VANHOLME, R., B. DEMEDTS, K. MORREEL, J. RALPH and W. BOERJAN (2010): Lignin biosynthesis and structure. Plant Physiol **153**: 895–905.

VARGAS-HERNANDEZ, J. and W. T. ADAMS (1994): Genetic relationships between wood density components and cambial growth rhythm in young coastal Douglas-fir. Can J Forest Res **24**: 1871–1876.

WHETTEN, R. and R. R. SEDEROFF (1992): Phenylalanine ammonia lyase from loblolly pine: Purification of the enzyme and isolation of complementary DNA clones. Plant Physiol **98**: 380–386.

WHETTEN, R. and R. R. SEDEROFF (1995): Lignin biosynthesis. Plant Cell **7**: 1001–1013.

WHETTEN, R., Y. H. SUN, Y. ZHANG and R. R. SEDEROFF (2001): Functional genomics and cell wall biosynthesis in loblolly pine. Plant Mol Biol **47**: 275–291.

YANG, S. H. and C. A. LOOPSTRA (2005): Seasonal variation in gene expression for loblolly pines (*Pinus taeda*) from different geographical regions. Tree Physiol **25**: 1063–1073.

YU, Q., S. E. MCKEAND, C. D. NELSON, B. LI, J. R. SHERRILL and T. J. MULLIN (2005): Differences in wood density and growth of fertilized and nonfertilized loblolly pine associated with a mutant gene, cad-n1. Can J Forest Res **35**: 1723–1730.

YU, Q., B. LI, C. D. NELSON, S. E. MCKEAND, V. B. BATISTA and T. J. MULLIN (2006): Association of the cad-n1 allele with increased stem growth and wood density in full-sib families of loblolly pine. Tree Genet Genomes **2**: 98–108.

Genetic and phenotypic correlations among volume, wood specific gravity and foliar traits in white spruce (*Picea glauca* (Moench) Voss)

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Abstract

White spruce is highly valued by the forest products industry in North America. Through tree improvement efforts, selected genotypes can exceed wild sources by 30% in volume. Negative correlations between growth and wood specific gravity have been established, but differences in leaf morphology between high and low performing genotypes are less well understood. We sampled five trees from each of 30 families at each of two locations in a 25-year old progeny test in Minnesota. One wood core was collected from each tree to sample wood specific gravity (WSG), along with a branch collected from the upper crown to examine foliar traits. We confirmed negative correlations between stem volume and WSG, but several families combined high wood volume with only small reductions in WSG. Leaf area ratio and specific leaf area were positively, genetically correlated with volume growth but not correlated with WSG. Increased growth rates of selected genotypes may be attributed, in part, to shifts in allocation to leaves and in leaf morphology that may optimize light interception.

Key words: white spruce, genetic correlations, wood specific gravity, specific leaf area, genotype by environment interaction, foliar nitrogen.

Introduction

White spruce is highly valued by the wood products industry in North America and is widely planted across Minnesota. Seed orchards, comprised of genotypes selected for superior growth are a common source used for tree planting on public and private land. Selection for fast growth is highly effective: differences between orchard-grown seedlings and local wild sources approach 30% in wood volume (PIKE et al., 2006; WENG et al., 2010). Neg-

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ative correlations between growth rate and wood specific gravity indicate the likelihood that fast-growing genotypes may be less windfirm than wild seed sources (CHANG and KENNEDY, 1967; CORRIVEAU et al., 1987; DUCHESNE and ZHANG, 2004). However, the gains in wood volume are high enough that net wood production after kiln-drying is not necessarily reduced (STELLRECHT et al., 1974). Tradeoffs between growth rate and other wood traits such as fiber length (BEAULIEU, 2003; DUCHESNE and ZHANG, 2004) and veneer quality (ZHANG, 1995; ZHANG et al., 2004) have also been found for white spruce, highlighting the need to quantify tradeoffs that may result from selection.

Growth rates, across plant taxa, are related to a myriad of traits that influence photosynthesis, light interception, and respiration (Reich et al. 2003). In global datasets, leaf traits that covary positively with relative growth rates across taxa include specific leaf area (SLA; $cm^2 \cdot g^{-1}$), leaf area ratio (LAR; $cm^2 \cdot g^{-1}$) and leaf mass ratio (LMR; $g \cdot g^{-1}$) (Cornelissen et al. 2003). Because SLA and LAR measure leaf area, these traits reflect, to some extent, efficiencies in light capture for photosynthesis and may be useful for breeders whose aim is to improve tree growth.

Measurements of foliar traits are complicated by variation within a tree crown. For example, leaves with high SLA are more common in low-light environments associated with understory branches (LEGNER et al., 2014). SLA (or its reciprocal leaf mass area) also decreases with foliage height (MARSHALL et al., 2001; MARSHALL and MONSERUD, 2003; KOCH et al., 2004), and from distal to proximal branch positions (LEGNER et al., 2014). SLA is usually lower for leaves in the upper crown, vs lower crown, an adaptation that reduces water loss from transpiration amidst high heat radiation loads (LAMBERS et al., 2008). SLA also varies with ontogeny: in Sitka spruce needle length and widths increase asymptotically over time, so that older trees have needles that are shorter (relative to their width), and heavier than younger needles (STEELE et al., 1989). Small differences in leaf or needle morphology may lead to large differences in carbon-fixation across an entire plant (REICH et al., 1998), but few studies have measured genetic correlations between wood properties and leaf traits of mature trees in spite of well-established correlations between SLA and relative growth rate.

Nitrogen is an essential element in the photosynthetic apparatus of a plant, and therefore critical for growth. The concentration of foliar nitrogen in plant tissue is influenced by environmental factors such as soils, latitude and mean annual temperature (REICH and OLEKSYN, 2004; KANG et al., 2011). Foliar nitrogen also has genetic underpinnings as revealed in common gardens (OLEKSYN et al., 1998). For example, selected genotypes of interior spruce (*Picea glauca* (Moench) Voss x *Picea engelmanii* Parry ex Engelm.) showed higher efficiencies in nitrogen use (MILLER and HAWKINS, 2003; HAWKINS, 2007; MILLER and HAWKINS, 2007) compared to slower-growing genotypes. In loblolly pine, tall trees allocated proportionately more biomass to stem mass in high- vs low-nitrogen environments (LI et al., 1991). The nitrogen content of foliar tissue also varies stoichiometrically based on concentrations of available mineral pools (CHAPIN et al., 1986). We are interested in using a genetic analysis approach to determine if foliar N varies with tree size, but disentangling efficiencies in nitrogen use from site-induced stoichiometric limitations is beyond the scope of this study.

