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# Differential Growth and Rooting of Upland and Peatland Black Spruce, *Picea mariana*, in Drained and Flooded Soils

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**Abstract**

A reciprocal experiment was analyzed to determine whether 30 open-pollinated families of peatland and upland populations of black spruce [*Picea mariana* (Mill.) B.S.P.] sampled from a single area in north-central Alberta, Canada, performed consistently when grown in either flooded or well-drained soils (i.e., if there is a family x soil interaction or generally called genotype x environment interaction (GEI)). The data for the analysis consisted of five traits (height, root dry weight, shoot dry weight, root/shoot dry weight ratio and number of braches) describing growth and rooting performance of tree seedlings in flooded and drained soils (root environments) in a greenhouse for 16 weeks. A mixed-model analysis was used to characterize GEI. The analysis revealed an interesting contrast of GEI patterns between the peatland vs. upland populations: GEI was absent (as indicated by a perfect correlation between flooded and drained soils) in peatland population but present in the upland population. Our results from the characterization of GEI are also consistent with the well-known theory about selection in different environments that correlated responses due to indirect selection are in general less than direct responses. The contrasting patterns of GEI in peatland vs. upland populations may be reflective of different strategies of adaptation to the contrasting environmental conditions, with the peatland trees growing slowly but steadily and with the upland populations growing fast and very responsive to environmental changes.

*Key words:* Black spruce, genetic correlations, genotype-environment interaction, reciprocal experiment.

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## Introduction

The natural range of black spruce [*Picea mariana* (Mill.) B.S.P.] covers most of the Canadian territory. This conifer grows on a variety of topographical conditions, moisture regimes and edaphic types within the Canadian boreal forest (ROWE, 1972; FARRAR, 1995). The range-wide surveys of this species (MORGENSTERN, 1978; BEAULIEU et al., 2004) have showed clinal differentiation, but ecotypic differentiation with respect to peatland and upland conditions in individual locations has not been conclusively demonstrated (MORGENSTERN, 1969; FOWLER and MULLIN, 1977; O'REILLY et al., 1985; WANG and MACDONALD, 1992; YEH et al., 1993).

Within a single site, the growth conditions of black spruce trees vary considerably including water-logged peatlands to well-drained mineral-soils. If soil (root environment) is an important habitat factor in differentiation of upland and peatland black spruce, soil influences should be reflected in characteristics of the rooting system and growth potential as adaptations. Thus, in a reciprocal test, trees grown on the substrate closest to its original habitat type are expected to be superior to the other trees grown on a different substrate in characteristics of rooting system and growth potential. In this regard, the reciprocal experiment would help answer a longstanding question in plant and animal breeding: should selection be conducted in a good environment, giving maximal expression to the desired character, or should it be carried out under the conditions in which the organisms will eventually live and grow? Conceptually the answer to the question depends on the extent to which the trait exhibits interaction between genotypes and environments (GEI). If the rank order and relative magnitudes of phenotypic expression for genotypes affecting the trait are the same across a range of environments, then there is no GEI and it does not matter in which environment the selection is conducted. However, if the expression of the trait changes rank or magnitude among the different genotypes, there is GEI and it

might be best to select in the environment in which the organisms will ultimately be reared.

It has been shown (FALCONER, 1952, 1990; FALCONER and MACKAY, 1996) that the magnitude of GEI could be quantified by the cross-environment genetic correlation, $r_{GE}$, in which the same character measured in two environments is considered to be two different characters. Obviously, a perfect correlation means the absence of GEI whereas a zero or negative correlation means a large magnitude of GEI. Thus the decision on the appropriate environment in which to select comes from examining the relative magnitude of the indirect to the direct response to selection. The indirect or correlated response ($CR_B$) is the response of the trait in the environment ($B$) in which it is expected to perform, given selection in a different environment ($A$), while the direct response ($R_B$) is for selection in the environment in which the organisms will ultimately be reared. If selection intensities are equal in the two environments, then $CR_B > R_B$ if $r_{GE}h_A > h_B$, where $h_A$ and $h_B$ are the square roots of the heritabilities of the trait in environment $A$ and $B$, respectively. If the genetic correlation is low, selection should be conducted in the environment in which the selected genotype is expected to perform.

