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Appendix 1. – Expected mean square table for individual chamber analysis.

Individual growth chamber analysis.

Source of Variation*	Expected mean squares
M	$\sigma^2 + K1\sigma^2_{MC} + K2\sigma^2_{MP} + K5\phi_M$
Population (P)	$\sigma^2 + K1\sigma^2_{MC} + K2\sigma^2_{MP} + K3\sigma^2_C + K4\sigma^2_P$
Clone(P) (C)	$\sigma^2 + K1\sigma^2_{MC} + K3\sigma^2_C$
MP	$\sigma^2 + K1\sigma^2_{MC} + K2\sigma^2_{MP}$
MC	$\sigma^2 + K1\sigma^2_{MC}$
Error	σ^2

*) Source of variation as described previously in text.
Note: K1 = 2, K2 = 8, K3 = 4 and K4 = 16.

Variance Components, Heritabilities and Gain Estimates for Growth Chamber and Field Performance of *Populus tremuloides*: Growth Parameters

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Summary

Variance components, heritabilities and gain estimates are reported for 29 clones (five populations) of *Populus tremuloides* from Alberta, Canada, which were grown (two years) at two field sites (northern and southern Alberta) and in two controlled environment chambers (for 12 weeks). Results indicated more variation at the clone-within-population level than the population level. There was more variation accounted for by both clone-within-population and population in the growth chamber than in the field. Caliper: clone-growth chamber: 32%, field: 22% to 7%; caliper: population-growth chamber: 12%, field: 2%; height: clone-growth chamber: 7% to 27%; bud-burst: clone-growth chamber: 26%; root-to-shoot ratio: clone-growth chamber: 17%. Broad-sense clone mean heritabilities for caliper were also lower in the field (0.56 to 0.29), than in the growth chambers (0.80). Heritabilities in the growth chamber were: Bud-burst = 0.72, height = 0.74, root-to-shoot ratio = 0.59. Expected gains, estimated based on growth chamber data, were: 9% to 38% across populations for bud-burst, 11% to 24% for caliper, 12% to 22% for final height, and 8% to 19% for root-to-shoot. This study indicates that significant improvement in traits is possible in trembling aspen with a 17% selection intensity but that care must be taken in determining the size of the region that will comprise the population where selections are made.

Key words: trembling aspen, heritability, gain, variance components, growth chambers, field, morphology.

FDC: 232.11; 165.3; 165.5; 56; 176.1 *Populus tremuloides*.

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1.0 Introduction

Trembling aspen (*Populus tremuloides* MICHAUX) is the most widely distributed native tree species in North America (BURNS and HONKALA, 1990) and is a major component of the boreal forest. Its distribution in western Canada is largely controlled by moisture availability, particularly at its southern limit (HOGG, 1994). With an ever increasing demand on this timber resource by forest companies in western Canada, a need for quantitative genetic parameter estimates, such as baseline values of heritabilities and gains, has arisen. The study results presented here should assist the recent initiatives of the Western Boreal Aspen Cooperative, which is establishing a tree improvement program for aspen in Alberta (LI, 1995).

As is true of any tree improvement program, there are many years of investment required prior to accruing any benefits and therefore it is crucial that selections and testing consider future needs and possible environmental changes. Current general circulation models predict increases in atmospheric CO_2 and temperature (BOLIN et al., 1986), as well as reductions in soil moisture for the Canadian boreal forest (MANABE and WETHERALD, 1986). HOGG (1994) has shown that the regions at low elevations, where the majority of the future harvesting will be focussed, will be most affected by these changes in western Canada. Thus, the question of performance under moisture stress conditions could become very important over the next 30 to 50 years. A few studies (JIANG et al., 1989; WU et al., 1992) have indicated that early performance under controlled conditions may be reflective of later field performance and if testing and selections can be made based on growth chamber trials,

Table 1. – Latitude, longitude and elevation for 5 collection sites and 2 field plantations.

(C)ollection/(P)lantation	Location	Latitude	Longitude	Elevation(m)
C	Calgary	50°50'	114°05'	1006
P	Calgary	51°03'	114°05'	1112
C	Lougheed	52°44'	111°33'	663
C	Dapp	54°22'	113°55'	614
C	Little Buffalo	56°26'	116°08'	732
C and P	Meander River	59°30'	117°00'	320

Table 2. – Temperatures and photoperiod for growth chambers.

CHAMBER	Calgary (South)			Meander River (North)		
Week[1]	Night	Day (°C)	Daylight	Night	Day (°C)	Daylight
May 6-19	-1	17	15hr 16min	1	16	16hr 25min
May 20-Jun 2	3	22	15hr 56min	3	19	17hr 23min
Jun 3-16	4	21	16hr 23min	6	19	18hr 4min
Jun 17-30	6	23	16hr 33min	9	20	18hr 21min
Jul 1-14	8	24	16hr 24min	10	21	18hr 6min
Jul 15-28	9	24	15hr 58min	11	21	17hr 26min

[1]) Represents week in spring conditions.

the lag time between selections and realized gains could be significantly reduced. The aspen tree improvement program would benefit by reducing the time required to make selections.

We present results from a two-year field study and three-month growth chamber experiment using 29 male clones of aspen representing five populations from Alberta, Canada. Diameter at the root collar (caliper) was measured four times over two growing seasons in the field and bud-burst, height, caliper and root-to-shoot ratio were measured in the growth chambers. From these data, the distribution of genetic variation among populations and clones-within-populations was determined and variance components, broad-sense clone mean heritabilities, and gain estimates were calculated based on clonal selections within population.

2.0 Materials and Methods

2.1 Collections, propagation and experimental setup

Twenty-nine clones of trembling aspen were collected from five populations (5 to 6 clones per population) from Calgary (south) to Meander River (north), Alberta, Canada (*Table 1*), in Spring, 1992. Collections were restricted to male clones due to the high male-to-female ratio in Alberta (approximately 5:1, based on flowering incidence, pers. obs.). Clones were randomly picked within an area (> 1 km between selections) and thirty sections of surface lateral roots (10 cm to 30 cm long, 0.5 cm to 3 cm diameter), were collected by hand and kept dark and cool (2° C) until used for clonal propagation.