In white spruce, high growth rates are associated with reduced wood specific gravity and early bud-break (WILKINSON, 1977) but other traits, neutral to adaptation and value, may covary with wood volume growth as well. For example, shifts in allocation to leaf area that may accompany increases in wood production, could potentially serve as a proxy for future growth if age-age correlations were confirmed. In order to assess the possibility of indirect selection, we are testing the hypothesis that genetic correlations between foliar and wood traits will be positive and significantly different from zero. Our primary objective is to determine if specific leaf area and leaf area ratio, traits that should be selectively neutral, are associated with increased growth in mature white spruce. This information may assist tree improvement programs in making selections and elucidate trade-offs in wood specific gravity that result from selection for volume growth.

Materials and Methods

Description of the study sites

In 1986, a progeny test consisting of 292 open-pollinated half-sib families of white spruce was

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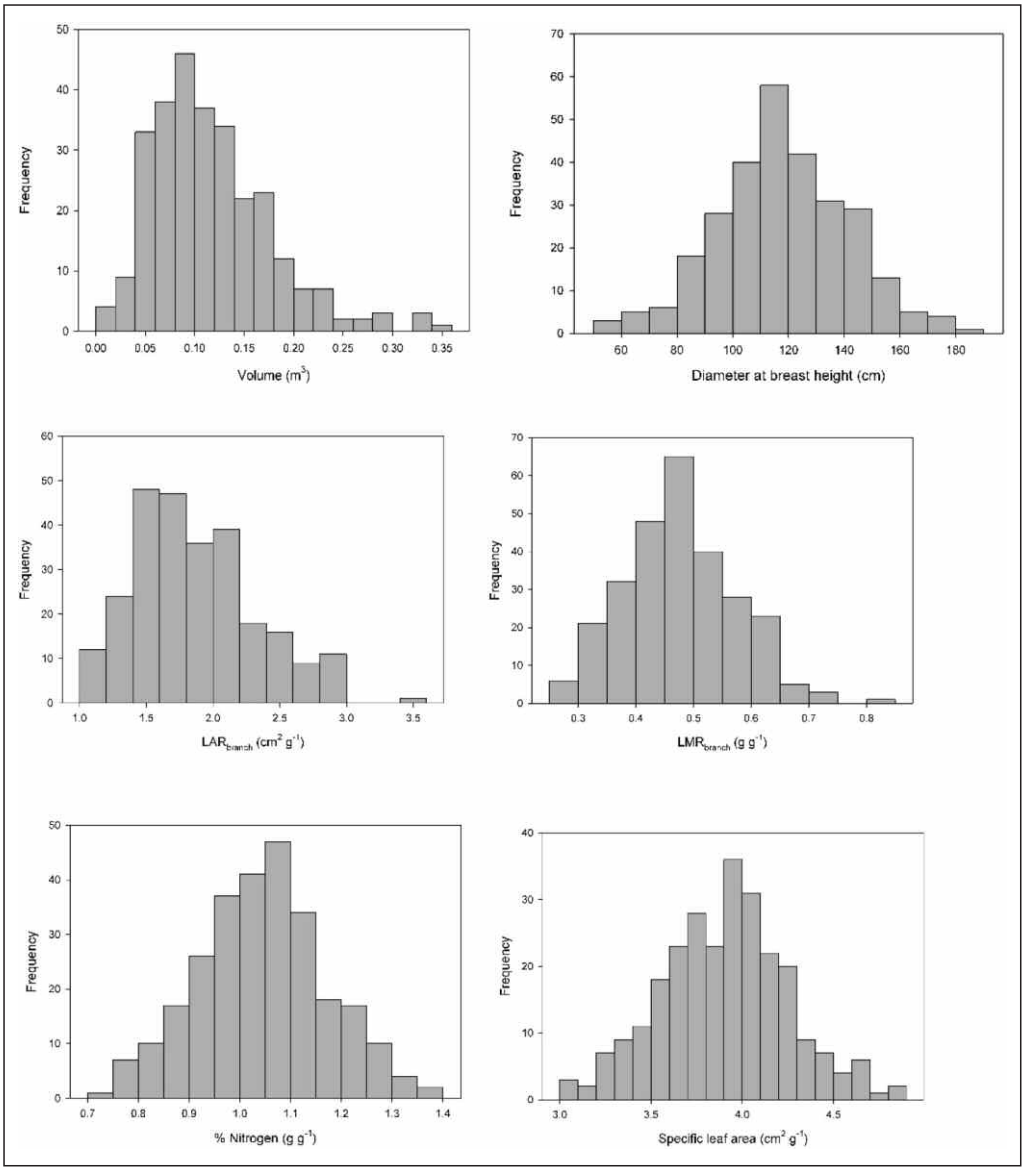
established at five sites in Minnesota. Two sites are the focus of this study: one located near Finland, MN (47°24'N, –91°14'W, MAT = 3.8 °C, MAP = 78 cm) hereafter referred to as "North" and one within the Nemadji State Forest in central Minnesota (46°24'N, –92°29'W, MAT = 3.8 °C, MAP = 78 cm) (State Climatology Office, MN DNR Division of Ecological and Water Resources) hereafter referred to as "South." These sites were selected because tree survival was high in the 25th year, exceeding 85% at both sites, and they represent distinct seed zones and floristic regions. Soils at each site were sampled and tested previously for macronutrients, texture, and organic matter. At North site, soil was a clay loam, pH 5.6, with 0.8% organic matter while South site had sandy loam soil with a pH of 5.5, also with 0.8% organic matter (KLEVORN, 1995).

