EARLY FIELD PERFORMANCE OF INTERIOR SPRUCE EMBLINGS[1]

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ABSTRACT—Somatic embryogenesis (SE) is a type of vegetative reproduction. It may offer an effective way of utilizing superior genetic material developed by tree improvement programs. Sixteen clonal tests or candidacy trials (CT) were established in the central interior of British Columbia between 1994 and 1998. The objective was to identify superior (growth and insect tolerance) SE clones from 52 interior spruce (*Picea glauca* (Moench) Voss, *P. engelmannii* Parry ex Engelm., and their naturally occurring hybrids) families. More than 48,000 individuals are in these CT. To date, survival has been excellent, greater than 95 percent, and exceeds 99 percent on the oldest CT. Clonal growth is affected more by environment than by genotype even on the oldest CT sites. This will delay the identification of superior SE clones. Attack by the spruce leader weevil (*Pissodes strobi* Peck) began this year in the older CT. It will be several years yet before weevil tolerant SE clones can be identified. In addition to biological issues, there are economic and social ones that must be addressed before operational scale deployment of SE emblings. The potential of SE technology is great but the validation process is exceedingly slow and costly.

INTRODUCTION

Vegetative reproduction is effective for utilizing superior genetic material developed by tree improvement programs. Significant advances in conifer vegetative propagation systems have been made over the past 25 years (Grossnickle and others 1996). These systems provide a means of bringing new genetic material into forestry programs (Libby and Rauter 1984) and a way to bulk up superior families (Gupta and Grob 1995, Kleinschmit and others 1993). By far the most significant means of vegetative propagation, today, is with rooted cuttings. Annual plantings exceed 65 million (Ritchie 1991, Talbert and others 1993). However there are significant limitations to this technology (Grossnickle 1998, Hackett 1985). Organogenesis is another means of vegetative reproduction but in conifers it has been used only on a limited operational scale (Frampton and Foster 1993, Ritchie and Long 1986, Smith 1997). Another vegetative protocol and the focus of this paper is somatic embryogenesis (SE) and it is described elsewhere (Grossnickle and others 1996, Tautorus and others 1991).

Compared to rooted cuttings technology, SE technology has the advantage that genotypes can be stored for a long time using cryopreservation (Cyr and others 1994) with little affect on genotype (Kartha and others 1988). The interior spruce (*Picea glauca* (Moench) Voss, *P. engelmannii* Parry ex Engelm., and their naturally occurring hybrids) seed orchard program of the BC Forest Service (Kiss 1968) was used as the source of parents with proven superior growth potential to assess the efficacy of SE technology in BC (Sutton and others 1993). Some of this parental material also has increased tolerance to the spruce leader weevil (white pine weevil *Pissodes strobi* Peck) (Alfaro 1996, Kiss and Yanchuk 1991). A large scale SE clonal testing program has evolved and it is expected that clonal selections for superior growth and tolerance to the weevil will occur about 5-7 and 7-10 years, respectively, after planting.

However, three issues must be addressed before SE technology can be successful operationally: 1) identification of clones with desired traits from sufficient superior families to have an effective population size of 10, 2) economic or cost benefit of SE embling clones compared to full sib or open pollinated seedlings of the same family, and 3) public acceptance of clonal forestry in BC. The paper will describe field testing of SE clones in candidacy or clonal test (CT) sites established between 1994 and 1998 in the central interior of BC, Canada, and briefly look at social and economic issues.