Roots were planted in trays in vermiculite and placed in a greenhouse. Emerging suckers (4 cm height) were cut and rooted in Spencer Lemaire 'Rootrainers' filled with Metromix. After two weeks rooted suckers were placed outside to harden-off. Stecklings from each clone were randomly assigned to the field or growth chamber experiment (THOMAS, 1996).

Field trials were established in mid-August 1992, at Meander River (north) (Hay River Tree Improvement Research Facility, Footner Lake Forest District) and Calgary (south) (University of Calgary Grounds Department Nursery) (*Table 1*) using a randomized complete block design with two blocks and three replicates (non-contiguous planting) per block per clone with 1.5 m spacing within and between rows (9 trees per row) for a total of 174 stecklings per site. A border row of surround trees was also planted with the same spacing. Sites were weeded twice each growing season. Both sites were fenced, although only Meander River was kept free of deer and rabbits. There was no supplemental watering or fertilizer applied at either site.

Stecklings assigned to the growth chamber experiment were maintained at 2° C with a 3 hour photoperiod and watered weekly for 6 months and then repotted into 130 mm (5") pots. A moisture treatment was applied through the use of two growing media (Metromix or 2:1 Metromix: graded sand [Manus Abrasives RAM silite #12, Edmonton, AB. Canada]). The sand reduced the water holding capacity by 25% by weight. All pots were also topped with 1 cm of sand. Slow release fertilizer (Osmocote 14-14-14) and micronutrients (dolomite lime, super phosphate, iron chelate and fritted trace elements) were also mixed into the potting media. After 60 days of growth, supplemental fertilizer (1 g/litre $MgSO_4$, 0.05 g/litre Fe, 0.5 g/litre 20-20-20) was applied every 5 days until harvest.

The experiment included two growth chambers programmed to represent the average (30-year) temperatures and photoperiod for the spring period (May 6 through to July 28) for each field site. The diurnal temperature and photoperiod programming was adjusted every two weeks (*Table 2*). There were three replicates per moisture treatment per clone in each chamber. Pots were re-randomized after each height measurement (four times) (LEE and RAULINGS, 1982). The growth chamber experiment ran for 12 weeks (83 days).

2.2 Data collection

At the field sites, caliper (mm) was measured on each steckling four times in June and August of 1993 and 1994. Height data were not used because of deer browsing. In the growth chambers, bud-burst was evaluated after two weeks using a score of 0 to 4 (no development – full flush), height (cm) was measured five times (Days 0, 27, 41, 62 and 83), caliper (mm) on Day 83, and root-to-shoot ratios were calculated based on dry weights (roots, shoots and leaves) at harvest.

2.3 Statistical models and data analyses

Individual field caliper measurements were analysed for both sites combined using analysis of variance according to the following linear model:

$$1]\ Y_{ijklm} = \mu + L_i + B_{j(i)} + P_k + C_{l(k)} + L_iP_k + L_iC_{l(k)} + B_{j(i)}P_k + B_{j(i)}C_{l(k)} + e_{m(ijkl)}$$

where Y_{ijklm} is an observation on the mth tree from the lth clone in the kth population in the jth block of the ith location, μ is the overall mean, L_i: effect of the ith location (i = 1,2), $B_{j(i)}$: effect due to the jth block in the ith location (i = 1,2), P_k: effect of the kth population (k = 1, ..., 5), $C_{l(k)}$: effect of the lth clone nested in the kth population (l = 1, ..., 6), L_iP_k: interaction of the ith location and the kth population, $L_iC_{l(k)}$: interaction of the ith location and the lth clone nested in the kth population, $B_{j(i)}P_k$: interaction of the jth block in the ith location and the kth population, $B_{j(i)}C_{l(k)}$: interaction of the jth block in the ith location and the lth clone nested in the kth population and $e_{m(ijkl)}$ is the random error. Location was considered to be a fixed effect while all other terms were considered random effects. See *Appendix 1a* for source of variation and expected mean squares. Subsequently, the two sites were analysed individually according to model [2]:

$$[2]\ Y_{jklm} = \mu + B_j + P_k + C_{l(k)} + B_jP_k + B_jC_{l(k)} + e_{m(jkl)}$$

where terms are as above except block is not nested in location and all terms were considered random.

All growth chamber data were analysed using analysis of variance based on model [3]:

$$[3]\ Y_{ijklm} = \mu + CH_i + M_j + P_k + CH_iM_j + CH_iP_k + C_{l(k)} + CH_iC_{l(k)} + M_jP_k + M_jC_{l(k)} + CH_iM_jP_k + CH_iM_jC_{l(k)} + e_{m(ijkl)}$$

where Y_{ijklm} is an observation on the mth tree from the lth clone in the kth population in the jth moisture and ith chamber, μ is the overall mean, CH_i: effect due to the ith chamber (i = 1,2), M_j is the effect due to the jth moisture (j = 1,2), P_k: effect due to the kth population (k = 1, ..., 5), CH_iM_j: interaction of the ith chamber and the jth moisture, CH_iP_k: interaction of the ith chamber and the kth population, $C_{l(k)}$: effect due to the lth clone in the kth population (l = 1, ..., 6), $CH_iC_{l(k)}$: interaction of the ith chamber and the lth clone in the kth population, M_jP_k: interaction of the jth moisture and the kth population, $M_jC_{l(k)}$: interaction of the jth moisture and the lth clone in the kth population, $CH_iM_jP_k$: interaction of the ith chamber and the jth moisture and the kth population, $CH_iM_jC_{l(k)}$: interaction of the ith chamber and the jth moisture and the lth clone in the kth population and $e_{m(ijkl)}$ is the random error. For this analysis moisture and chamber by moisture were considered to be fixed effects, while all other terms were considered to be random effects. Variance components were calculated by hand using the appropriate mean square values and corresponding expected mean square coefficients generated from PROC GLM, RANDOM/TEST options (SAS Inc., 1985). Negative variance components were assumed to be zero (FINS et al., 1992). Since treatments at the chamber level were not replicated, chamber differences were only examined graphically.