In this paper, we will present the results of a reciprocal experiment in greenhouse that was designed to evaluate the differential growth and rooting responses of peatland and upland populations of black spruce sampled from north-central Alberta, Canada grown in flooded and drained soils. An earlier analysis focused on the assessment of isozyme variation and mean performance of the two populations in the two soil environments (YEH et al., 1993). However, our current analysis of the reciprocal experiment will focus on determining if there is a perfect correlation between environments (no GEI) and if there is the 'carry-over' effect of selection from one environment to another (FALCONER, 1990; HILL and MACKAY, 2004). A mixed-model approach (YANG, 2002; CROSSA et al., 2004) will be adopted for such analysis.

## Materials and Methods

*Reciprocal Experiment*

Population sampling, cone collection and seed extraction were described in YEH et al. (1993), but here we recapitulate essential details. Two natural stands of black spruce located two kilometers apart, one from a peatland site (55° 9'N; 114° 15'W) and the other from an upland site (55° 8'N; 114° 14'W) were selected. The peatland stand had an open-canopy of black spruce interspersed with tamarack whereas the upland stand had a closed canopy of white spruce, black spruce, aspen, and balsam poplar. Within each stand, cones were collected from 30 trees that were spaced at least 30 meters apart. Seed was extracted, dewinged, and cleaned with individual tree identities being kept.

The reciprocal experiment was carried out in greenhouse with the upland and peatland populations each grown in flooded and drained soils. Twenty-four trays, each consisted of 60 deep-5, Spencer-Lemaire containers (22 x 25 x 125 mm deep) were filled with moistened horticultural peat moss. Several seeds from the same half-sib family (i.e., seeds from the same mother tree) were planted in a randomly selected container in each of the trays. Twelve of the trays were placed in a container flooded to within 30 mm from the top of the growing containers (flooded treatment). The other twelve trays were grown in drained condition (drained treatment). Ten days after seedling emergence, seedlings in each container were randomly selected and removed until only one seedling per cell remained. In total this experiment had 1440 tree seedlings [2 populations x 2 growing conditions x 30 families x 12 replications (blocks)]. During the course of the experiment, the trays marked with the drained treatment were watered daily whereas the trays marked with the flooded treatment were maintained by controlling evapotranspiration through daily addition of deionized water to ensure the desired level of flooding (≥ 95 mm deep). Seedlings were grown for 16 weeks at 18 hours photoperiod, at 22 °C. The 16-week period approximated one growing season under greenhouse conditions and was also a best compromise between more seedling growth and constraint on root development due to the small size of the containers. Seedlings under both flooded and drained treatments were fertilized bi-weekly with 20N-20P-20K fertilizer supplemented by added micronutrients. At harvest, seedling height, shoot dry weight, root dry weight, and root/shoot ratio were recorded. The number of branches of >1 cm was also counted to provide an assessment of a different aspect of tree growth other than height and dry matter production.

*Characterization of Genotype-Environment Interaction*

For convenience, we labeled the four population-soil combinations (or simply environments) in the reciprocal experiment as follows: 1 = peatland, flooded; 2 = peatland, drained; 3 = upland, flooded; and 4 = upland, drained. We used the population-soil combinations to allow for the opportunity to model both population differentiation (correlations between the populations) and GEI (correlations between soils). A mixed model (LYNCH and WALSH, 1998; YANG, 2002; CROSSA et al., 2004) was fitted to the data from the reciprocal experiment,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\\ \mathbf{y}_4\end{bmatrix} = \begin{bmatrix}\mathbf{1}\mu_1\\ \mathbf{1}\mu_2\\ \mathbf{1}\mu_3\\ \mathbf{1}\mu_4\end{bmatrix} + \begin{bmatrix}\mathbf{G}_1 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{G}_2 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{G}_3 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{G}_4\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\\ \mathbf{g}_3\\ \mathbf{g}_4\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\\ \mathbf{e}_3\\ \mathbf{e}_4\end{bmatrix} \quad (1)$$

where $\mathbf{y}_1$ is an $n_1$ x 1 vector of mean values (over 12 replications) for the $i$th environment with $n_1$ being the number of families for the ith environment (in the present case, $n_1 = n_2 = n_3 = n_4 = 30$); $\mu_i$ is the mean of the $i$th environment and $\mathbf{1}$ is an $n_i$ x 1 vector of ones; $\mathbf{g}_i$ is a vector of family effects with incidence matrix $\mathbf{G}_i$ relating $\mathbf{g}_i$ to $\mathbf{y}_i$; and $\mathbf{e}_i$ is a vector of residuals.