THEORETICAL

Why field test if all the parents other than some from the BIOTIA series been chosen for the program based on superior growth and weevil tolerance? There are several reasons: a) a very small number of genotypes from each family has successfully progressed from the laboratory on to

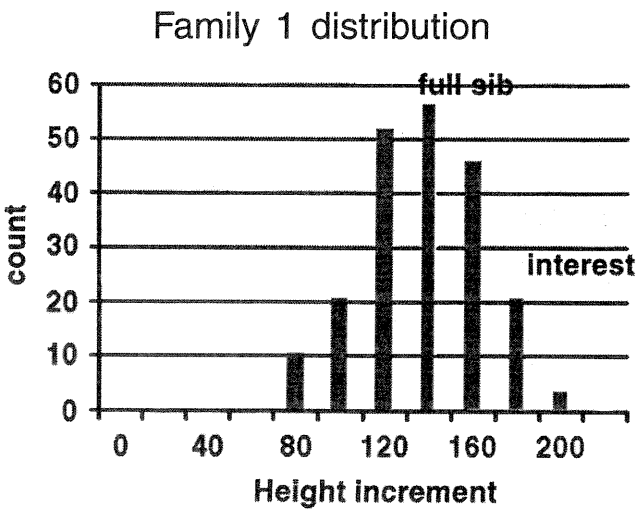

Figure 1—Count of the first year height increments for Family 1 (PG001 X PG021).

[1]Hawkins, C.D.B. 1999. Early field performance of interior spruce emblings. In: Landis, T.D.; Barnett, J.P., tech. coords. National proceedings: forest and conservation nursery associations—1998. Gen. Tech. Rep. SRS-25. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 122-128.

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the nursery and out to the field; b) it is necessary to determine if family clonal means, for any trait, approximate a normal distribution (fig. 1); c) full sib or seedling controls are expected to be near the centre of the normal distribution for a family and this must be determined through testing; d) clones selected for operational deployment should be at the extreme right of the normal distribution; and e) need to ascertain clonal weevil tolerance under field rather than laboratory conditions. The only way to meet these objectives with any degree of certainty is through field testing.

The goal of the SE, CT program is to identify the superior clone for growth and for weevil tolerance from each of the families plus those clones that display desirable silvicultural characteristics such as tolerance to low air temperatures, cold wet soils, or competing vegetation. Ideally after growth and weevil results are determined from the described CT series about 70 clones (five percent selection intensity) would be selected to form the base population. Seedlots with a minimum of 30 clones and an effective population size of 10 (Anonymous 1998) would be constructed from the base population for operational deployment. A more likely scenario is a second phase of CT where the top 15-20 percent of the clones from the present CT are tested on a wider range of sites. The base population of 50-70 clones would then be chosen from this smaller phase two test population.

Regardless of test scenario, it will take a minimum of 5-8 years from plantation establishment to attain reliable growth estimates. Estimates of weevil tolerance will take longer, probably a minimum of 8-10 years. Therefore while the

Table 1—Candidacy test sites: year of establishment, stocktype planted, locale, biogeoclimatic (BEC) subzone, parental origins, number of families and total number of clones planted per familv including full sib seedlings

Year planted	Stocktype	Sitename	Site BEC	Parental source	Number families[a]	Number clones
1994	1+0 313B	Hungary Creek 4	ICHvk	BIOTIA	12	113
1995	1+1 PBR	Hungary Creek I	ICHvk	BIOTIA	12	158
1995	1+1 PBR	Huble Road	SBSwk1	BIOTIA	12	206
1996	1+0 415B	Aleza Lake	SBSwk1	PG95[b]	15 (24)	268 (290)
1996	1+0 415B	Tumuch	ICHvk	PG95	15 (24)	306 (329)
1996	1+0 415B	Indian Point	SBSwk1	PG95	15 (23)	312 (334)
1997	1+0 415B	Aleza Lake	SBSwk1	PG-ENA	7 (29)	109 (278)
1997	1+0 415B	Hungary Creek 1.5	IVHvk	PG-ENA	6 (30)	97 (263)
1997	1+0 415B	Arctic Lake	SBSwk	PG-ENA	7 (28)	94 (268)
1997	1+0 415B	2700 Road Quesnel	SBSmw	PG-ENA	7 (30)	64 (166)
1997	1+0 415B	Marie North	SBSmc2	PG-ENA	4 (14)	49 (87)
1998	1+0 415B	Weldwood 6000	ICHdk	QL[c]	18 (34)	452 (497)
1998	1+0 415B	Riverside Likely	ICHmk3	QL	18 (37)	654 (705)
1998	1+0 415B	Weldwood TFL 5	SBSdw	QL	18 (34)	513 (558)
1998	1+0 415B	Catfish Creek	ICHwk3	QL	18 (34)	436 (477)
1998	1+0 415B	Missinka	SBSvk	QL	18 (33)	485 (528)

[a] Number of families and clones are new for that year while the numbers in parentheses indicates the total number of families and clones planted.
[b] All sites planted after 1995 have clones from previous years to serve as benchmarks among the years.
[c] Quesnel Lakes, no clones were produced from families 3 1 and 32 (see table 2).