Broad-sense heritabilities were calculated on a clone mean basis. The following equation was used for the field data based on individual sites :

$$[4]\ H^2_C = \frac{\sigma^2_C}{\frac{K2\sigma^2_C}{K2} + \frac{K1\sigma^2_{BC}}{K2} + \frac{Error}{K2}}$$

where K2 is the coefficient associated with the variance due to clone-within-population (σ^2_C) and K1 is the coefficient associated with the variance due to the block by clone-within-population interaction term (σ^2_{BC}) (see *Appendix 1b*). When heritability was calculated on a combined site basis, the variance due to location by clone-within-population (σ^2_{LC}) and its associated coefficient (K3) divided by the clone variance coefficient (K5), was included in the denominator (*Appendix 1a*).

For the growth chamber data heritabilities were calculated using equation [5]:

$$[5]\ H^2_C = \frac{\sigma^2_C}{\frac{K6\sigma^2_C}{K6} + \frac{K5\sigma^2_{CHC}}{K6} + \frac{K3\sigma^2_{MC}}{K6} + \frac{K1\sigma^2_{CHMC}}{K6} + \frac{Error}{K6}}$$

where K6 is the coefficient associated with the variance due to clone-within-population (σ^2_C), K5 is the coefficient associated with the variance due to the chamber by clone-within-population interaction (σ^2_{CHC}), K3 is the coefficient associated with the variance due to moisture by clone-within-population interaction (σ^2_{MC}) and K1 is the coefficient associated with the variance due to chamber by moisture by clone-within-population interaction (σ^2_{CHMC}). Expected mean square tables are in *Appendix 1c* with the corresponding coefficients. For all analyses sources of variation were considered significant at α = 5%.

Gains were calculated using growth chamber data on a clone-within-population basis selecting the best clone out of 5 or 6, (17% selection intensity) and using the broad-sense clone mean heritability estimates. The following general formula was used for gain calculations (FALCONER, 1989):

$$[6]\ \text{Expected Gain} = \text{Selection differential} \times H^2_c$$

where the selection differential = the mean of the best clone in a population minus the overall population mean and H^2_c = associated broad-sense clone mean heritability estimate. Equation [6] gives the actual gains possible if selections were made with the populations sampled.

Standard errors (s.e.) were calculated for the broad-sense heritability (H^2) estimates following the general formula of NYQUIST (1991):

$$[7]\ \text{s.e. } H^2 = X/Y[(\sigma^2_X / X^2) + (\sigma^2_Y / Y^2) - (2\text{Cov}(X,Y) / XY)]^{1/2}$$

where: X and Y are linear functions of mean squares, i.e.: $X = 1/K_c\,(MSc) - 1/K_c\,(MScd)$ and K_c = the coefficient for clone, MSc = the mean square for clone and MScd = the error mean square for clone. The Y function would be comprised of the terms found in the denominator of the heritability equation. (σ^2_x) and (σ^2_y) are the variances of X and Y, i.e.: (σ^2_x) = $(1/K_c)^2(\sigma^2_{MSc}) + (-1/K_c)^2(\sigma^2_{MScd})$ where $\sigma^2_{MSc} = 2(MSc)^2/(dfc + 2)$ and $\sigma^2_{MScd} = 2(MScd)^2/(dfcd + 2)$ and dfc = degrees of freedom for clone, dfcd = degrees of freedom for the clone error term. The (σ^2_y) term was calculated in the same way, again using the terms found in the denominator of the heritability equation.

Field *versus* growth chamber performance was compared using regression analysis: Calgary (August 1994) caliper *versus* Calgary growth chamber (Day 83) caliper and height.

Table 3. – Heritabilities (H^2_c) (s.e.), and percent variation (% Var.) for the 4 caliper measurements at the 2 field sites (combined).

	June 1993 H^2_c=0.60 (0.14)	Aug 1993 H^2_c=0.00 (0.00)	June 1994 H^2_c=0.04 (0.37)	Aug 1994 H^2_c=0.24 (0.24)
Source[1]	% Var.	% Var.	% Var.	% Var.
L		*	*	**
P	0	2.9	0.5	0
LP	0	0.9	4.6	3.5
B(L)	0	18.2**	22.5**	7.9**
C(P)	18.4*	0	0.6	3.5
LC(P)	2.3	13.9*	9.7	0
PB(L)	0	0	0	0
B(L)C(P)	12.4*	13.8*	15.2**	7.7
Error	66.9	50.4	46.9	77.3

[1]) Source effects where L is location (no variance component presented since this term was considered a fixed effect), P is population, LP is location by population, B(L) is block-within location, C(P) is clone-within-population, LC(P) is location by clone-within-population, PB(L) is population by block-within-location, and B(L)C(P) is block-within-location by clone-within-population.

*) $p < 0.05$, **) $p < 0.01$.

3.0 Results

3.1 Field analyses

Relatively little of the variance in caliper in the combined field site analysis was accounted for by clone-within-population (0% to 18.4%) or population (0% to 2.9%). The corresponding error variance ranged from 46.9% to 77.3% (*Table 3*). However, the block-within-location by clone-within-population interaction was significant for the first three sampling dates indicating that during early establishment clone performance was variable between the two sites. Neither population nor location by population (0% to 4.6% of the variance) were significant (*Table 3*) for any test date.

Broad-sense clone mean heritability varied from 0 to 0.60 over two growing seasons (*Table 3*). There low values and the significant effect of location are likely attributable to very dry spring conditions at the Meander River site at the start of both growing seasons and browsing by deer at the Calgary site. The consequence at Meander River was heavy mortality in the first year and minimal growth thereafter (*Figure 1*). Although population was not significant, heritability calculations were based on clone-within-population because of the 9° range in latitude from which the populations were collected and to allow for a comparison with growth chamber calculations where population accounted for substantially more of the variation.