Vectors $\mathbf{g}_i$'s and $\mathbf{e}_i$'s were considered random, normally distributed, and independent of each other, with zero mean vectors and variance-covariance matrices being:

$$\mathrm{Var}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\\ \mathbf{g}_3\\ \mathbf{g}_4\end{bmatrix} = \mathbf{I}_g \otimes \mathbf{\Sigma}_g = \mathbf{I}_g \otimes \begin{bmatrix}\sigma^2_{g1} & \rho_{12}\sigma_{g1}\sigma_{g2} & \rho_{13}\sigma_{g1}\sigma_{g3} & \rho_{14}\sigma_{g1}\sigma_{g4}\\ \rho_{12}\sigma_{g1}\sigma_{g2} & \sigma^2_{g2} & \rho_{23}\sigma_{g2}\sigma_{g3} & \rho_{24}\sigma_{g2}\sigma_{g4}\\ \rho_{13}\sigma_{g1}\sigma_{g3} & \rho_{23}\sigma_{g2}\sigma_{g3} & \sigma^2_{g3} & \rho_{34}\sigma_{g3}\sigma_{g4}\\ \rho_{14}\sigma_{g1}\sigma_{g4} & \rho_{24}\sigma_{g2}\sigma_{g4} & \rho_{34}\sigma_{g3}\sigma_{g4} & \sigma^2_{g4}\end{bmatrix} \quad (2)$$

and

$$\mathrm{Var}\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\\ \mathbf{e}_3\\ \mathbf{e}_4\end{bmatrix} = \mathbf{I}_e \otimes \boldsymbol{\Sigma}_e = \mathbf{I}_e \otimes \begin{bmatrix}\sigma^2_{e1} & 0 & 0 & 0\\ 0 & \sigma^2_{e2} & 0 & 0\\ 0 & 0 & \sigma^2_{e3} & 0\\ 0 & 0 & 0 & \sigma^2_{e4}\end{bmatrix} \quad (3)$$

where $\mathbf{I}_g$ and $\mathbf{I}_e$ are identity matrices of order 30, $\sigma^2_{gi}$ is the family variance for the $i$th environment, $\rho_{ij}$ is the genetic correlation between the $i$th and $j$th environments and $\sigma^2_{ei}$ is the residual variance which is the sum of error variance and one $n$th replication variance for the $i$th environment ($\sigma^2_{ei} = \sigma^2_i + {}^1/_n\sigma^2_{ri}$, where $n = 12$ in our study). The use of family means rather individual tree observations allowed us to focus on characterizing GEI but it might result in a slightly downward bias of estimated genetic parameters such as heritability with the bias being negligible when $n$ is large.

The unstructured family variance-covariance matrix ($\Sigma_g$) as given in equation (2) would have provided an opportunity to simultaneously characterize and test for GEI and population differentiation. The allowance for nonzero genetic correlations between the two populations would be reasonable given that (i) the peatland and upland populations sampled were only two kilometers apart; (ii) the estimated outcrossing rates were high for individual isozyme loci and across the loci and (iii) the estimated population differentiation ($G_{ST}$) across all isozyme loci was typically low just as like in other conifers (YEH et al., 1993). More specifically, correlations of $\rho_{12}$ and $\rho_{34}$ measured the consistency of peatland and upland populations over two contrasting environments (flooded vs. drained soils), respectively; correlations of $\rho_{13}$, $\rho_{14}$, $\rho_{23}$ and $\rho_{24}$ measured the extent to which the peatland and upland populations were differentiated. However, our preliminary mixed-model analysis showed that fitting to the full (unstructured) model of family variance-covariance matrix ($\Sigma_g$) in equation (2) would often result in a failure to converge. To circumvent this problem, we chose an alternative strategy by fitting a reduced model with the block diagonal matrix,

$$\boldsymbol{\Sigma}_g = \begin{bmatrix}\sigma^2_{g1} & \rho_{12}\sigma_{g1}\sigma_{g2} & 0 & 0\\ \rho_{12}\sigma_{g1}\sigma_{g2} & \sigma^2_{g2} & 0 & 0\\ 0 & 0 & \sigma^2_{g3} & \rho_{34}\sigma_{g3}\sigma_{g4}\\ 0 & 0 & \rho_{34}\sigma_{g3}\sigma_{g4} & \sigma^2_{g4}\end{bmatrix}. \quad (4)$$

This new strategy was built on our earlier observation that the peatland population significantly differed from the upland population for the five quantitative traits measured (YEH et al., 1993). Thus, we used the block diagonal matrix $\Sigma_g$ in equation (4) as our baseline model for subsequent analyses to focus on the characterization of GEI.

