



Morice & Lakes Innovative Forest Practices Agreement

Genetic Improvement:

Interactions with Silviculture and Disease

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Introduction

Background

Tree improvement is widely viewed as a cost-effective method of increasing forest productivity. The interior lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) breeding program in B.C. is well established, and predicted gains in individual tree growth rates are substantial and verifiable. Orchards to produce genetically improved seed for the Bulkley Valley have been established. Planting of lodgepole pine in the Bulkley Valley low elevation Seed Planning Unit (SPU), which includes much of the Lakes and Morice TSAs, averages 15.2 million seedlings annually (Forest Genetics Council of B.C., 2002). Of this, Seed Orchard 219, at the Vernon Seed Orchard (VSOC) site, is expected to produce seed sufficient for 2.6 million seedlings in 2003, and 8.8 million at maturity; a new high-gain BV low orchard at VSOC is expected to produce an additional 4.5 million plantable seedlings at maturity (*ibid.*). Interior lodgepole pine accounts for 41.5% of total seedling requests in B.C. (Ministry of Forests, 2001).

Tree improvement programs often consider genetics separately from other factors affecting growth, such as silvicultural practices and disease. It is assumed that tree growth on one or a few test sites will reflect the true genotypic potential.

Genetics and silviculture

Silvicultural factors may affect the performance of genetically improved stock. Some previous studies (Brouard and John, 2000), show that the best genotypes under one set of conditions may not be optimal under different conditions. Stocking density, for example, could affect the ranking of genotypes, through genetic differences in competitive ability. However, progeny tests are generally planted at a single spacing on all sites, so that the effect of spacing cannot be determined.

Genetics and disease

Lodgepole pine hosts the largest array of fungal pathogens of any B.C. timber species. Stand infection levels with comandra rust (*Cronartium comandrae* Peck.) are high in much of the Lakes TSA, according to Woods *et al.* (2000), who examined many stands of age 15-25 years, and observed rust incidence as high as 60.6%.

Survival and growth of young lodgepole pine are severely reduced by comandra rust (Geils and Jacobi, 1993). In one study by Hiratsuka *et al.* (1988), trees with bole infections suffered 87.2% mortality. Woods *et al.* (2000) estimate that rotation age volume losses to stem rusts in lodgepole pine-dominated stands could be as high as 7.2%. Although this estimate includes losses to western gall rust (*Endocronartium harknessii* (J.P.Moore)

Hirat.), the authors suggest that comandra “has a far greater impact on volume than [western gall rust]” in stands where both occur (Woods *et al.*, 2000), since western gall rust is frequently non-lethal, while comandra, once present, is likely to kill the tree.

Comandra rust requires an alternate host to complete the infection cycle; the rust cannot be spread directly from tree to tree. *Geocaulon lividum*, or bastard toadflax, is the alternate host in B.C. and Alberta; it is widespread through the Lakes and Morice TSAs and much of B.C. The distribution of the disease is likely attributable to a combination of alternate host distribution and weather patterns, since high air moisture content facilitates infections. Control of the alternate host is not feasible.

The degree of genetic control of disease resistance can only be estimated in stands with a known genetic structure, such as progeny tests. In these tests, where families from a wide geographic range are planted together in a “common garden,” assessments can reveal whether certain families are more resistant to a given disease than others, and whether trees from some areas of the province are more disease-resistant than trees from other areas. Existing Ministry of Forests progeny tests in the Bulkley Valley provided an excellent opportunity for obtaining this information.

Identification of resistant genotypes can help in developing deployment strategies for improved stock in disease-prone areas.

The questions

This project continues to focus on several questions relating to genetics, silviculture and disease resistance of lodgepole pine in the Morice and Lakes TSAs.

1. Should the choice of families for operational deployment depend on factors such as plantation spacing and variation in crown structure among families?
2. Results (John, 2003), suggest that some pine trees are “naturally” resistant to comandra rust, and that this resistance has a genetic basis. Furthermore, levels of resistance seem to have a geographical linkage, in that pine genotypes from some regions show higher resistance than genotypes from other areas. How big are these genetic differences?
3. Are genetic differences in disease resistance constant across planting (test) sites, or are they site-specific?