The progeny test was designed as a randomized complete block design with five blocks and one four-tree row plot per family in each block at five sites. Each family was planted at a minimum of three sites, and some families were replicated at all five sites. All trees in the progeny test were planted in May 1986 at roughly 1.2 x 2.4 meter spacing. Tree heights were measured in 1990 and 1993, five and eight growing seasons, respectively, after planting (KLEVORN, 1995). In 2000, both sites were thinned from four- to two-tree row plots, removing alternate trees in the row plots to increase spacing to 2.4 x 4.8 meters. Tree heights and diameters were measured again in 2005 and



S-Figure 1. – Histograms for all traits by individual tree (n ~ 300).

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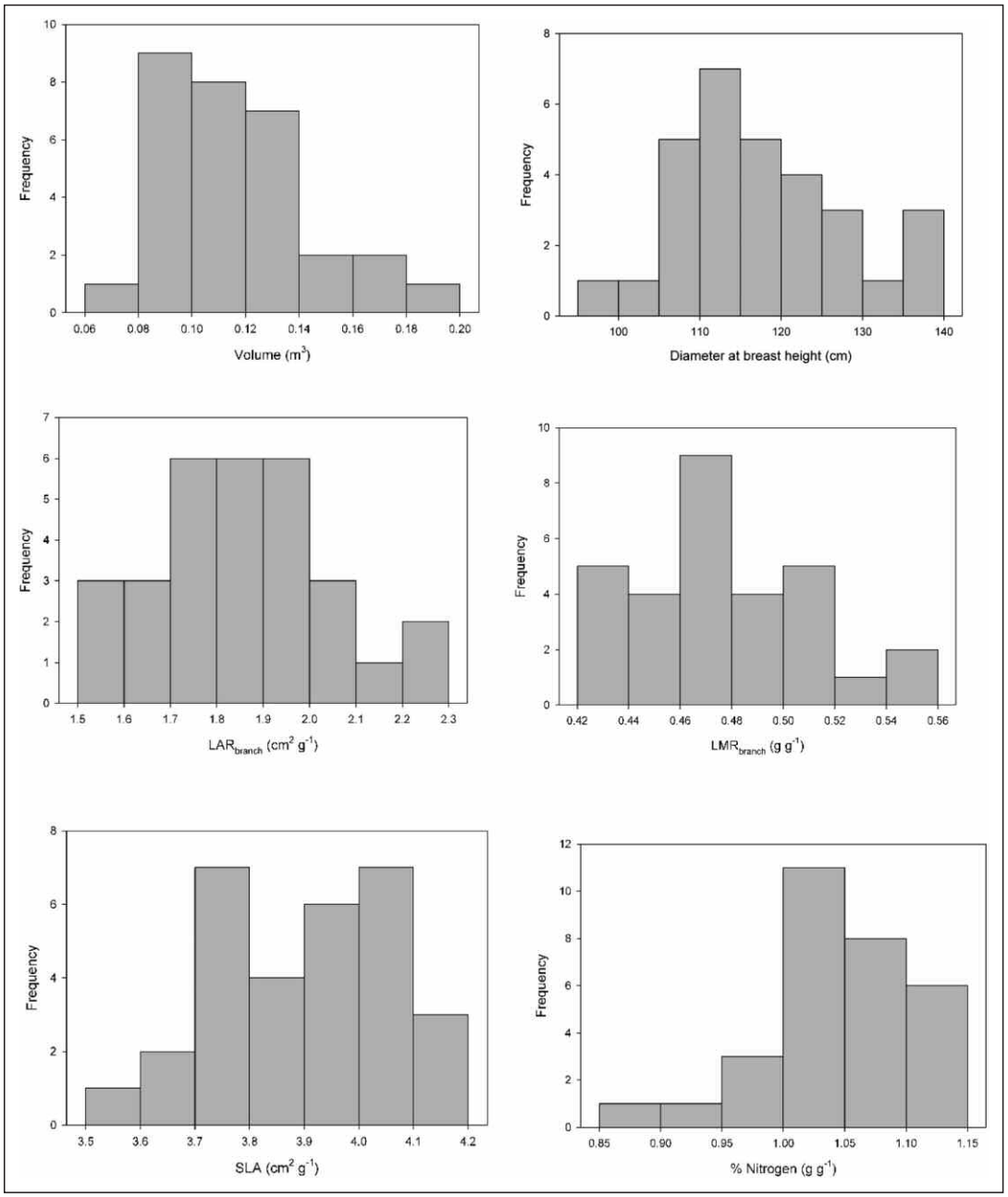
2010 after 20 and 25 growing seasons, respectively. Volume was calculated for each tree (Equation 1) after (Ek, 1985), where BA is the basal area:

$$\text{Volume}(m^3) = ((0.42 + 0.006 * 30 - \text{Height}) * \text{Height} * \text{BA}) * 0.0283 \quad \text{Equation 1}$$

Tree volumes (year 25) were transformed to the cube-root to improve variance heterogeneity, and averaged by family across sites using ordinary least-squared (OLS) means.

Criteria for sample selection

We selected 300 trees for intensive study representing 30 families (five trees per site per family), divided evenly among each of three initial selection tiers based on their least-squared mean volumes at 25 years of age (10 genotypes per tier): top (ranks 1-91), middle (119-193), and low (202-292). Families were selected randomly within each tier, requiring only that the selected families were replicated at the two study sites. Families from the top tier are most



S-Figure 2. – Histograms of family means (30 families) for all traits, ten each from top, middle and low tiers (see *Table 1* caption for description of tiers). N = 10 for each family.

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commonly represented in seed orchards, while those from middle or lower tiers are generally excluded because of low performance. One tree was selected from each of the five two-tree row plots at each site, favoring co-dominant trees with no stem deformations, for a total of five trees per family per site. When both trees in the row-plot were co-dominant one tree was selected at random. When neither tree met our criteria that replication was skipped but in most cases at least one suitable tree was available. In the end, we sampled 284 trees.

Selection tiers (herein referred to as tiers) were refined further using breeding values calculated as twice the best linear unbiased predictor (BLUP) from the 25-year volumes. Families were sorted in ascending order, and divided evenly into three post hoc tiers each with ten families (top, middle and low). The post hoc tiers allowed us to more accurately correlate wood volume with wood specific gravity and foliar traits, and replaced the initial tier assignments.