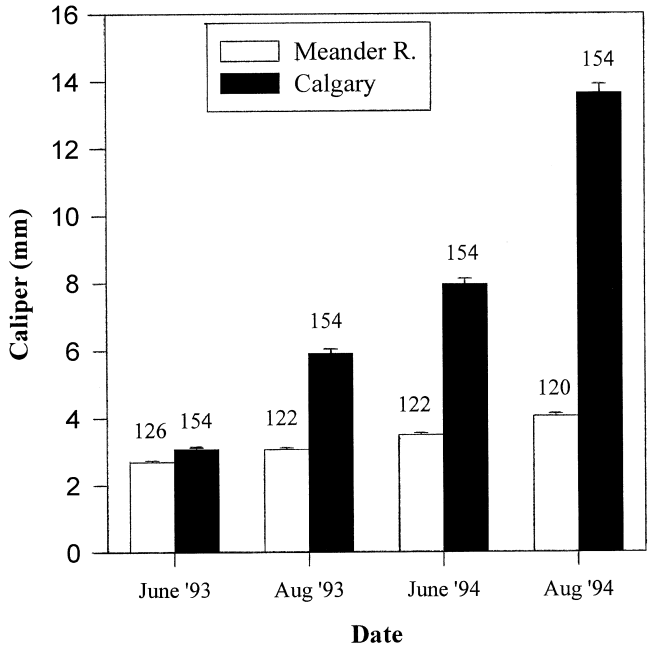

Figure 1. – Caliper (mean + s.e.) for 2 growing seasons at the 2 field sites. N given above bars for each site and date.

Analysis by individual site revealed heritabilities of 0.29 to 0.56 over the two growing seasons for Calgary (*Table 4*) and 0 to 0.46 for Meander River (not presented). Gain estimates are therefore not presented for the field experiment. Except for the first measurement date, the Meander River population (farthest north) showed the poorest performance with Calgary (farthest south) also showing relatively poor performance (*Table 5*). Other populations out-performed the Calgary population at its site of origin, with Little Buffalo ranking first (*Table 5*).

3.2 Growth chamber analyses

In the growth chamber experiment, population explained substantially more of the variation than in the field experiment and was significant for all four height measurements and root-to-shoot ratio. Clone-within-population was significant for bud-burst, height (Days 41, 62 and 83), caliper and root-to-shoot ratio. The amount of variation explained by clone increased over time for height and was higher than that explained by population for the remaining three traits (*Table 6*). These analyses show that, for some traits, variation is distributed both among and within populations of aspen, and proportionally more variation is explained by population when testing is done under controlled environmental conditions.

In contrast to the field results, the Meander River population out-performed the other four populations in the growth chambers, whereas the Calgary population still performed poorly (*Figures 2 and 3*). The Calgary and Little Buffalo populations had the latest bud-burst (population not significant) (*Table 7a*) and also showed poorer height growth (*Figure 2a, b*) in both chambers. The Meander River and Dapp populations were the tallest and had the largest caliper at harvest (*Figure 3a, Table 7d*). Meander River also had the highest root-to-shoot ratio in both chambers (*Figure 3b, Table 7e*).

The drier moisture treatment (Metromix plus sand) resulted in reduced height growth (Days 27 and 83) and a significant ($p < 0.05$) increase in root-to-shoot ratio (*Table 6c*).

Table 4. – Source of variation, percent variation (% Var.) and heritabilities (H^2_c) (s.e.) for caliper over 2 growing seasons at the Calgary site.

	a. June 1993 H^2_c=0.56 (0.17)	b. August 1993 H^2_c=0.51 (0.19)	c. June 1994 H^2_c=0.49 (0.20)	d. August 1994 H^2_c=0.29 (0.28)
Source[1]	% Var.	% Var.	%Var.	%Var.
P	0	4.4	5.5	2.3
B	0	18.4**	21.8**	7.7*
PB	0	0	0	0
C(P)	21.7**	15.9*	14.7*	7.1
BC(P)	5.3	12.7*	13.5*	7.4
Error	73.1	48.5	44.5	75.5

[1]) Source effects where P is population, B is block, PB is population by block interaction, C(P) is clone-within-population, and BC(P) is block by clone-within-population interaction.

*) $p < 0.05$, **) $p < 0.01$.

Table 5. – Heritability (H^2_c), population means (overall mean), clone mean range, and best clone-within-population for 4 field caliper measurements (mm) taken over 2 growing seasons at the Calgary site.

a. June 1993 H^2_c = 0.56	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	3.0	3.0	3.1	3.1	3.1
Clone mean range	2.5-3.4	2.5-3.6	2.3-3.4	2.9-3.6	2.2-3.5
Best clone	5	6	6	2	5
b. August 1993 H^2_c = 0.51	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	5.7	6.2	6.1	6.4	5.0
Clone mean range	4.1-7.3	5.2-7.1	4.9-8.6	4.9-7.6	4.7-5.4
Best clone	5	4	1	4	5
c. June 1994 H^2_c = 0.49	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	7.8	8.3	8.0	8.7	6.9
Clone mean range	6.0-9.6	7.6-9.4	6.4-10.7	8.3-10.4	6.2-8.1
Best clone	5	4	1	4	5
d. August 1994 H^2_c = 0.29	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	13.6	14.2	13.6	14.4	12.4
Clone mean range	10.5-16.5	12.3-15.7	11.2-16.0	11.9-17.1	11.8-13.3
Best clone	5	4	1	4	4

3.3 Heritabilities and gains

Broad-sense clone mean heritability for bud-burst was 0.72. Clone-within-population accounted for 26.2% of the variance with population accounting for 6.2% (*Table 6a*). The heritability calculated for caliper was 0.80 with clone-within-population accounting for 32.1% ($p = 0.002$) of the total variation and population accounting for 12.0% ($p = 0.07$) (*Table 6b*). Clone-within-population (17.4%) and population (13.6%) both accounted for a significant proportion of the variation in root-to-shoot ratio (*Table 6c*). Heritability estimates for height for Days 27, 41, 62 and 83 were 0.47, 0.81, 0.77 and 0.74 respectively (*Table 6d, e, f, g*). Population and clone-within-population were significant for Days 41, 62 and 83. Chamber by clone-within-population was significant for bud-burst ($p = 0.007$), root-to-shoot ratio ($p = 0.02$) and Day 62 height ($p = 0.03$).