Project history

This project began as a component of the Babine Enhanced Forest Management Pilot Project (EFMPP) in the fourth quarter of the 1998-99 fiscal year. Initially the project focussed on silviculture-genetics interactions. Disease resistance objectives were incorporated in June 1999, following observation of high levels of comandra, stalactiform and gall rust on one 14-year-old progeny test site, and discussion with Ministry of Forests (MoF) and Babine Forest Products staff, and academics. The project was carried over into the Lakes & Morice IFPA in April, 2001.

Prior work (1999 – 2002)

This incremental project builds on results from earlier EFMPP and IFPA work, summarised below.

Overall project

- Article on project prepared for, and published in, *TICtalk*, the newsletter of the B.C. Forest Genetics Council (John, 2000);
- Presentation made at three Burns Lake public information sessions.

Genetics and silviculture

- Obtained, analysed and summarised MoF harvest index data;
- Developed and field tested crown form rating system;
- Assessed 3,216 trees (134 families) for upper and lower crown form;
- Entered, compiled, analysed and interpreted crown form data;
- Selected six families of three contrasting crown form types for field trials, and seed of these six families sent to PRT Telkwa nursery for stratification and sowing;
- Seedlings grown, lifted and stored in preparation for May, 2001 planting;
- Field trial structure defined;
- Planting site selected and site prepped;
- Seedlings established May, 2001.

Genetics and disease

- Comandra assessment complete (10,194 trees); data entered and compiled;
- Comandra data analysed, and susceptible and resistant families identified;
- Geographic variation confirmed through cluster analysis;
- Controlled crosses made among some resistant genotypes;
- Seedlots collected from 50 orchard parents;

- Preliminary trial design prepared.

Ongoing work

Genetics and silviculture

Earlier analyses of data from existing MoF trials confirmed the existence of genetic variation in harvest index, or proportion of biomass contained in the bole of the tree. This was followed by assessments of crown architecture in existing progeny tests in the Bulkley Valley. All trees of each of 134 families were assessed on the Chowsunket and Gramophon MOF progeny test sites for branch length in upper and lower crown. Average upper and lower crown branch length scores were calculated for each family. Based on these assessments, three contrasting crown types were identified (see Table 1), and six families, two of each type, were selected (see Table 2). Variances of these scores also influenced the choice of families; an attempt was made to choose less variable families. Also, all families chosen had ranked in the top 40 families for breeding value at age 14.

Table 1. Crown form traits of three contrasting crown types.

Crown form traits	Type 1	Type 2	Type 3
Harvest index	<ul style="list-style-type: none"> high harvest index 	<ul style="list-style-type: none"> intermediate harvest index 	<ul style="list-style-type: none"> low harvest index
Branch length and distribution	<ul style="list-style-type: none"> short branches in both upper and lower crown 	<ul style="list-style-type: none"> long branches in upper crown, shorter branches in lower crown 	<ul style="list-style-type: none"> long branches in both upper and lower crown
Distribution of biomass	<ul style="list-style-type: none"> most biomass clearly allocated to tree bole 	<ul style="list-style-type: none"> intermediate biomass allocation 	<ul style="list-style-type: none"> large proportion of biomass allocated to tree branches

Table 2. Families included in espacement trials.

family	crown type	mean upper crown score*	mean lower crown score
480	1	1.96	2.25
422	1	1.95	2.10
488	2	2.91	2.41
482	2	2.60	2.35
286	3	3.19	3.52
252	3	2.95	3.37

* crown scores are an indication of average branch length, from 1 (very short) to 4 (very long)

Using seedlings grown from remnant seed of the six selected families, an espacement study has been established on one test site in the Bulkley Valley. This study will investigate the relative performance of families with different patterns of biomass allocation and crown architecture when grown under a wide range of planting densities.

The trial was laid out and planted on May 29-30, 2001, at a test site near the Decker Lake sawmill. Surplus stock was planted nearby for future mortality replacement. Thirty-six plots of 64 trees each, at spacings of 0.6 to 2.4 m (0.36 m²/tree to 5.76 m²/tree), were established. Planting to replace mortality was done in May 2002, and measurements will begin when crown competition is imminent, likely in the fall of 2003. Further fill planting may be done, if feasible.

The espacement study implemented under this project may influence the design of future genetic tests, through recognition of the role of crown architecture in influencing growth performance. This may suggest that trials should be tailored to specific silvicultural conditions, rather than relying on the assumption that performance is invariant with respect to factors such as spacing.