Table 2—Embling family, parents used in cross and parental rank for growth and weevil tolerance if known. Rank for BIOTIA and PG material is based on 15 years of 173 parents and for Quesnel Lakes (QL) it is 10 year data from 142 parents

Source	Family	Female	Rank growth	Rank weevil	Male	Rank growth	Rank weevil
BIOTIA	G	PG001	29	14	PG144	19	34
BIOTIA	H	PG001	29	14	PG127	67	118
BIOTIA	I	PG002	4	20	PG096	78	97
BIOTIA	J	PG002	4	20	PG094	102	130
BIOTIA	L	PG010	46	32	PG146	45	82
BIOTIA	M	PG059	165	146	PG021	11	3
BIOTIA	N	PG059	165	146	PG073	167	138
BIOTIA	Q	PG084	21	57	PG088	68	18
BIOTIA	R	PG090	158	132	PG041	168	142
BIOTIA	T	PG113	60	83	PG143	3	36
BIOTIA	U	PG113	60	83	PG140	36	15
BIOTIA	W	PG171	170	91	PG173	173	145
PG95	1	PG001	29	14	PG021	11	3
PG95	2	PG001	29	14	PG029	8	1
PG95	5	PG001	29	14	PG087	1	10
PG95	10	PG001	29	14	PG167	9	9
PG95	23	PG167	9	9	PG161	2	7
PG95	65	PG087	1	10	PG021	11	3
PG95	73	PG087	1	10	PG161	2	7
PG95	75	PG087	1	10	PG167	9	9
PG95	107	PGO87	1	10	PG138	5	2
PG95	119	PG021	11	3	PG029	8	1
PG95	125	PG021	11	3	PG161	2	7
PG95	127	PG021	11	3	PG167	9	9
PG95	142	PG029	8	1	PG161	2	7
PG95	143	PG029	8	1	PG167	9	9
PG95	186	PG161	2	7	PG029	8	1
PG-ENA	3	ENA0663	-	-	ENA0866	-	-
PG-ENA	4	EBA0872	-	-	PG145	6	-
PG-ENA	6	ENA0866	-	-	PR0063	-	-
PG-ENA	7	ENA1659	-	-	ENA1649	-	-
PG-ENA	8	PR0063	-	-	ENA0866	-	-
PG-ENA	13	ENA1659	-	-	ENA1645	-	-
PG-ENA	77	PG087	1	10	ENA1645	-	-
QL[a]	14	QL4731	14	1	-	-	-
QL	15	QL1846	26	1.5	-	-	-
QL	16	QL1665	52	4	-	-	-
QL	17	QL1856	1	4.5	-	-	-
QL	18	QL1816	62	9.5	-	-	-
QL	19	QL1848	28	11	-	-	-
QL	20	QL1819	59	11.5	-	-	-
QL	21	QL1857	16	11.5	-	-	-
QL	22	QL4781	2	12.5	-	-	-
QL	24	QL1871	24	13	-	-	-
QL	25	QL1951	8	14	-	-	-
QL	26	QL4729	42	17	-	-	-
QL	27	QL1843	5	17.5	-	-	-
QL	28	QL1870	10	17.5	-	-	-
QL	29	QL1837	25	18.5	-	-	-
QL	30	QL4728	3	19	-	-	-
QL	31	QL4757	56	19	-	-	-
QL	32	QL4790	9	20	-	-	-

[a] No clones made it to the field from QL families 31 and 32 in 1998.

potential of SE technology is great, the validation process is costly and exceedingly slow.