The analysis of model (1) was implemented through the use of SAS MIXED procedure (SAS Institute 1999) for studying genetic correlations between measurements of the same trait at different environments in relation to characterization of GEI. We modified the SAS programs of YANG (2002) and CROSSA et al. (2004) for our implementation. The restricted maximum likelihood (REML) method was used for estimation of variance components by including the option of METHOD=REML in the PROC MIXED statement. Family covariance structures (variances and correlations) were estimated by specifying the SUBJECT and TYPE options in the RANDOM statements, whereas the heterogeneity of error variances between the environments was allowed with the GROUP option in the REPEATED statement. The PARMS statement was needed to try different sets of initial values for the possibility of multiple peaks in the likelihood surface and for enhancing the chance and rate of convergence of likelihood function. The REML estimation guaranteed nonnegative estimates of variance components, but out-of-bound estimates of family correlations between the environments were avoided by including the UPPERB option in the PARMS statement [in our present study, this option was not needed as none of correlation estimates were outside the acceptable range of -1 to +1]. The block diagonal matrix $\Sigma_g$ was estimated by specifying the TYPE = UNR option in the RANDOM statements and by issuing the HOLD option in the PARMS statement to force zeros at the off-diagonal blocks. The PROC MIXED procedure used the iterative technique based on a ridge-stabilizing Newton-Raphson algorithm to solve highly nonlinear REML equations. The iteration continued until the convergence criterion of $10^{-8}$ was achieved.

We tested reduced models by imposing the constraints of $\rho_{12} = 1$ or $\rho_{34} = 1$ or both on the unstructured correlation matrix $\Sigma_g$ (UNR) to determine if the family correlation between flooded and drained soils would be significantly less than unity in peatland and upland populations. For each model analyzed, the SAS PROC MIXED generated -2 times the residual log-likelihood (-2RLL) and other fit statistics including Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (BIC). Unlike penalty-free -2RLL, AIC and BIC are essentially log likelihoods penalized for the number of covariance parameters estimated. Such penalties impose more stringent significance levels (instead of fixed 0.05 or 0.01 probability) for the LR test when a more complex covariance structure with more parameters being estimated is selected, and make the LR test "dimensionally consistent" (when sample size goes to $\infty$, the false positive rate approaches zero) as explain in BURNHAM and ANDERSON (1998). BIC imposes a heavier penalty than AIC but the two criteria often concur with each other in identifying the best model. The likelihood ratio (LR) statistic was used to test for adequacy of a covariance structure. It was constructed by comparing likelihoods for a 'full' model (model with fewer constraints and more parameters) and for a 'reduced' model (model with more constraints and fewer parameters) from the REML estimation, $LR = -2(RLL_{\text{reduced model}} - RLL_{\text{full model}})$. Under the null hypothesis that the full model is not different from the reduced model, the LR statistic is distributed approximately as a $\chi^2_{df}$ with $df$ degrees of freedom where $df$ is the difference between the 'effective' numbers of covariance parameters ($CP_e$) estimated for the two models. Given the relationship between -2RLL and AIC, viz., $AIC = -2RLL + 2CP_e$, $CP_e$ was calculated as $CP_e = [AIC-(-2RLL)]/2$.

*Calculation of Genetic Parameters*

The heritability of family means at the *i*th environment ($h_i^2$) was calculated as

$$h_i^2 = \sigma_{gi}^2 / \left(\sigma_{gi}^2 + \sigma_{ei}^2\right), \quad (5)$$

where $\sigma_{gi}^2$ and $\sigma_{ei}^2$ are variance components for family means and residuals at the *i*th environment as estimated using the REML method. We assessed the relative efficiency of selection on the performance of trees grown in their own environments (direct selection) vs. selection in different environments where the performance of trees is either easier to evaluate or leads to maximum expression (indirect selection) (FALCONER and MACKAY, 1996; HILL and MACKAY, 2004), i.e., the ratio of genetic responses from indirect and direct selections at the *i*th group ($CR_i/R_i$) as,

$$\frac{CR_i}{R_i} = \rho_{ij}\sqrt{\frac{h_j^2}{h_i^2}} \quad (6)$$

where subscript *i* indexes the environment in which the improved population will live whereas subscript *j* indexes the environment in which the selection is made.