Protocol for collection of plant material

All plant material was collected between July and August 2008, after growth extension was completed but before trees were fully dormant. One wood core and one branch sample per tree were collected as described below for assessments of wood specific gravity and leaf traits, respectively. The wood core was collected at 1.3 meters above the ground, from bark to pith with bark plug removed, using a 12 mm-diameter standard increment borer (Haglof®). The core was then placed into a precut PVC tube, and sealed with a cork for transportation to the lab. Within six hours of collection, the volume of each core was assessed using water displacement technique (SMITH, 1954; SIMPSON, 1993). Each core was submerged in a flask of tap water with known volume, and the amount displaced was recorded to the nearest 1 mg. The cores were dried at 100 °C for a minimum of five days and stored in a desiccant chamber until it reached room temperature, then removed and immediately weighed to the nearest 0.0001 grams. Wood specific gravity was calculated as the green volume divided by dried mass.

One branch, selected from the upper third of the south side of the tree crown was collected using pole pruners from each selected tree. Each branch, approximately one meter in length and containing multiple cohorts of needles, was refrigerated (1°C) until processing. Approximately 40 needles excised from the previous year growth were scanned (Hewlett Packard Scanjet 6100C). Needle images were inspected for foreign particles that were manually erased, along with shadows, from each image. Using ImageJ software, the projected surface area of each composite needle sample was calculated (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011). After scanning, needles were dried at 65 °C for at least four days prior to weighing. Specific leaf area (SLA) was calculated as leaf area per gram leaf, $cm^2 \cdot g^{-1}$.

Branch sections that contained one-year old needles were excised and dried at 65 °C for a minimum of five days. After drying, dried woody material and needles were separated and weighed to the nearest 0.001 gram. The dried

Table 1. – Least-squared means for traits among tiers (low, middle and top). Tiers were assigned based on breeding values for tree volume at year 25 so that families in the top tier had the highest breeding values. Five trees were sampled per family at each of two sites. Traits include tree height (m), diameter at breast height (dbh; mm), volume (m^3), and wood specific gravity (WSG), Leaf area ratio ($cm^2\ g^{-1}$; LAR_{branch}), leaf mass ratio (g; LMR_{branch}), specific leaf area of ($cm \cdot g^{-1}$; SLA) and foliar % nitrogen (%N) were all sampled from last year's foliage from a branch collected from the upper crown. Means between top and low tiers were significantly different using Tukey's test for height, dbh, volume, and LAR. Middle tier families were similar to top and lower tiers for all traits.

		Low	Middle	Top
Height	Mean	7.45	7.60	8.00
	SE	1.39	1.38	1.36
DBH	Mean	111	116	125
	SE	2.8	2.8	2.8
Volume	Mean	0.100	0.114	0.136
	SE	0.007	0.007	0.007
WSG	Mean	0.371	0.359	0.356
	SE	0.004	0.004	0.004
LAR_{branch}	Mean	1.83	1.82	1.93
	SE	0.06	0.06	1.06
LMR_{branch}	Mean	0.470	0.467	0.486
	SE	0.011	0.011	0.011
SLA	Mean	3.87	3.90	3.96
	SE	0.05	0.05	0.05
Foliar % N	Mean	1.02	1.06	1.06
	SE	0.02	0.02	0.02

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woody material was also weighed to the nearest 0.001 gram. After weighing, a sample of needles was ground and assessed for elemental nitrogen by sample mass (%N) on a COSTECH Analytical ECS 4010 (C. McFadden, University of Nebraska). Branch leaf area ratio (LAR_{branch}, $cm^2 \cdot g^{-1}$) was calculated as leaf area (using the leaf area from the SLA as a sample for the branch) divided by the dried mass (needle plus woody stem) of the branch. Branch leaf mass ratio, $g \cdot g^{-1}$ (LMR_{branch}), was calculated as the needle mass / needle+stem mass for the sample branch.

Statistical analysis

All data distributions and variances were checked for each trait with SAS/STAT (Proc univariate, SAS Institute, Inc. 2011 version 9.3). Two outlier points that fell outside three standard deviations of the normal distribution were removed. Distribution of the remaining points was approximately normal, so no additional data transformations were attempted (*S-Figures 1* and *2*).

We used mixed linear models in SAS/STAT (Proc mixed) to compare the sites and tiers using the model (Equation 2):

$$Y_{ijkl} = \mu + Site_i + Tier_j + Site*Tier_{ij} + Block\ (Site)_{i(k)} + Fam(Tier)_{l(j)} + Site*Fam(Tier)_{i(j)k} + e_{ijkl} \quad \text{Equation 2}$$

where Y_{ijkl} is the observed value of the l^{th} tree in the i^{th} site, j^{th} tier, l^{th} family, and the k^{th} block, with e_{ijkl} as the pooled error. Site, tier, and block(site)) were set as fixed variables. Families, selected at random from within each tier, were designated as random, along with site*family interactions. Tiers were compared using Tukey's HSD adjusted for multiplicity for all traits (tree height, diameter, volume, LAR_{branch}, LMR_{branch}, SLA, and foliar %N). Degrees of freedom were adjusted with Kenward-Roger correction (KENWARD and ROGER, 1997).

Site*family interactions were compared by removing tier and tier*site interactions:

$$Y_{ijkl} = \mu + Site_i + Block(Site)_{i(k)} + Fam_l + Site*Fam_{il} + e_{ikl} \quad \text{Equation 3}$$

Site, family, block(site), family and site*family interactions were set as fixed effects. The significance of this g x e interaction was tested against the mean square error with 29 and 284 degrees of freedom.

Table 2. – Least-squared means, standard errors across families for traits (see *table 1* for descriptions) at each of the two sites (approximately five trees per each of 30 families at each site). Variance components for family (V_f), family by site interaction (V_{fxs}) and residual error (V_e) are shown. Individual (h^2_i) and family heritabilities (H^2_f) and their standard errors were calculated with Delta method and averaged across sites with equations 5 and 6 (in text).