ESTABLISHMENT

Generally all CT have been established on mesic sites in the various biogeoclimatic ecosystem classification (BEC) units utilized. The appropriate seedling control material (full sib or open pollinated) was planted for each family on all the CT.

1994 and 1995

The first CT was established in the spring of 1994 (table 1) with 12 families from the BIOTIA series of crosses (table 2). The parents of these families were high, mid and low ranked. Due to the poor nursery quality of the material scheduled for planting in 1994, much of it was grown as a bareroot transplant in 1994. In the spring of 1995, two more CT were established using the BIOTIA transplant material (table 1). The above CT were all single tree plot design with 10 randomly allocated ramets per genetic entry. All future CT were also single tree plot design with random allocation of ramets.

1996

The PG95 (Prince George 1995 nursery culture) material was established on three sites in the spring of 1996 (table 1). The parents of this series of crosses were chosen for their superior growth and tolerance to the spruce leader weevil (table 2). The quality of the SE material in some families was as good as that of the seedling controls. However, in others quality was still lacking as it had been for the earlier BIOTIA material. The three sites were subjected to a growing season frost in early July 1996 and again in early June 1998. Subsequently they have gone into severe planting check. Selection for growth will be delayed by at least three years for the PG95 material.

1997

In the spring of 1997, the PG-ENA (Prince George - Eastern North America parents) clones were planted on five sites (table 1). All parents in this series of crosses had demonstrated good growth and tolerance to the spruce leader weevil (Kiss 97/01, retired spruce breader, BC Forest Service, Vernon, BC) (table 2). For the first time, the quality of the SE material at planting was equal or superior to that of the seedling controls. These plantations did not appear to be affected by the early June 1998 frost.

1998

Five CT were established across the central interior of BC with material from the former Quesnel Lakes (QL) seed planning zone in the spring of 1998 (table 1). These parents were selected primarily for tolerance to the spruce leader weevil and secondarily for growth potential (table 2). Again the quality of the SE material was equal or superior to that of the seedling controls. It is too soon to assess the impact of the June 1998 frost on these sites.

Overview

A total of 16 CT were established between 1994 and 1998. There are more than 48,000 single tree plots from 1400+ clones within 52 families in test. The size of the CT are variable. They range in size from about 1000 to 7000 emblings and seedlings per CT. Immediately after planting for each CT, groundline stem diameter (GSD) was determined. In the fall of the year of planting, height at planting and fall height and fall GSD are measured. This is the base data for all CT on all sites. CT sites are visited in the spring and fall of the first three years and annually in the fall thereafter to assign a health or vigor score to each individual in the CT.

Stock quality differences observed between clones and seedlings on the CT established in 1994-1996 may be due to nursery culture or genetics. This has two ramifications. It slows down the testing (takes longer for stock to equilibrate) and it may result in early over estimates of seedling growth potential when compared to appropriate SE clones. This should not be a concern for the CT established in 1997-1998.

At the same time as the CT were established, demonstration plantations called clonal block (CB) sites were also planted. The base unit of a CB contains about 200-300 ramets of a single clone planted at operational spacing. Generally at each site, a minimum of 10 SE clones and an operational and a wild seedlot were planted, a dozen base units. There are more than 25 CB in the central interior of BC containing more than 100,000 emblings and seedlings. Survival plots were established in each base unit of a CB. The CB will be discussed elsewhere (report in preparation, D Summers and C Hawkins unpublished data).

RESULTS AND DISCUSSION (PRELIMINARY)

Surprisingly on all CT sites, regardless of when established or quality of planting stock, survival was excellent. It exceeded 95 percent on all sites and on some sites it still exceeds 99 percent. The oldest site, Hungary Creek 4 planted with the poorest quality stock, is in the latter category. These results may be due, in part, to the aggressive control of competing vegetation on the CT sites.