Genetics and disease

The Chowsunket test site of the MoF Bulkley Valley progeny trials was assessed for comandra rust incidence in 2000 (see Figure 1). Since this trial, established in 1985 and 1986, was not designed for the purpose of disease assessment, numbers of seedlings/family and their spatial arrangement were not adequate to ensure high accuracy of family rankings. Despite these design implications, variation in infection rates among families was large and significant. These analyses have been reported elsewhere (John, 2001).



Figure 1. Diamond-shaped stem canker of comandra rust on young lodgepole pine in the Bulkley Valley (*photo: S. John*)

Analyses

Analyses completed in 2002 explored a possible geographic basis for observed family variation. Data from 309 families, established in both planting years, was aggregated for these analyses of infection rates and geographic information.

Initial analyses attempted to relate variation in rust susceptibility to latitude, longitude and elevation, both singly and in various linear combinations. Regression analyses failed to show any strong linear relationship between infection rate and geographic variables. The highest R^2 value was obtained by regressing infection rate on latitude alone; this value was only 0.07. For infection rate and elevation, the calculated R^2 was a surprisingly low 0.00013.

Cluster analyses were then performed, using SAS PROC FASTCLUS (SAS, 1987), to assign tested families into logical geographic groups. A number of iterations were run, with numbers of clusters ranging between 5 and 100.

The objectives of the iterations were to:

- find a reasonably small number of clusters that would separate the data into distinct groups; and
- find a clustering that explains a reasonable amount of the variation in infection rate.

Clearly, a larger number of clusters will yield a higher R^2 value, while a reasonably small number of groupings (fewer than, say, 30) will ensure that most groups will contain a significant number of entries, and enable a more precise estimate of mean infection rate for each cluster. The number of clusters chosen represents a compromise between these two objectives.

An analysis of variance was performed at each iteration, using the simple model

$$y_{ij} = \mu + c_i + \varepsilon_{ij}$$

where:

y_{ij} = the infection rate of family j in the cluster i ;

μ = the overall mean infection rate;

c_i = the mean deviation in infection rate of cluster i from the overall mean; and

ε_{ij} = error.

The R^2 value from the ANOVA was used as an indication of the gain in value from separating data points into more clusters. A large gain (0.280 to 0.318) in the R^2 value was noted in increasing from 8 to 9 clusters. With more than 9 clusters, R^2 values increased much more slowly up to 100 clusters ($R^2 = 0.56$); thus 9 was felt to be a reasonable number of clusters. Only results for 9 clusters are presented here.

The ANOVA is shown in Table 3.

Table 3. Analysis of variance: cluster model

Source	DF	Sum of squares	Mean Square	F Value	Pr > F
Cluster	8	0.97992521	0.12249065	17.51	<0.0001
Error	300	2.09831895	0.00699440		
Corrected total	308	3.07824416			
R-Square		c.v.	Root MSE	Infection mean	
0.318339		50.84129	0.083633	0.164497	

Family origin locations, identified by cluster number, were plotted by latitude and longitude to give a graphic indication of variation in infection rates by source across the range of family origins (see Figure 2). Note that

most (235 out of 309) observations are hidden behind other observations in the same cluster (e.g. cluster 4, with 10 members, and cluster 7, with 11 members, are each represented by a single point). Note also that longitude is plotted decreasing to the right, to give a map-like display.

Infection and location data were summarised by cluster (see Table 4). Infection rate is clearly influenced by geographic source, with the highest infection rates occurring in clusters at low latitudes and longitudes (clusters 1, 3 and 4), and the cluster with the lowest infection rate (cluster 7) located at intermediate latitude and longitude.

Results of these analyses are reported in John (2003).