Gain estimates for bud-burst were relatively high (average 15.7%) ranging from 8.8% (Lougheed) to 37.7% (Little Buffalo) (*Table 7a*). For height, gain calculated for Days 62 and 83 was similar (~16%) and clone performance was relatively consistent with three out of the five populations showing that the same clone would be selected at either time (*Table 7b, c*). Mean gain increase was 16.8% for caliper (range 10.6% to 23.8%) and 11.4% for root-to-shoot ratio (range 7.9% to 19.4%) (*Tables 7d, e*).

4.0 Discussion

4.1 Genetic variation and performance

In trembling aspen, a significant amount of quantitative genetic variation exists at the clone level, as has been found previously with isozyme studies of this species (CHELIAK and DANCIK, 1982; LUND et al., 1992). However, over the course of two growing seasons, with minimal tending of the field sites, the heritabilities and variances explained by clone-within-population decreased. The effect of environmental variation within these sites was increasing over time as the carry-over effect of the controlled conditioning of the steckling production in the greenhouse declined. Nonetheless, by the end of the first growing season at the Calgary site, the top performing clone-within each population was established although the field ranking differed substantially from that in the growth chambers. The Little Buffalo population was consistently superior (caliper) in the field (Calgary) while in the growth chambers, the Meander River population out-performed the other four populations for all traits measured. The Calgary

Table 6. – Source of variation, heritability (H^2_c) (s.e.), variance components (Var. Comp.), and percent variance (% Var.) for bud-burst (Day 14), caliper, root-to shoot ratio, and height (Days 27, 41, 62 and 83) measured in the growth chamber experiment.

	a. Bud-burst H^2_c=0.72 (0.09)		b. Caliper H^2_c=0.80 (0.08)		c. Root-to-shoot ratio H^2_c=0.59 (0.15)	
Source[1]	Var. Comp.	%Var.	Var. Comp.	%Var.	Var. Comp.	%Var.
CH						
P	0.1	6.2	0.2	12.0	<0.01*	13.6
C(P)	0.3**	26.2	0.6**	32.1	<0.01*	17.4
CHP	0	0	0	0	0	0
CHC(P)	0.1	9.8	0.1	6.8	<0.01*	13.9
M					**	
CHM						
MP	0	0	0.04	2.6	0	0
MC(P)	0	0	0	0	0	0
CHMP	0	0	0	0	0	0
CHMC(P)	0	0	0.04	2.0	<0.01	2.3
Error	0.7	57.8	0.8	44.6	<0.01	52.8

	d. Height, Day 27 H^2_c=0.47 (0.19)		e. Height, Day 41 H^2_c=0.81 (0.07)		f. Height, Day 62 H^2_c=0.77 (0.08)		g. Height, Day83 H^2_c=0.74 (0.09)	
Source[1]	Var. Comp.	%Var.	Var. Comp.	%Var.	Var. Comp.	%Var.	Var. Comp.	%Var.
CH								
P	13.4*	18.7	17.3**	30.4	32.1**	23.5	30.7*	13.1
C(P)	4.6	6.5	13.1**	23.0	33.9**	24.9	62.4**	26.7
CHP	0.04	0.1	0	0	0	0	0.2	0.1
CHC(P)	1.02	1.4	0.8	1.4	7.0*	5.1	14.3	6.1
M	*						*	
CHM								
MP	0	0	0.3	0.5	0	0	0	0
MC(P)	0	0	0.9	1.6	2.9	2.2	8.7	3.7
CHMP	0.4	0.5	0.2	0.4	2.3	1.7	3.0	1.3
CHMC(P)	0	0	0	0	0	0	0	0
Error	52.1	72.8	24.3	42.7	58.2	42.7	114.5	49.0

[1]) Source effect terms where CH is chamber, CHP is chamber by population, CHC(P) is chamber by clone-within-population, M is moisture, CHM is chamber by moisture, MP is moisture by population, MC(P) is moisture by clone-within-population, CHMP is chamber by moisture by population, and CHMC(P) is chamber by moisture by clone-within-population. *) $p < 0.05$, **) $p < 0.01$. Note: CH, M and CHM are fixed effects.

population performed relatively poorly for all traits measured in both field and growth chamber. This poor performance may indicate that this is a genetically depauperate region where a few disperse clumps of aspen remain in the midst of extensive agricultural development. Alternatively, the higher elevation of this site, combined with a highly variable climate (warm chinook winds during the winter and high probability of both late and early frosts) may have resulted in selection for individuals with a conservative growth strategy.

The lack of significance of population in the field analyses suggests clone selections could be made across a wide ranging area with less emphasis on population structure and allowing for an increased intensity of selection. In the combined site analysis however, location by population accounted for as much variation as clone suggesting that population performance should be assessed at more locations. The growth chamber results showed population did account for a significant amount of the variation for most traits measured and this may be more reflective of performance under intensive forestry culture.

4.2 Heritabilities and gain estimates

Heritability estimates from the field trial are reasonably consistent with those presented in the literature, despite the inherent variability of these estimates across different studies and populations. Growth chamber values were generally higher than field values reflecting enhanced genetic expression under increased environmental control. Relatively high heritabilities are expected for broad-sense clone mean calculations, since both the additive and non-additive genetic components are included (BECKER, 1985; FALCONER, 1989). Broad-sense heritability values for height in trembling aspen have been reported as 0.52 (VAN BUIJTENEN et al., 1959); 0.45 (BARNES, 1969); 0.33 (triploids, EINSPAHR et al., 1963) and 0.69 (EINSPAHR et al., 1967). For diameter, values in the literature range from 0.14 (VAN BUIJTENEN et al., 1959), to 0.36 (BARNES, 1969) and 0.45 (EINSPAHR et al., 1967).