Trait	North (SE)	South (SE)	V_f	V_{fxs}	V_e	h_i^2 (SE)	H_f^2 (SE)
Height	7.27 (0.08)	8.19 (0.11)	0.11	0.00	1.13	0.34 (0.17)	0.35 (0.15)
DBH	113.2 (1.8)	122.5 (2.1)	51.8	0.00	474	0.39 (0.19)	0.52 (0.14)
Volume	0.035 (0.001)	0.045 (0.001)	0.0004	0.00	0.0031	0.44 (0.20)	0.55 (0.13)
WSG	0.365 (0.002)	0.359 (0.003)	0.00007	0.000005	0.0009	0.28) (0.19)	0.42 (0.22)
LAR_{branch}	1.78 (0.05)	1.97 (0.06)	0.009	0.002	0.189	0.19 (0.21)	0.32 (0.28)
LMR_{branch}	0.46 (0.03)	0.48 (0.01)	0.003	0.00	0.009	0.12 (0.15)	0.24 (0.23)
SLA	3.83 (0.07)	4.01 (0.09)	0.116	0.00	0.104	0.40 (0.20)	0.53 (0.14)
Foliar % N	1.01 (0.01)	1.08 (0.01)	0.0007	0.00	0.0120	0.19 (0.26)	0.25 (0.29)

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Table 3. – Genetic correlations (above the diagonal with standard errors in parentheses), and Pearson correlations (below the diagonal). Pearson correlations with $p<0.05$ are boldfaced; genetic correlations are boldfaced if the absolute value differs from zero (absolute value minus standard error is greater than zero). Trait abbreviations are described in *Table 1*. Genetic correlations that have zero in the numerator are not calculable (NC).

	Volume	WSG	LAR_{branch}	LMR_{branch}	SLA	%N
Volume	---	**-0.59 (0.31)**	**0.90 (0.08)**	**0.73 (0.42)**	**0.99 (0.06)**	-0.12 (0.38)
WSG	**-0.27**	---	-0.21 (0.49)	-0.24 (0.45)	**0.99 (0.01)**	NC
LAR_{branch}	**0.36**	-0.07	---	**0.89 (0.12)**	**0.78 (0.07)**	-0.47 (0.46)
LMR_{branch}	**0.33**	-0.08	**0.93**	---	**0.38 (0.52)**	NC
SLA	**0.19**	-0.04	**0.59**	**0.26**	---	NC
%N	0.10	**-0.17**	**0.13**	-0.04	**0.40**	---

Individual (h^2_i) and family (H^2_f) heritabilities were calculated with SAS (Proc mixed) with a model containing site (fixed term), block (site), family, and site∗family as random terms, and errors calculated with Delta method (LYNCH and WALSH, 1998) in Proc IML (Personal communication, Dr Fikret Isik, NC State University, July 2015) with the equations:

$$h_i^2 = \frac{4*V_f}{V_f+V_{b(s)}+V_{fs}+V_e} \qquad \text{Equation 4}$$

$$H_f^2 = \frac{V_f}{V_f+{V_{b(s)}}/{BS}+{V_{fs}}/{S}+{e}/{NBS}} \qquad \text{Equation 5}$$

where V_f, $V_{b(s)}$, $V_{f(s)}$, and e represent the variance due to family, block within site, family∗site, and residual error, respectively. The number of blocks (B=5), sites (S=2), families (F=30) and trees per plot (N=1) are used for estimations of family heritability (Equation 5).

Pearson correlations (phenotypic) were obtained using SAS/STAT (Proc corr) for all trait combinations and evaluated at $p<0.05$. Genetic correlations were calculated from covariance components in SAS Proc mixed, with heterogenous variances for family and sites (Personal communication, Dr Fikret Isik, NC State University). The genetic correlation, r_g, was calculated for traits x and y:

$$r_g = \frac{cov_{Fxy}}{\sqrt{V_{Fx} \cdot V_{Fy}}} \qquad \text{Equation 6}$$

where $covF_{xy}$ is the cross-product differences of traits x and y for each half-sib family; V_{Fx} is the variance among half sib families for trait x and V_{Fy} is the variance among half sib families for trait y. Best linear unbiased predictors (BLUPs) were calculated for each family using the model in Equation 1, and converted to breeding values by multiplying each BLUP by two, assuming families are true half-sibs (WHITE and HODGE, 1989). Standard errors for predictions from SAS output were used for illustration. Standard errors for genetic correlations were calculated using the Delta Method (LYNCH and WALSH, 1998) in Proc IML (Personal communication, Dr Fikret Isik, NC State University). Genetic gains were calculated by multiplying breeding values by family heritabilities, then dividing by the grand mean (WHITE and HODGE, 1989).

Results

Selection tiers differed significantly for tree height, diameter, volume, and LAR_{branch}:

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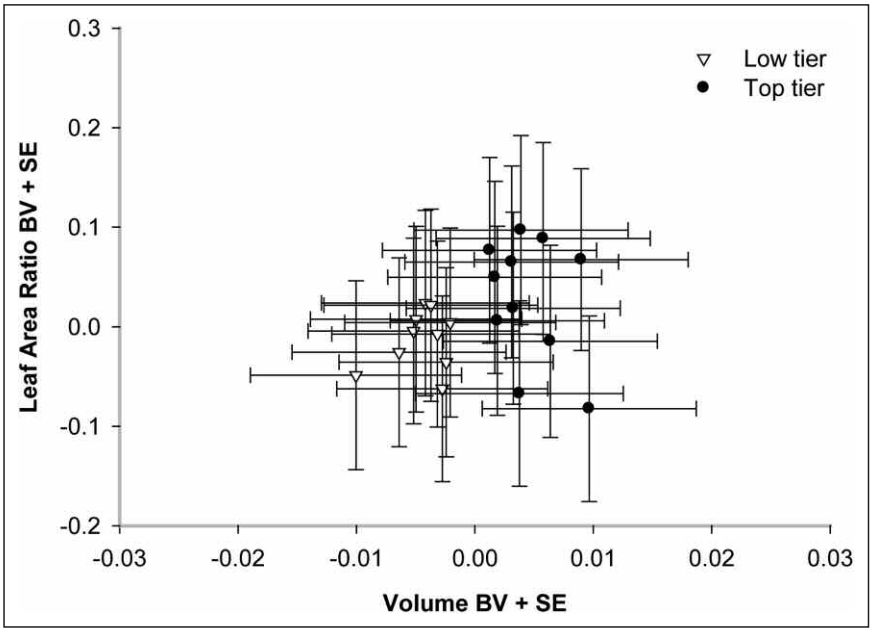

Figure 1. – Breeding values (BV) for 20 open-pollinated families, ten each from low and top-tiers, calculated from 25-year tree volumes at two locations of a progeny test. Breeding values were calculated as 2*BLUPs for 25-year volume and leaf area ratio. Tier assignments are described in *Table 1*. Leaf Area Ratio ($cm \cdot g^{-1}$) was calculated from a single branch sample collected per tree. Standard errors for predicted values were obtained from SAS Proc mixed output.