## Results and Discussion

*Mean Performance*

Seedling growth was significantly faster in upland populations than in peatland populations regardless of whether it was aboveground (height, shoot and branches) or underground (root) growth (*Table 1*). However, the root/shoot dry weight ratio was significantly greater in peatland than in upland, suggesting that the peatland populations may grow slower than the upland populations but they may have allocated relatively more biomass to the roots. The flooded condition was significantly more conducive to the aboveground growth but significantly less conducive to the underground growth than the drained condition. The most remarkable of all differences between the two growing conditions was that seedling root/shoot dry weight ratio was 29% and 26% lower under flooding than under drained environment for peatland and upland populations, respectively.

The faster aboveground and underground growth in upland population is probably a result of adaptation to competition with cohabitating plants of other species. In mixed-wood stands (upland population), black spruce is usually in the shade of faster growing species such as aspen, balsam poplar and white spruce. Fast growing upland black spruces are certainly advantageous in escaping suppression by neighboring plants. On the other hand, in typical peatland natural stands in Alberta, black spruce was open grown and light was not limiting (LIEFFERS, 1986). Thus, there is less competition for fast growth in the peatland population.

Greater root/shoot ratio in peatland than in upland populations may be a direct result of differential growth patterns revealed between the two populations. Biomass allocation tends to be greater to the organ or structure that is most limiting to growth (HUNT and NICHOLLS, 1986). In our study, the root system of peatland trees may be less competitive for water/nutrients and thus needs to be relatively larger than that of upland trees, whereas upland trees need to have greater top growth in order to adapt to their usual understory environment where light is limiting.

*Genotype-Environment Interaction*

The estimates of family variances were greater in the peatland population than in upland population regardless of whether the seedlings were grown in flooded or drained soils for all traits except for the root/shoot dry weight ratio (*Table 2*). High variability in growth in peatland populations of black spruce has been suggested before (LIEFFERS, 1986; YEH et al., 1993). A similar trend

*Table 1.* – Least square means and contrast differences (± standard errors) for five traits of peatland and upland black spruce grown under flooded and drained soils.

| Population | Soil | Height (mm) | Root (mg) | Shoot (mg) | Root/shoot (× 1000) | Branches (no.) |
|---|---|---|---|---|---|---|
| **Least square means** | | | | | | |
| Peatland | Flooded | 48.63 ± 1.10 | 31.64 ± 1.08 | 141.97 ± 5.89 | 234.55 ± 4.30 | 3.13 ± 0.17 |
| Peatland | Drained | 42.75 ± 0.71 | 37.56 ± 1.00 | 120.44 ± 3.57 | 328.66 ± 5.17 | 2.63 ± 0.10 |
| Upland | Flooded | 51.58 ± 0.76 | 34.95 ± 0.87 | 158.22 ± 4.13 | 229.93 ± 3.95 | 3.68 ± 0.11 |
| Upland | Drained | 45.11 ± 0.45 | 38.83 ± 0.78 | 133.50 ± 2.50 | 310.54 ± 5.90 | 2.96 ± 0.10 |
| **Contrasts**[a] | | | | | | |
| Peatland vs. upland | | -5.31 ± 1.88** | -4.59 ± 2.23* | -29.31 ± 9.72** | 22.75 ± 10.06* | -0.89 ± 0.28** |
| Flooded vs. drained | | 12.35 ± 1.21** | -9.81 ± 1.45** | 46.26 ± 6.84** | -174.71 ± 9.50** | 1.22 ± 0.20** |

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

[a] The two single-degree-of-freedom contrasts were calculated based on the following sets of coefficients: 1 1 –1 –1 for peatland vs. upland; and 1 –1 1 –1 for flooded vs. drained.