LI and WYCKOFF (1993) reported a volume gain of 25% based on vegetative propagation of highly selected aspen trees from controlled crosses. Increases in genetic gains for 15-year height of interspecific hybrids of trembling aspen (full-sib progeny) and *P. tremula* were approximately 30% above standard site index height values for unimproved aspen from different sites (LI and WYCKOFF, 1993). There are few, if any, estimates of gain for unimproved pure aspen since most tree improvement studies have concentrated on selected clones or parents, triploids and hybrid performance. Not surprisingly, the values we report for genetic gains of unimproved material are lower

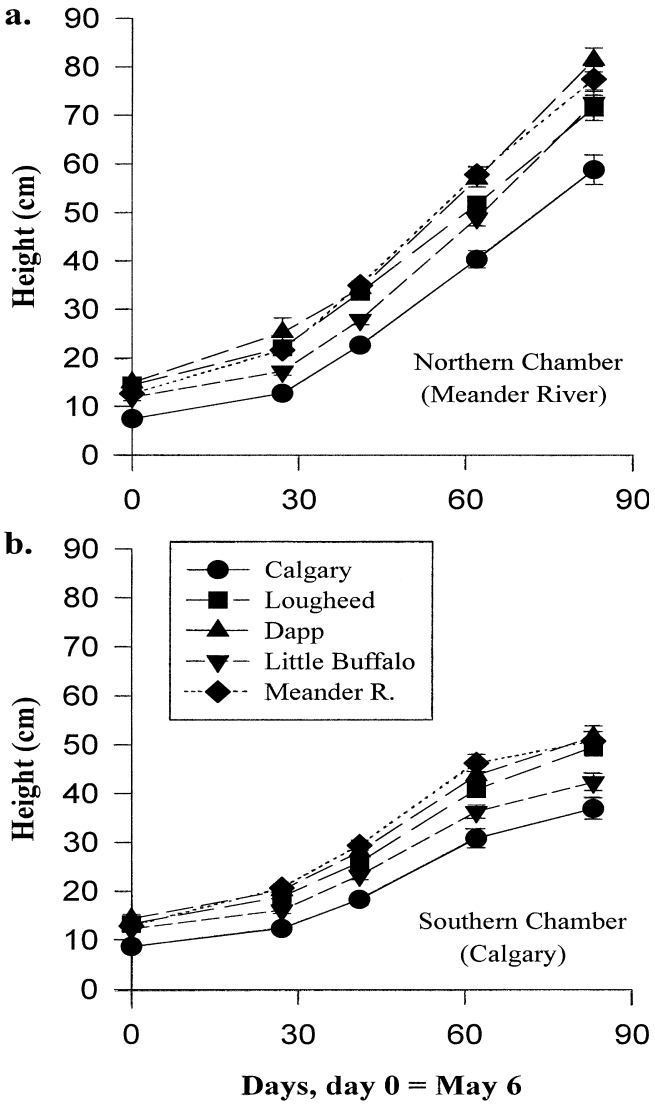

Figure 2. – Heights (s.e.) by population and growth chambers a) Northern and b) Southern. Summer solstice = day 45.

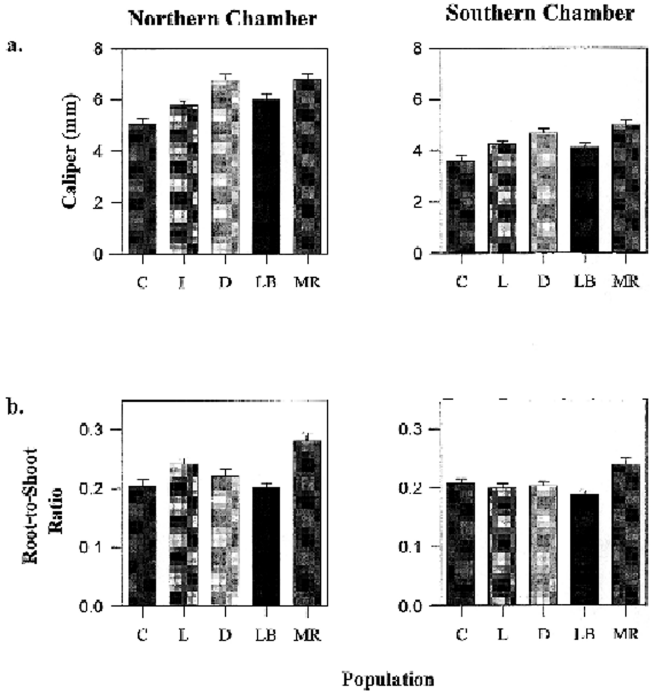

Figure 3. – a) Final caliper (mean + s.e.) and b) Root-to-shoot ratio (mean + s.e.) by growth chamber and population. Where C = Calgary, L = Lougheed, D = Dapp, LB = Little Buffalo and MR = Meander River.

(16% for height and caliper in the growth chamber with a 17% selection intensity). Still they indicate that significant advances can be made using pure aspen.

4.3 Field versus growth chamber performance

Although the absolute amounts of variation in caliper accounted for by population are substantially different between the growth chamber and field trials (growth chamber: 12%, p = 0.07 and field: two-year analysis 2.3%, p = 0.29), the relative amounts were similar (growth chamber, 27.2% and field, 22.8%). There was little evidence of correlation in clone performance between the field and growth chamber. A regression of clone means for caliper from the field and growth chamber had an r^2 = 0.1 and p = 0.1 (August 1994 and Day 83 for the Calgary site and chamber). Further, there was no relationship between the Calgary field caliper (August 1994) and Calgary growth chamber height (Day 83, r^2 = 0.02) as indicated by rank differences for populations between trials. Both experiments have limitations with respect to the growing period and a longer field experiment would have been preferred. Early selection trials for aspen show that, for height increment, years four and five provide the best correlations with 20-year growth performance, although hypoxylon resistant families could not be correctly identified until year 10 (Li et al., 1993).