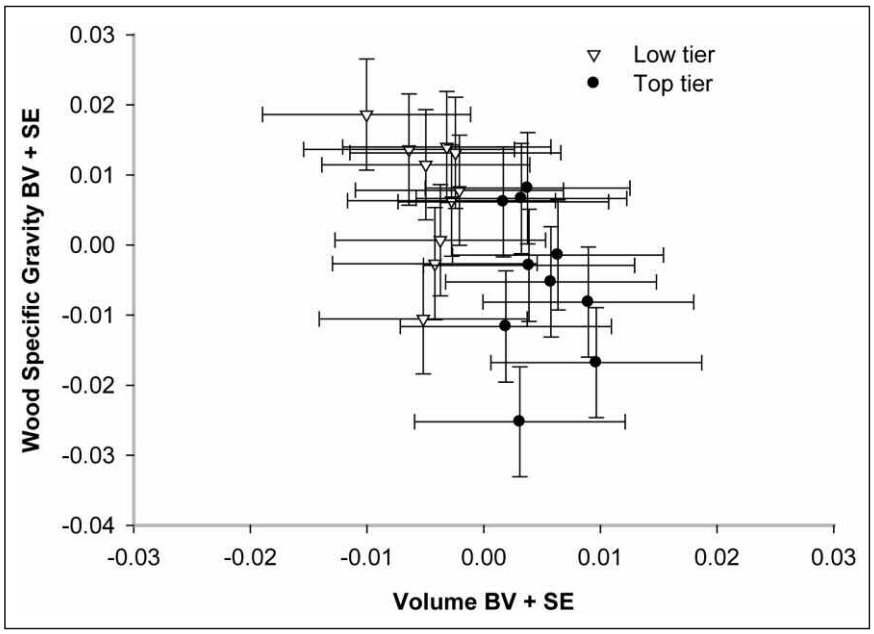

Figure 2. – Breeding values (BV) for 20 families, ten each from low and top-tiers calculated from 25-year tree volumes at two locations of a progeny test. Breeding values were calculated as 2*BLUPs for 25-year volume and leaf area ratio. Tier assignments are described in *Table 1*. Breeding values (BV) for wood specific gravity (WSG) were calculated using volumetric method from one core collected per tree at each of two sites (five samples per family per site). Standard errors for predicted values were obtained from SAS Proc mixed output.

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Table 4. – Genetic gains (%) for tree volume (after 25 growing seasons), wood specific gravity (WSG), leaf area ratio of an upper branch (LAR_{branch}), and specific leaf area (SLA) by tier relative to grand means for the trees sampled across two sites. Tier assignments are described in *Table 1*. Genetic gains were calculated as the % difference of the breeding value*family heritability (H^2_f), relative to the grand mean (across sites, n ~ 300). No genotype by environment interaction was significant for any trait.

Tier	Volume	WSG	LAR_{branch}	SLA
Low	-24.7%	0.3%	-0.4%	-2.3%
Mid	-1.9%	-0.1%	-0.3%	-0.4%
Top	26.6%	-0.2%	0.7%	2.7%

top-tier trees were significantly larger (volume, height, diameter) with larger values of LAR_{branch} than the low tier (*Table 1*). Mid-tier families were intermediate to top and low tiers for all traits.

Trees at South site were significantly taller with larger volumes than the North site (*Table 2*). In addition, foliar %N, and SLA were significantly higher at South than North site. Wood specific gravity (WSG), LAR_{branch} and LMR_{branch} did not differ significantly between sites (*Table 2*). Site by family (i.e. g x e) interactions were not significant for any variable.

Individual heritabilities ranged from 0.12 (LMR_{branch}) to 0.43 (volume). Family heritabilities ranged from 0.24 (LMR_{branch}) to 0.55 (volume). Standard errors were used to evaluate whether correlations differed from zero. All family heritabilities, except %N, were more than 1 SE from zero.

Tree volume was positively correlated, both phenotypically (r_p) and genetically (r_g), with LAR_{branch}, LMR_{branch} and SLA_{branch} (*Table 3*, *Figure 1*). Genetic correlations between tree volume and WSG were negative and significantly different from zero (*Table 3*, *Figure 2*). Correlations between WSG and foliar traits (LAR_{branch}, LMR_{branch}) did not differ from zero, and were incalculable with SLA because of zero covariance in the numerator. Genetic correlations were also incalculable between %N and WSG, SLA, and LMR.

Genetic gains were calculated for each family and averaged by tier (*Table 4*). Gains for top-tier families were 26.6% in volume relative to the mean of families, and 0.7% for LAR_{branch}. Losses in WSG were relatively small (–0.2%) for top tier families with gains of 0.3% for low-tier families. Gains for SLA were 2.7% for top tier families.

Discussion

Selection for volume growth in this limited population of white spruce occurred in tandem with increases in LAR and SLA, supporting our hypothesis that top-tier families possess needles that may be more efficient at light interception than low-tier families. This single-year assessment of leaf area would be further supported for tree breeders if repeated measures of LAR or SLA were consistently higher for larger trees over the course of the rotation. It is likely that we avoided ontogeny effects since our measurements occurred after year 10, noteworthy as the asymptote for SLA in Sitka spruce (Steele et al., 1989).

Significant positive genetic correlations between tree size and SLA occurred in spite of variation inherent to sampling leaf area in conifers. We attempted to limit sampling variation by excising branches consistently from the same approximate height and aspect, rejecting branches with broader and less xeric shade leaves, and excising the same cohort of needles from every tree (previous years' needles). We could not avoid other sources of variation, notably that spruce needles are approximately rhomboid (Niinemets et al., 1995), so that two-dimensional images of needles to derive SLA may under represent leaf area. In addition, low image-quality of scans required manual removal of shadows from each needle/image. Bigger trees are associated with higher foliage biomass and projected leaf area in spruce (Power et al., 2014), but our study is among the few to assess genetic parameters for these traits. Leaf mass (LMR), which can be assessed with greater precision because it does not include leaf area, was less heritable than leaf area (LAR or SLA), and weakly correlated with growth traits, supporting the premise that leaf area is more related to growth than leaf mass.