*Table 2.* – Restricted maximum likelihood estimates of variances and correlations (± standard errors) from the unstructured correlation model for five traits of peatland and upland black spruce grown under flooded and drained soils.

| Parameter[a] | Height | Root | Shoot | Root/shoot | Branch |
|---|---|---|---|---|---|
| **Peatland population** | | | | | |
| $\sigma_1^2$ | 23.02 ± 9.47 | 17.55 ± 9.11 | 628.67 ± 272.85 | 355.14 ± 145.87 | 0.39 ± 0.22 |
| $\sigma_2^2$ | 13.91 ± 4.02 | 15.64 ± 7.85 | 222.64 ± 100.20 | 443.11 ± 210.76 | 0.20 ± 0.08 |
| $\rho_{12}$ | 0.81 ± 0.13 | 1.00 ± 0.25 | 0.91 ± 0.20 | -0.22 ± 0.30 | 0.99 ± 0.21 |
| **Upland population** | | | | | |
| $\sigma_3^2$ | 7.72 ± 4.60 | 15.31 ± 5.97 | 261.46 ± 134.28 | 284.64 ± 123.00 | 0.20 ± 0.10 |
| $\sigma_4^2$ | 3.53 ± 1.58 | 14.12 ± 4.82 | 99.62 ± 49.06 | 453.57 ± 273.34 | 0.19 ± 0.08 |
| $\rho_{34}$ | 0.22 ± 0.36 | 0.25 ± 0.25 | 0.02 ± 0.36 | 0.44 ± 0.33 | -0.15 ± 0.31 |

[a] $\sigma_1^2$, $\sigma_2^2$, $\sigma_3^2$ and $\sigma_4^2$ are, respectively, the family variances for the four population-soil combinations in the reciprocal experiment as follows: 1 = peatland, flooded; 2 = peatland, drained; 3 = upland, flooded; and 4 = upland, drained. $\rho_{12}$ and $\rho_{34}$, are the family (genetic) correlations between flooded and drained soils for peatland and upland populations, respectively.

for lower variability in the upland population (though not always significant) was observed for most of the characters. This is probably the result of correlated responses to selection for height growth through the pleiotrophic action of genes (FALCONER and MACKAY, 1996).

The estimates of genetic correlations between the peatland families grown on flooded and drained soils were not significantly less than unity for all traits except the root/shoot dry weight ratio, judging from the fact that they were not different from the hypothesized value of 1 by more than 2 standard errors. The insignificant departure from perfect genetic correlation in the peatland population ($\rho_{12} = 1$) suggests the absence of crossover GEI. In contrast, the estimated genetic correlations of the upland families were all significantly less than unity ($\rho_{34} < 1$), suggesting the presence of family x soil interaction in the upland population. These contrasting results in the peatland and upland populations were confirmed by the LR chi-square tests.

The contrasting patterns of GEI in peatland vs. upland populations reported above might reflect the facts that peatland and upland black spruce stands reside in different ecological conditions and thus have different strategies of adaptation to the environmental conditions. From an ecological perspective, growth is probably under selection due to possible competitions for light aboveground and/or for nutrients/water underground. In other words, tree growth depends on its ability to escape suppression by its neighboring plants (light competition), and extract nutrients/water (nutrient/water competition) underground. In peatland sites throughout much of western Canada, black spruce commonly occurs in pure stands or in association with tamarack (*Larix laricina*). Despite the apparent lack of competition for light but because of the stressful rooting environment of water saturated substrates, growth rates of black spruce are slow; stands usually remain open-grown long after sexual maturity (LIEFFERS, 1986). In mineral-soil sites (upland sites), on the other hand, the species is found mixed with the other species such as white spruce, trembling aspen and balsam poplar that often overtop black spruce. Thus, the stress related to shade might be the limiting factor for growth and survival of black spruce in these mixed-wood upland sites. Thus, it appears that the adaptation strategy of peatland populations is to grow slowly but steadily whereas that of upland populations is to grow fast and very responsive to environmental changes. In the future, it may be desirable to design a greenhouse or 'common garden' experiment where peatland and upland families are tested across an array of light intensities and/or water stress levels to examine the stability of the two types of black spruce.

*Carry-Over Effect*

The estimates of family heritability were generally higher in the peatland population than in the upland population regardless of whether the seedlings were grown under flooded or drained conditions (*Table 3*). These results are of course expected given greater among-family variances in peatland than in upland populations coupled with no family x soil interaction in either population.