4.4 Future performance

The early establishment phase of newly deployed aspen stock, exposes them to highly variable environmental conditions (e.g., drought, frost, disease) (Li et al., 1993). Although a mature aspen may have a large network of roots (beyond 3 m^2, Tew, 1967), young plants (< 5 years) have limited access to moisture. Under very dry conditions the stecklings at Meander River site showed virtually no height (not shown) or caliper growth (*Figure 1*) until late in the second season.

A higher root-to-shoot ratio may be a more desirable trait when selecting clones in order to decrease mortality of stock after planting (Wyckoff et al., 1995) by mitigating moisture stress (Burns and Honkala, 1990; Mitton and Grant, 1980). Alternatively, for commercial purposes, it may be more economical to simply produce larger stock for planting with a root capacity which can better buffer against environmental variability and moisture stress.

Several studies have suggested that male aspen clones occupy environments of comparatively lower water availability than females (Freeman et al., 1976; Lloyd and Webb, 1977; Grant and Mitton, 1979). In turn, female aspen clones have been shown to have higher radial growth rates (Grant and Mitton, 1979) and greater basal area (Sakai and Burris, 1985) despite reproductive costs. These studies suggest that clonal trials of known sex will be necessary to assess differential performance for optimization of gains in a clonal tree improvement program, particularly for regeneration on marginal or drier sites. Despite the 1:1 ratio of males: females reported for aspen in other areas (Mitton and Grant, 1980; Anonymous, 1959; 1971), the collection range in Alberta clearly did not follow this pattern (pers. obs. BRT, BPD) and other studies have shown a male-bias sex ratio with increasing elevation (Grant and Mitton, 1979). If indeed male aspen do occupy

Table 7. – Heritability (H^2_c), population means, clone mean range, best clone-within-population, and gain estimates for bud-burst, height (cm) Days 62 and 83, caliper (mm) and root-to-shoot ratio (Day 83) for traits measured in the growth chambers.

a. Bud-burst $H^2_c = 0.72$	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	3.0	3.3	3.1	2.5	3.4
Clone mean range	2.2-3.6	2.7-3.8	2.3-4.0	1.8-3.9	2.9-4.0
Best clone	4	2	2	6	1 or 3
Gain %	13.1	8.8	21.3	37.7	12.8
b. Height, Day 62 $H^2_c = 0.77$	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	35.9	46.4	50.4	42.6	52.1
Clone mean range	30.4-44.7	42.8-56.3	41.4-63.2	35.2-49.0	46.9-59.9
Best clone	4	5	2	5	6
Gain %	19.0	16.5	19.7	11.7	11.5
c. Height, Day 83 $H^2_c = 0.74$	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	48.5	60.7	66.5	57.7	64.1
Clone mean range	41.6-63.2	55.7-72.9	54.9-82.6	45.8-67.8	55.9-74.1
Best clone	4	5	6	5	3
Gain %	22.4	14.9	17.9	13.0	11.6
d. Caliper, Day 83 $H^2_c = 0.80$	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	4.4	5.0	5.7	5.1	5.9
Clone mean range	3.5-5.7	4.4-5.7	4.3-7.4	3.8-6.1	4.9-6.7
Best clone	2	4	2	5	6
Gain %	23.8	10.6	23.0	16.0	10.6
e. Root-to-shoot ratio, Day 83 $H^2_c = 0.59$	POPULATION				
Item	Calgary	Lougheed	Dapp	Little Buffalo	Meander R.
Overall mean	0.21	0.22	0.21	0.20	0.26
Clone mean range	0.16-0.23	0.20-0.26	0.17-0.25	0.17-0.23	0.19-0.35
Best clone	2	5	4or3	3	6
Gain %	7.9	9.0	9.3	11.4	19.4

drier areas than females, then moisture availability in the areas of the boreal forest where our collections were made, and harvesting occurs, may be causing differential survival by sex. With the current climate change predictions of increased air temperatures and reduced soil moisture for the boreal forest in the next 30 to 50 years (HOGG, 1994), sex differences may become more important over the next rotation.

4.5 Conclusions

This study confirms, at a quantitative level, the high degree of clonal variation previously reported for trembling aspen (CHELIAK and DANCIK, 1982; CHONG et al., 1994; GRANT and MITTON, 1979; LUND et al., 1992; MITTON and GRANT, 1980). The heritability estimates and gain values should provide a baseline for direct comparisons with future trials and more accurate predictions of potential gains for the clonal aspects of the pure aspen component of the tree improvement initiatives currently underway in Alberta (LI, 1995). Despite the lower gain values of pure aspen compared to hybrids, this part of the breeding program may become extremely important if planting of improved material onto public lands is limited to native species and local sources. Our results clearly show significant levels of genotype by environment interaction suggesting that both testing and deployment need to be done on similar sites.

A more controlled field testing regime, and longer testing period, is likely to reveal a significant relationship between field and growth chamber performance, thus reducing the amount of time required to assess clonal performance. The consistency in both heritability and gain values for the final two height measurements in the growth chamber and similar ranking of clones-within-populations is encouraging and may prove to be useful if a good correlation between field and growth chamber performance could be obtained. A strong silvicultural commitment, combined with planting onto previously cleared agricultural lands with improved progeny and clones, could achieve the goal of anticipated gains, for pure aspen, which forest companies are looking to for future harvests.