Our results reaffirm that differences of 0.011 m^3 in volume (*Table 1*), approximately 30% of the grand mean (across sites), are possible by selecting top- instead of low tier families in first-generation white spruce orchards. Predicted gains for volume (26%) were similar to realized gains in field trials in spite of under-

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sampling genotypes from the extreme tails of our initial population. Families were chosen for study based on ranks generated from ordinary least-squared means (OLS), which is less refined (and more confounded with environmental variation) than the ad hoc breeding values. Individual heritabilities for tree height in our dataset exceeded those reported in a previous analysis of the full dataset at year eight (KLEVORN, 1995), which is consistent with earlier work demonstrating clear increases of heritablility with age in white spruce (NIENSTAEDT and RIEMENSCHNEIDER, 1985). Heritability calculations for tree heights from our relatively limited population (h^2_i 0.3, H^2_f 0.4), are similar to other studies in the lake states (HOLST and TEICH, 1969; WILKINSON, 1977; NIENSTAEDT and RIEMENSCHNEIDER, 1985).

Tree volume and WSG were negatively genetically correlated, mirroring other studies in the Lake States (MERRILL and MOHN, 1985; CORRIVEAU et al., 1991; BEAULIEU, 2003). However, our data supports the contention that high growth rates are not necessarily a harbinger of reduced WSG (GASPAR et al., 2009) since three of the top-tier families are predicted to have WSG at or above the mean (*Figure 2*). Selection for genotypes that combine high volume and high WSG appears plausible for advanced first generation seed orchards. Genetic gains and losses for WSG were small in magnitude, less than 1% (*Table 4*). It is possible that our top tier families did not represent extreme values, or that losses in WSG are expected to be low in white spruce. Genetic correlations between wood specific gravity and foliar traits (LAR, LMR, SLA) were not different from zero possibly due to the small sample size that increased sampling errors. Our findings recapitulate that the potential to make genetic improvements for volume growth in white spruce is high, but tradeoffs with WSG may be relatively small.

Heritability estimates for WSG were lower than expected compared to other studies (POLGE and ILLY, 1967; CORRIVEAU et al., 1991; ZHANG et al., 2004), and may be attributed to several factors. Our sample size (five trees per family per site) was small for this type of analysis, a limitation that was partially remedied by a combining data across the two locations. In addition, saturation is a prerequisite for the „maximum moisture content“ method in assessing WSG (SMITH, 1954), but we relied on latent moisture because each sample was placed in a sealed cylinder and assessed volumetrically within a few hours of sampling. Inconsistency in moisture at processing likely increased variability, reducing overall heritability estimates.

The absence of g x e interactions is not surprising since the sites were less than 200 kilometers apart, in latitude. An analysis of the full dataset (292 families across five sites) did not report significant g x e interactions for tree height at year 8. The two sites we chose to study were located in distinct ecological classifications: North site was dominated by spruce and fir, and South site was surrounded by deciduous northern hardwoods. The absence of g x e interaction in our dataset should not be extrapolated to a larger area since our sampling does not approach the range of locales where white spruce is found in this region.

Nitrogen content, in foliage of sample branches, was largely a function of environment, and did not correlate genetically with growth or wood traits. Foliar Nitrogen was generally higher at the South than the North site (*Table 2*) reflecting local differences in nutritional cycling. Another study attributed increased plant nitrogen content on full-sib seedlings with fast-growth in a controlled study of interior spruce (MILLER and HAWKINS, 2003), but did not obtain genetic parameters. Our study on large trees lacks precision and replication to draw a similar conclusion.

Conclusions

Genetic and phenotypic correlations between volume and foliar traits (SLA, LMR_{branch}, LAR_{branch}) were significant and positive, supporting the hypothesis that trees selected for increased wood volume also possessed leaf traits that may increase light interception, a mechanism that may improve photosynthesis and subsequent carbon fixation. Significant negative correlations between volume and wood specific gravity were found, but several top tier families combined high volumes with above average wood specific gravity. Genotype by environment interactions were not significant for any trait between two ecologically distinct sites supporting earlier findings that only one breeding population may be necessary for the state of Minnesota. Future studies that elucidate correlations leaf area and tree volume over time are recommended to determine if specific leaf area or leaf are ratio may be used as predictors for future volume growth.

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Literature Cited

BEAULIEU, J. (2003): Genetic variation in tracheid length and relationships with growth and wood traits in eastern white spruce (*Picea glauca*). Wood Fiber Sci **35**: 609–616.

CHANG, C. I. and R. W. KENNEDY (1967): Influence of specific gravity and growth rate on dry wood production in plantation-grown white spruce. For Chron **43**: 165–173.

CHAPIN, F. S. I., P. M. VITOUSEK and C. K. VAN (1986): The nature of nutrient limitation in plant communities. Am Nat **127**: 48–58.

CORNELISSEN, J. H. C., S. LAVOREL and E. GARNIER et al. (2003): A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot **51**: 335.

CORRIVEAU, A., J. BEAULIEU and G. DAOUST (1991): Heritability and genetic correlations of wood characters of Upper Ottawa Valley white spruce populations grown in Quebec. For Chron **67**: 698–705.

CORRIVEAU, A., J. BEAULIEU and F. MOTHE (1987): Wood density of natural white spruce populations in Quebec. Can J For Res **17**: 675–682.

DUCHESNE I. and S. Y. S. ZHANG (2004): Variation in tree growth, wood density, and pulp fiber properties of 35 white spruce (*Picea glauca* (Moench) Voss) families grown in Quebec. Wood Fiber Sci **36**: 467–475.

EK, A. R. (1985): A formula for the total cubic foot stem volume of small trees in the Lake States. North J Appl For **2**: 3.

GASPAR, M. J., J. L. LOUSADA and J. C. RODRIGUES et al. (2009): Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal? For Ecol Manage **258**: 115–121.

HAWKINS, B. J. (2007): Family variation in nutritional and growth traits in Douglas-fir seedlings. Tree Physiol **27**: 911–919.