For all traits except for root/shoot ratio, ratios of responses to indirect selection and to direct selection

*Table 3.* – Estimates of heritability of family means (± standard errors) for five traits of peatland and upland black spruce grown under flooded and drained soils.

| Heritability[a] | Height | Root | Shoot | Root/shoot | Branch |
|---|---|---|---|---|---|
| **Peatland population** | | | | | |
| $h_1^2$ | 0.638 ± 0.206 | 0.506 ± 0.184 | 0.605 ± 0.201 | 0.639 ± 0.206 | 0.478 ± 0.179 |
| $h_2^2$ | 0.908 ± 0.246 | 0.523 ± 0.187 | 0.584 ± 0.197 | 0.552 ± 0.192 | 0.672 ± 0.212 |
| **Upland population** | | | | | |
| $h_3^2$ | 0.440 ± 0.171 | 0.674 ± 0.212 | 0.511 ± 0.185 | 0.608 ± 0.201 | 0.544 ± 0.191 |
| $h_4^2$ | 0.585 ± 0.198 | 0.769 ± 0.226 | 0.533 ± 0.189 | 0.434 ± 0.170 | 0.664 ± 0.210 |

[a] $h_1^2$, $h_2^2$, $h_3^2$ and $h_4^2$ are heritabilities for the four population-soil combinations in the reciprocal experiment as follows: 1 = peatland, flooded; 2 = peatland, drained; 3 = upland, flooded; and 4 = upland, drained.

*Table 4.* – Ratios of responses to indirect selection and to direct selection (CR/R) for five traits evaluated for black spruce seedlings of peatland and upland population grown in flooded and drained conditions.

| Environment of selection | Environment of assessment | Height | Root | Shoot | Root/shoot | Branches |
|---|---|---|---|---|---|---|
| **Peatland population** | | | | | | |
| Drained | Flooded | 0.678 | 0.980 | 0.926 | -0.238 | 0.836 |
| Flooded | Drained | 0.966 | 1.013 | 0.893 | -0.206 | 1.175 |
| **Upland population** | | | | | | |
| Drained | Flooded | 0.190 | 0.231 | 0.016 | 0.526 | -0.135 |
| Flooded | Drained | 0.253 | 0.264 | 0.016 | 0.376 | -0.165 |

were close to one in the peatland population whereas the ratios were quite lower in the upland population (*Table 4*). This occurs regardless of whether an environment (flooded or drained) was considered as the one of selection or assessment. The negative ratios for root/shoot ratio in the peatland population and branches in upland population were a direct consequence of negative family correlations, indicating little carry-over effect. With only two exceptions, correlated responses are smaller than direct responses and thus selection is often most effective if it is conducted in the environment for which the improvement is sought (FALCONER, 1952; FALCONER, 1990; FALCONER and MACKAY, 1996; HILL and MACKAY, 2004). However the advantages of direct selection over indirect selection were less convincing in the peatland population than in upland population. In other words, it is practically no different whether the selection of peatland families is carried out in flooded or drained condition. In contrast, with low genetic correlations as observed in the upland population, the selection should be conducted directly in the environment in which the families are expected to perform unless the heritability or the selection intensity in the other environment is much higher.

*Limitations and Outlook*

A period of 16 growing weeks in the greenhouse can hardly be considered sufficient for a definite assessment of the concordance of growth patterns across different environments in black spruce. For our reciprocal experiment to be more valuable for practical tree improvement programs, we not only need to determine the consistency of tree performance across environments (flooded vs. drained soils) at the same ages as we did here but also across different environments (greenhouse and field conditions) at different ages (juvenile vs. maturity). For example, the inference made about the concordance and carry-over effect of selection between environments may not go very far if the juvenile growth in the greenhouse is only weakly correlated with the field performance. Indeed, there are evidences of weak genetic correlations between nursery or greenhouse experiments and field trials in black spruce (MULLIN and PARK, 1994; MULLIN et al., 1995). However, since the flooded and drained

treatments in our reciprocal experiment mimicked the peatland and upland environment conditions, respectively, such imitation would strengthen the correlation between the juvenile growth in controlled environments and more mature growth in the field conditions due to reduced GEI between juvenile and mature environments as shown in other conifer species (e.g., SONESSON et al., 2002). While further studies are definitely needed determine if there is a high correlation between controlled environment and field conditions, our results would be useful whenever such high correlation exists.