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Appendix 1. – Expected mean square tables for combined field site analysis (a), individual site analysis (b) and combined growth chamber analysis (c).

a. Combined field site analysis

Source of Variation*	Expected mean squares
Location (L)	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{BP} + K3\sigma^2_{LC} + K4\sigma^2_{LP} + K7\sigma^2_{B} + K8\phi_{L}$
Block (L) (B)	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{BP} + K7\sigma^2_{B}$
Population (P)	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{BP} + K3\sigma^2_{LC} + K4\sigma^2_{LP} + K5\sigma^2_{C} + K6\sigma^2_{P}$
Clone (P) (C)	$\sigma^2 + K1\sigma^2_{BC} + K3\sigma^2_{LC} + K5\sigma^2_{C}$
LP	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{BP} + K3\sigma^2_{LC} + K4\sigma^2_{LP}$
LC	$\sigma^2 + K1\sigma^2_{BC} + K3\sigma^2_{LC}$
BP	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{BP}$
BC	$\sigma^2 + K1\sigma^2_{BC}$
Error	σ^2

The Ki values varied due to missing data but were equal to or less than the following: K1 = 3, K2 = 13, K3 = 5, K4 = 24, K5 = 9, K6 = 48, K7 = 62.

b. Individual site analysis

Source of Variation*	Expected mean squares
Population (P)	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{C} + K3\sigma^2_{PB} + K5\sigma^2_{P}$
Block (B)	$\sigma^2 + K1\sigma^2_{BC} + K3\sigma^2_{BP} + K4\sigma^2_{B}$
PB	$\sigma^2 + K1\sigma^2_{BC} + K3\sigma^2_{BP}$
Clone (P) (C)	$\sigma^2 + K1\sigma^2_{BC} + K2\sigma^2_{C}$
BC	$\sigma^2 + K1\sigma^2_{BC}$
Error	σ^2

The Ki values varied due to missing data but were equal to or less than the following: K1 = 3, K2 = 6, K3 = 16, K4 = 76, K5 = 31.

c. Combined growth chamber analysis

Source of Variation*	Expected mean squares
Chamber (CH)	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K5\sigma^2_{CHC} + K7\sigma^2_{CHP} + K8\phi_{CHM} + K11\phi_{CH}$
Population (P)	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP} + K5\sigma^2_{CHC} + K6\sigma^2_{C} + K7\sigma^2_{CHP} + K9\sigma^2_{P}$
Clone(P) (C)	$\sigma^2 + K1\sigma^2_{CHMC} + K3\sigma^2_{MC} + K5\sigma^2_{CHC} + K6\sigma^2_{C}$
CHP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K5\sigma^2_{CHC} + K7\sigma^2_{CHP}$
CHC	$\sigma^2 + K1\sigma^2_{CHMC} + K5\sigma^2_{CHC}$
M	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP} + K8\phi_{CHM} + K10\phi_{M}$
CHM	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K8\phi_{CHM}$
MP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP}$
MC	$\sigma^2 + K1\sigma^2_{CHMC} + K3\sigma^2_{MC}$
CHMP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP}$
CHMC	$\sigma^2 + K1\sigma^2_{CHMC}$
Error	σ^2

*) Source of variation as described previously in text.

The Ki values varied due to missing data but were equal to or less than the following: K1 = 3, K2 = 17, K3 = 6, K4 = 33, K5 = 6, K6 = 12, K7 = 32, K9 = 64. For THOMAS et al., 1997b: K1 = 2, K2 = 8, K3 = 4, K4 = 16, K5 = 4, K6 = 8, K7 = 16, K9 = 32.

Effect of Genotype on Micropropagation of Walnut Trees (*Juglans regia*)

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Summary

Embryos of *Juglans regia* originating from elite trees, selected for their wood quality were cultured on DKW medium supplemented with 4.44 µM 6-benzylaminopurine (BA) and 0.005 µM indole-3-butyric acid (IBA). Results on the effects of cytokinin (BA) and auxin (IBA) on shoot development indicated that elongation is enhanced with 2.22 µM BA while the formation of new axillary shoots is favored by 4.44 µM BA and 0.005 µM IBA. It was, also, observed that different genotypes had different requirements for growth regulators.

Significant differences were observed among the multiplication rates of twelve different clones of *J. regia*. The effect of genotype is obvious in the rooting phase, as well. Some clones exhibit high rooting ability (95%) and some low (5%).

Key words: *Juglans regia*, micropropagation, genotype, multiplication, rooting.

FDC: 165.44; 176.1 *Juglans regia*.

Abbreviations: BA: 6-benzylaminopurine
IBA: indole-3-butyric acid
DKW: DRIVER and KUNIYUKI Walnut medium

Introduction

Persian walnut (*Juglans regia*) is a valuable cultivated forest species of economic importance both for the production of nuts and wood and according to MALVOLTI *et al.*, (1994), great variation, has been observed, in morphological and biochemical characters among distinct geographic ecotypes. At present, although, the tree improvement programmes for *Juglans* species have made some progress (e.g. new promising clones and varieties have been produced) problems in vegetative propagation of selected walnut trees are holding back the improvement of the species and thereby their wider use (ZOBEL and TALBERT, 1984).

Generally, the most popular way of walnut vegetative propagation is that of grafting which is, however, labor-intensive, time-consuming and costly. On the other hand, propagation by cuttings is very difficult due to their low rooting ability (MCGRANAHAN *et al.*, 1988; LAND and CUNNINGHAM, 1994).

In the last decade, other more sophisticated techniques (micropropagation, embryogenesis etc.) have been investigated for the successful large scale propagation of walnut. RODRIGUEZ and SANCHEZ-TAMES (1981) and RODRIGUEZ (1982a, b) were among the first to report establishment of walnut cultures *in vitro* and to describe the development ol shoots or roots from cultured walnut embryos. Later, a large amount of work was conducted on different walnut species using different types of explants, media, culture conditions and rooting techniques, with encouraging results (JAY-ALLEMAND and CORNU, 1986; GRUSELLE *et al.*, 1987; CORNU and JAY-ALLEMAND, 1989; JAY-ALLEMAND *et al.,* 1992).

Most of the above work was based on a medium developed by DRIVER and KUNIYUKI (1984) (DKW medium) for the *in vitro* culture of *Juglans* spp..