouse studies of seedling morphology and ecophysiology in response to a range of environments.

Acknowledgements

This study was supported by a Natural Sciences and Engineering Research Council (Canada) (NSERC) Forestry Transition Grant to V. J. Lieffers and F. C. Yeh, by a grant from the University of Alberta Central Research Fund and by a NSERC Operating Grant to S. E. M. We thank Uldis Silins and Matt Simons for help in the field and Alison Burns for lab assistance. Dr. F. C. Yeh, Dr. R. J. Bayer, and Dr. V. J. Lieffers provided helpful comments on the manuscript.

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