The presence of maternal effect may be another concern with evaluating the juvenile growth as in our study. The maternal effect is often indicated by strong dependence of germination rate and juvenile growth on seed size or initial seed mass but the difference in seed size is not always consistent with the difference between fast- vs. slow-growing families (WANG et al., 1994). Thus, the significant effect of seed size or mass as a covariate would suggest that nongenetic effect of see mass is crucial for seedling survival and growth performance. In our present study, we did not record the seed size prior to sowing and thus were unable to correct for potential maternal effect. In general, the maternal effect causes a time lag in response to selection. Thus, the differentiation of the growth as observed in our reciprocal experiment is expected to be even greater when seed reserves are depleted in subsequent growing seasons.

## Conclusions

Our study focuses on the characterization of GEI from the mixed-model analysis of the reciprocal experiment and its implications for genetic improvement of black spruce. The inability to fit the unstructured variance-covariance structure [equation (2)], likely due to the limited number of families per population, prevented us from simultaneously assessing GEI and population differentiation, which would otherwise have been permissible with our reciprocal experiment. Nevertheless, our mixed-model analysis showed an interesting contrast of GEI patterns between the peatland vs. upland populations: GEI was absent (as indicated by a perfect correlation between flooded and drained soils) in peatland population but present in the upland population. Our results from the characterization of GEI also support FALCONER's (1990) modified rule about selection in different environments that correlated responses due to indirect selection are in general less than direct responses. The contrasting patterns of GEI in peatland vs. upland populations may be reflective of different strategies of adaptation to the contrasting environmental conditions, with the peatland trees growing slowly but steadily and with the upland populations growing fast and very responsive to environmental changes.

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# Genetic Performance and Maximizing Genetic Gain Through Direct and Indirect Selection in Cherrybark Oak

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## Abstract

In 1987, an open-pollinated test of cherrybark oak (*Quercus pagodae* Raf.) was established on a loess site in Carlisle County, Kentucky. The test contained 37 half-sib families representing eight provenances from Louisiana, Mississippi, Tennessee, and Virginia. Height measurements were taken at ages one, three, five, ten, and fifteen, and diameter at ages five, ten, and fifteen. Significant differences existed among provenances and among families within provenances. Seed sources from the west-central Mississippi area performed better for both diameter and height, yet no overall geographic trend was apparent. The top three families were all from the Warren Co., Mississippi source while two of the top three diameter families were from Washington Co., MS and the third was from Warren Co., Mississippi. Survival among the eight provenances was constant from age one to ten. A drop in survival was shown between ages 10 and 15, probably a result of inter-tree competition. Height and diameter growth between ages five and 10 was nearly double that prior to age five and between ages 10 and 15. Family heritabilities for height and diameter were calculated for each measurement year. Family heritabilities for diameter ranged from 0.55 to 0.70 while height ranged from 0.50 to 0.70. Strong age-age correlations for height, diameter, and volume were found indicating good trait predictability from early measurements. Genetic gain equations were used to identify the optimum selection age and trait for maximizing age 15 volume. Early selection of families within provenances should yield gains in height, diameter, and volume.

*Key words:* Cherrybark oak, provenance, genetics, selection, genetic gain

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## Introduction

Cherrybark oak (*Quercus pagoda* Raf.) is an important and highly valued southern hardwood timber species. The species range is from Maryland south to Florida, west to eastern Texas, and north to southern Illinois (STEIN et al., 2003). Usually this oak occurs in well-drained alluvial, lowland soils as well as loess bluffs. Reaching reproductive maturity between 15 to 20 years of age, this species is generally grown commercially for 40 to 60 years for timber production.

Previous studies have shown growth differences exist among provenances (GREEN et al., 1991 and SCHOENIKE et al., 1982). Neither of these studies identified any geographic trend in provenance productivity. Substantial variation among families within provenances was also observed by GREEN et al. (1991) which led to the recommendation that provenance and individual family considerations should be made during selection. Similar results were found in water oak (*Q. nigra* L.) in a provenance study that covered most of the geographical region in this study (ADAMS, 1989). While these studies found differences in growth characteristics among provenances, YUCEER et al. (1998) sampled cherrybark oak seed sources on an east to west transect within Mississippi and found no significant variation in seedling emergence and survival.

The objective of this study was to identify growth differences among various provenances. Determination of potential genetic gains through selection was conducted through estimation of heritability and calculation of genetic gain. The ability to indirectly select for maximization of volume at age 15 was studied.

## Materials and Methods

### *Plant Material and Experimental Design*

A cherrybark oak provenance-progeny test was established in 1987 on MeadWestvaco property located in