HOLST, M. J. and A. H. TEICH (1969): Heritability estimates in Ontario white spruce. Silvae Genet **18**: 23–27.

KANG, H., H. ZHUANG and L. WU et al. (2011): Variation in leaf nitrogen and phosphorus stoichiometry in *Picea abies* across Europe: An analysis based on local observations. For Ecol Manage **261**: 195–202.

KENWARD, M. G. and J. H. ROGER (1997): Small sample inference for fixed effects from restricted maximum likelihood. Biometrics **53**: 983–997.

KLEVORN, R. E. (1995): Genotype-environment interaction for height growth in white spruce. University of Minnesota, Masters of Science Thesis, St Paul USA.

KOCH, G. W., S. C. SILLETT, G. M. JENNINGS and S. D. DAVIS (2004): The limits to tree height. Nature **428**: 851–854.

LAMBERS, H., F. S. CHAPIN and T. L. PONS (2008): Plant Physiological Ecology, Second Edition.: Springer Science + Business media. New York, NY.

LEGNER, N., S. FLECK and C. LEUSCHNER (2014): Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance. Trees **28**: 263–280.

LI, B., H. L. ALLEN and S. MCKEAND (1991): Nitrogen and family effects on biomass allocation of loblolly pine seedlings. Forest Science **37**: 271–283.

LYNCH, M. and B. WALSH (1998): Genetics and Analysis of Quantitative Traits (1st ed). Sinauer Associates, Inc.: Sunderland, MA

MARSHALL, J. D. and R. A. MONSERUD (2003): Foliage height influences specific leaf area of three conifer species. Can J For Res **33**: 164–170.

MARSHALL, J. D., G. E. REHFELDT and R. A. MONSERUD (2001): Family differences in height growth and photosynthetic traits in three conifers. Tree Physiol **21**: 727.

MERRILL, R. E. and C. A. MOHN (1985): Heritability and genetic correlations for stem diameter and branch characteristics in white spruce. Can J For Res **15**: 494–497.

MILLER, B. D. and B. J. HAWKINS (2007): Ammonium and nitrate uptake, nitrogen productivity and biomass allocation in interior spruce families with contrasting growth rates and mineral nutrient preconditioning. Tree Physiol **27**: 901–9.

MILLER, B. D. and B. J. HAWKINS (2003): Nitrogen uptake and utilization by slow-and fast-growing families of interior spruce under contrasting fertility regimes. Can J For Res **33**: 959–966.

NIENSTAEDT, H. and D. RIEMENSCHNEIDER (1985): Changes in heritability estimates with age and site in white spruce, *Picea glauca* (Moench) Voss. Silvae Genet **34**: 34–41.

NIINEMETS, U. and O. KULL (1995): Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: variation in needle morphology. Tree Physiol **15**: 307–15.

OLEKSYN, J., J. MODRZÝNSKI and M. G. TJOELKER et al. (1998): Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. Funct Ecol **12**: 573–590.

PIKE, C., J. WARREN and A. DAVID (2006): Annual Report of the Minnesota Tree Improvement Cooperative. University of Minnesota, St Paul USA.

POLGE, P. H. and G. ILLY (1968): Héritabilité de la densité du bois et corrélations avec la croissance étudiées à l'aide de tests non destructifs sur plants de Pins maritimes de quatre ans. Silvae Genet **17**: 173–181.

POWER, H., R. SCHNEIDER and F. BERNINGER (2014): Understanding changes in black (*Picea mariana*) and white spruce (*Picea glauca*) foliage biomass and leaf area characteristics. Trees **28**: 345–357.

REICH, P. B. and J. OLEKSYN (2004): Global patterns of plant leaf N and P in relation to temperature and latitude. Proc Natl Acad Sci USA **101**: 11001–11006.

REICH, P. B., M. G. TJOELKER and M. B. WALTERS et al. (1998): Close association of RGR, leaf and root

DOI:10.1515/sg-2015-0015
edited by Thünen Institute of Forest Genetics

morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Funct Ecol **12**: 327–338.

REICH, P. B., I. J. WRIGHT and J. CAVENDER-BARES et al. (2003): The evolution of plant functional variation: traits, spectra, and strategies. Int J Plant Sci **164**: S143–S164.

SIMPSON, W. T. (1993): Specific gravity, moisture content, and density relationship for wood. Gen. Tech. Rep. FPL-GTR 76, Madison WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 13 p.

SMITH, D. M. (1954): Maximum moisture content method for determining specific gravity of small wood samples. Report No. 2014, Madison WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

STEELE, M. J., M. P. COUTTS and M. M. YEOMAN (1989): Developmental changes in Sitka spruce as indices of physiological age I. Changes in needle morphology. New Phytol. **113**: 367–375.

STELLRECHT, J. W., C. A. MOHN and W. CROMELL (1974): Productivity of white spruce seed sources in a Minnesota tree planting. Minnesota For Res Notes No. 251.

WENG, Y., K. TOSH and M. FULLARTON (2010): Determining and projecting realised genetic gains: results from early-stage spruce improvement programmes in New Brunswick, Canada. New Zeal J For Sci **40**: 5–17.

WHITE, T. L. and G. R. HODGE (1989): Predicting breeding values with applications in tree improvement. Kluwer Academic Publishers, Dordrecht. The Netherlands.

WILKINSON, R. C. (1977): Inheritance of budbreak and correlation with early height growth in white spruce (*Picea glauca*) from New England. NE Exp Stn Res Pap-391, U.S. Department of Agriculture, Forest Service, Upper Darby, PA.

ZHANG, S. Y. (1995): Effect of growth rate on wood specific gravity and selected mechanical properties in individual species from distinct wood categories. Wood Sci Technol **29**: 451–465.

ZHANG, S. Y., Q. YU and J. BEAULIEU (2004): Genetic variation in veneer quality and its correlation to growth in white spruce. Can J For Res **34**: 1311–1318.

Use of genetic markers to build a new generation of *Eucalyptus pilularis* breeding population

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Abstract

Tree improvement generally proceeds by incremental gains obtained from recurrent selection in large diverse populations but is slow due to long generation times and delay till trees reach assessment age. This places a premium upon extracting data from historic introductions used to found landraces when reinstating modern breeding programs. The value of such resources, however, may be

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