Table 3—ANOVA model for comparison among sites after the same number of growth periods. All sources in the model are significant, aŏ = 0.05

	HR vs HC1 2 years		HR vs HC4 3 years		HR vs HC1 vs HC4 2 years	
Source	df	F	df	F	df	F
Site	1	1595.5	1	2939.2	2	2156.4
Family	11	29.0	11	15.4	11	20.5
Clone (family)	136	14.0	85	13.7	78	20.1
Site X family	11	5.3	11	4.5	22	3.0
Site X clone (family)	136	2.9	85	2.9	155	2.9
Error	4900		3594		4372	

On at least two sites, vegetation control was done in the summer of the year of planting. Stock vigor, health or quality was good for both seedlings and SE emblings and not different between them but differences among sites were considerable. The range among sites for stock that was healthy is 70 - 86 percent. Survival and quality were not different between SE emblings and representative seedlings on a given CT site.

Analysis of variance for the BIOTIA sites, when compared after an equivalent number of growth periods on site, indicates model main effects were significant (table 3). More importantly, all interactions were significant. The interaction between genotype and environment (Site X Clone(Family)) is of particular interest. For example, after 3 growing seasons clone T689 ranked 162 at Huble Road but was ranked in the top 10 (9th) clones at Hungary Creek 4. This indicates some clones are not spatially stable and they will not be selected if the difference remains.

When comparing Huble Road and Hungary Creek 4, after 3 years growth on site for both, broad sense heritabilites were low 0.05 and 0.24 respectively. Pooled H^2 for these two sites was 0.13. This suggests that 10-15 percent of any clone mean for height is due to true genetic differences among clones. Conversely, 85-90 percent of the variation in clone mean is due to environment. Therefore at this point in time, BIOTIA clone means are not reliable for the selection of superior clones. The younger CT plantations have not had sufficient time or growth for any selection to be considered.

Generally full sib seedling grew faster than their BIOTIA clonal counterparts. This may reflect the seedlings better quality and larger size at planting. The larger stock at planting usually was still larger after three growth periods at the Huble Road site (fig. 2). Removing seedlings from the plot does not change the relationship, the taller emblings at planting are generally still taller after three seasons. This relationship was not as good at Hungary Creek 4 or 1, some of the smaller individuals at planting performed as well as the taller ones and vice versa (poorer). This probably reflects differences among sites; that is clone by site interactions. Again, this reinforces the observation it is too soon for clonal selections.

The susceptibility of interior spruce to the leader or white pine weevil (*Pissodes strobi* Peck) depends on several factors in addition to spruce genotype (SE clone): local weevil population dynamics, site elevation and aspect, and BEC subzone (for example weevil hazard is generally low in the SBSmc2 but can range from low to extreme in the SBSwk1). The weevil requires 785 degree days above 7.2°C to complete its life cycle in an interior spruce plantation (Alfaro 1996). In some BEC subzones, such as the SBSvk this requirement will be met some years and not in others. The spruce seedling (embling) probably needs to be taller than 1.5 m to be susceptible to weevil attack (Turnquist and Alfaro 1996). To date, summer 1998, weevil attack has just started in the BIOTIA CT. However, the attack levels are still too low to identify tolerant or susceptible clones. This information will be forthcoming as the plantations grow in size and local weevil populations

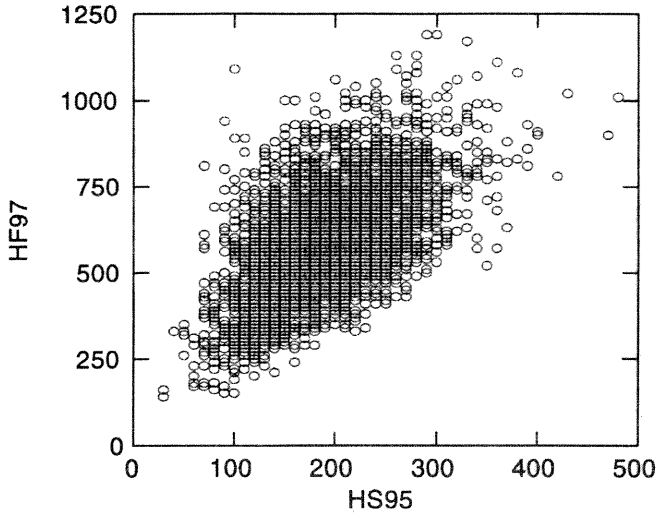

Figure 2—Height after 3 growing seasons (HF97) versus height at planting (HS95) for 12 BIOTIA families at Huble Road.

increase. Again, as with height growth, more time is required before superior clones will be identified.