GENETIC IMPROVEMENT

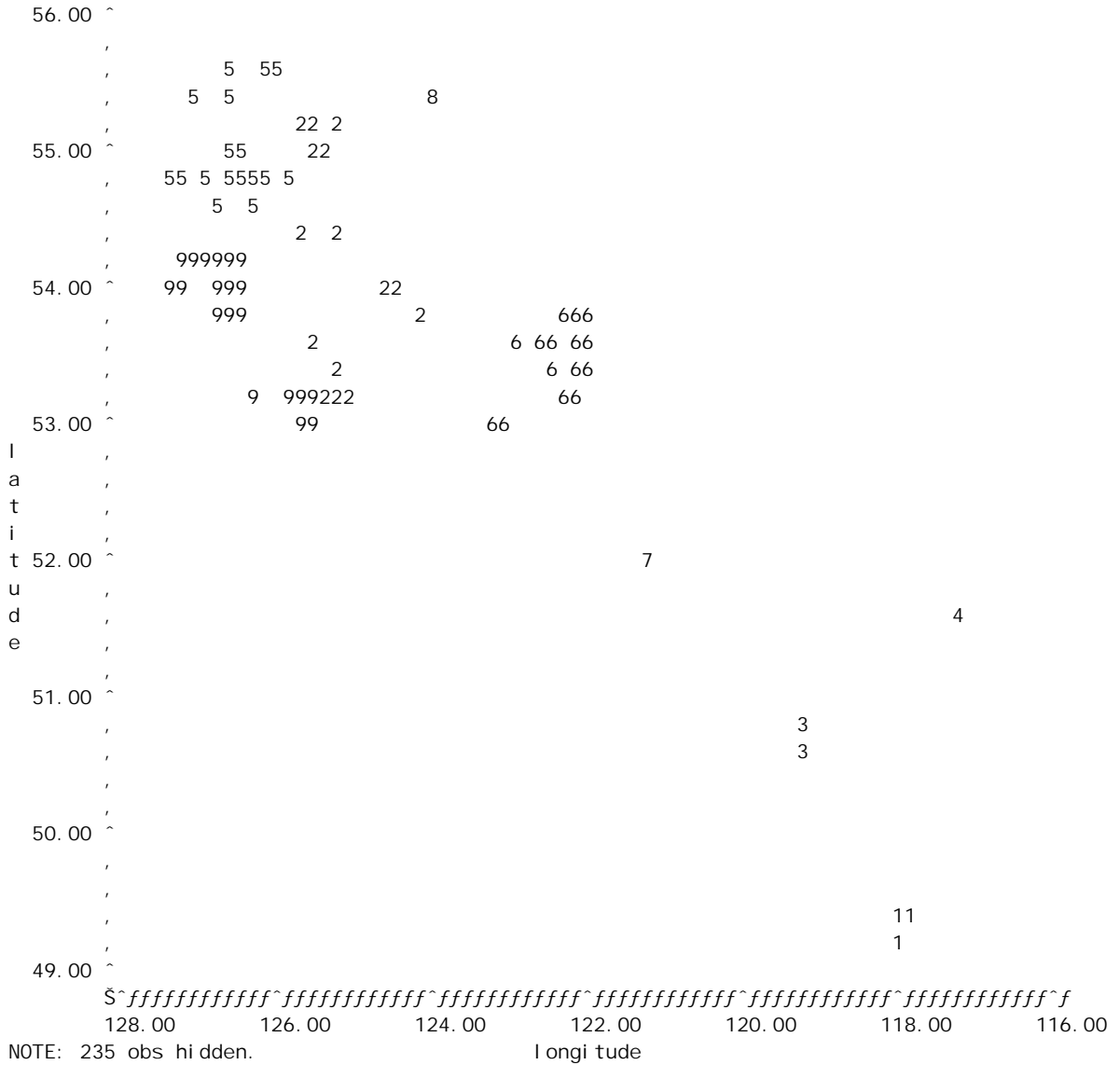


Figure 2. Nine geographic clusters of origins of tested families.

Data and earlier results from this study have already been used by MoF genetics staff to identify several provenances as resistant to comandra rust. Single-family estimates of resistance are imprecise, with wide confidence intervals. Cluster means would provide a more solid basis for predicting provenance-based resistance levels.

Table 4. Cluster size, location and mean infection rates.

Cluster	Count	Mean latitude	Mean longitude	Mean infection
1	10	49.28	117.96	0.359

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2	48	54.18	124.97	0.142
3	10	50.69	119.21	0.250
4	10	51.54	117.21	0.321
5	63	55.03	126.46	0.194
6	95	53.52	122.56	0.138
7	11	51.96	121.19	0.073
8	1	55.40	124.03	0.188
9	61	53.95	126.57	0.137
all	309			0.164

The next steps

A new trial has been designed specifically to test comandra resistance of genotypes established in the Bulkley Valley seed orchard (#219) located at the VSOC orchard site, and to allow more accurate ranking of families. Seedlots representing 65 clones were available for testing by the spring of 2003 (see Table 5). Forty-seven clones are represented by open-pollinated family collections made in the orchard, 18 are represented by open-pollinated family collections made from original parent trees in the wild, and 37 clones have both types of family seedlots available, for a total of 102 family seedlots. In addition, seed is available from nine full-sib families (Table 6) from controlled crosses made in 2001 among putatively resistant parents, based on earlier results from this project (John, 2002). Two wild seedlots (Table 7) of known susceptible provenance and 13 operational seedlots (Table 8) from the target deployment area for Orchard 219 (Bulkley Valley low) were also included, for a total of 126 seedlots.