ADMINISTRATIVE CONCERNS

The basic or first comparison for SE emblings is how much better is the SE embling clone than the full sib seedling from the same family. Based on a 415B stocktype which appears to be adequate for spring plant deployment on most sites in the central interior, the incremental increase in cost for full sib material is about 5-10 cents. This is considerably less than the present incremental cost of SE emblings at the nursery gate of 60-80 cents. Clearly, if incremental costs for SE emblings do not decrease, to justify a large operational SE program, there has to be SE benefits to which economic worth can be assigned beyond enhanced growth. Earlier free to grow, green up and adjacency considerations are three factors which could increase the relative worth of the SE propagule. Tolerance to the spruce leader weevil will increase the economic value of SE material. However, seed orchard full sib seedlings will also have some degree of tolerance to the weevil, and again, it comes down to the difference between seedlings and emblings from the same family.

Until the testing program is well underway, at least five more field seasons, the growth potential value that can be assigned to any SE clone is the same as that of full sib seedlings from the same family. Therefore in the short term, on an operational scale, SE technology cannot compete economically with full sib seedling lots from the same family or even with orchard select families. However, once the superior SE clones have been identified and the incremental SE costs have decreased (similar to that of rooted cuttings), there is a good probability that a small SE operational program for interior spruce in the BC central interior will be justified.

SE deployment is a form of clonal forestry. Public concerns have been expressed about forest health, ecosystem function and reduced genetic diversity certainly with regards to clonal forestry. In British Columbia, the Forest

Practice Code ensures that technical standards are in place to ensure adequate genetic diversity is maintained in seed and vegetative lots derived from seed orchards. The basis for these standards resides in many publications, for example Roberds and Bishir (1997). Further, Carson (1997) refutes the claim that forest health problems are more likely in a clonal forest. Rather, he (Carson 1997) suggests more forest health problems will arise from poor forest management than from atypical genotype representation in a clonal forest. Concerns about ecosystem function for a clonal forest with adequate genetic diversity is no different from concerns about ecosystem function for any plantation regardless of its seed origin. The public likely will have to be convinced of the safety of an operational SE program before operational deployment on any scale will be accepted in BC. This can be achieved through a concentrated, well focused extension program.

SUMMARY AND CONCLUSIONS

The CT for all interior spruce parental sources are established with about 48,000 individuals identified in the field. Environment still contributes significantly to observed clonal variation in the oldest CT site. This may result in a longer time frame, than projected at the beginning, for testing. In the older CT, few clones are performing better than the full sib seedlings from the same family. This may reflect SE stock quality issues at planting. It could result in fewer clones to select from for operational deployment. The full sib seedling - SE embling concern does not appear to be a factor in the more recent CT.

Weevil attacks were observed for the first time in BIOTIA CT in 1998. This should result in preliminary BIOTIA clonal weevil ratings by about 2000 or 2001. Unfortunately the 1996 CT have been hit with growing season frosts and it may be 2003 - 2005 before useful weevil ratings come from these CT. It is too soon to predict when weevil ratings will be available from the 1997 and 1998 CT.

Today, the economics associated with SE technology does not justify operational deployment. However there are some very good SE clones in the CT. As these clones are confirmed and selected, economic worth will be assigned to other traits or characteristics, such as weevil tolerance or green up. The economics then will probably justify a small SE operational program in the central BC interior.

Public concerns about clonal forestry need to be addressed through a coordinated extension program to ensure the opportunity to deploy SE material operationally.

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