Seed is under stratification at the time of writing (March 2003) and will be hand sown in 410 styroblocs, using two blocks per seedlot, for a total of 224 cavities per seedlot, at the Landing Nursery, Vernon BC. Lift is planned for November 2003, for a 2004 spring planting.

Three field trial sites, chosen for high likelihood of comandra rust exposure, will be identified and confirmed during the 2003 field season, and field layout will be completed in fall 2003 or spring 2004. Seedlings will be established in a tightly spaced (1 m x 1 m) layout, using 100 single tree replicates in 25 interlocking sets.

Table 5. Families from orchard clones to be included in field trials.

orchard clone #	o.p. seed from orchard	o.p. seed from original wild selections
223	1	1
224		1

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orchard clone #	o.p. seed from orchard	o.p. seed from original wild selections
233	1	1
236	1	
250	1	1
258	1	1
266	1	
275		1
276	1	1
284	1	
291	1	
299	1	1
327		1
346	1	1
375	1	1
385	1	1
392	1	1
402	1	1
406	1	1
422	1	1
428		1
430	1	1
431	1	1
445	1	
472	1	1
478		1
479	1	1
480	1	1
483	1	1
484	1	1
488	1	1
490	1	1
491	1	1
495	1	1
499	1	
501	1	
502	1	
1616		1
1618		1
1629		1
1630	1	1
1666	1	
1679		1
1730	1	1
1732		1
1733	1	1
1736		1
1739	1	1
1740		1
1741	1	1

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orchard clone #	o.p. seed from orchard	o.p. seed from original wild selections
1745	1	1
1770	1	1
1772		1
1773	1	1
1775	1	1
1779	1	1
1791		1
1792		1
1795	1	1
1799	1	1
1804		1
1809		1
1820		1
1822	1	1
bulk *	1	
count	47	55

* bulk collection (seedlot # F2001219) from orchard clones with putative high resistance

Table 6. Controlled crosses between putatively resistant parents.

sowing seedlot #	parent 1	parent 2
118	1809	1617
119	1809	1834
120	1610	1619
121	433	1617
122	433	1619
123	1618	1611
124	1618	1616
125	1809	1616
126	1829	1611
count = 9		

Table 7. Operational seedlots from provenances of high susceptibility.

sowing s.l. #	seedlot	genetic class	provenance
116	44201	B+	Larch Hills
117	45786	B+	Champion Lakes
count = 2			

Table 8. Operational Bulkley Valley seedlots.

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sowing s.l. #	seedlot	genetic class	elevation (m)
103	33201	B	825
104	33209	B	800
105	33216	B	975
106	39411	B	1070
107	39544	B	945
108	42843	B	1050
109	47310	B	1040
110	47373		780
111	47391		800
112	47935	B+ ?	960
113	61033	A	887
114	61039	A	879
115	61041	A	877
count = 13			

Conclusion

Genetic variation exists in susceptibility to comandra rust infection. This variation has a geographic basis, and can be exploited in two distinct ways to produce resistant seedlots for deployment in high-risk areas:

1. resistant provenances can be identified as sources for wild seed collections; and
2. resistant genotypes established in seed orchards can provide custom orchard seedlots.

Based on results from this project, both of these options are being actively pursued by the Ministry of Forests and the forest industry.

Future Plans

2003/2004

- assess/measure espacement trial
- fill planting (if required and feasible)
- permanent monumentation
- grow and lift seedlings for comandra trial to confirm rust resistance of orchard families
- select and prepare test sites
- finalise design and lay out test sites

2004/2005

- establish comandra trial
- maintain espacement trial
- measure espacement trial

2005/2006 and subsequent years

- maintain and assess comandra and espacement trials
- analyse and report

Recommendations

- Ensure that tests established under this project are maintained and measured periodically through the short and medium term (up to 20 years).
- Ensure that applicable results are disseminated to appropriate user audience.

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