An industrial perspective on the use of advanced reforestation stock technologies

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Abstract

J.D. Irving, Limited is a forest products company with large forest land holdings in Eastern North America. The company has been active in tree improvement programs for many years for a number of conifer species. As well as using traditional seed orchards to produce improved seed, the company has also integrated vegetative propagation initially through rooted cuttings and then via somatic embryogenesis (SE), primarily of spruces. The objective of the SE program is to obtain tested varietal lines to be deployed in multi-varietal forestry plantations. Data are presented that show the genetic gain in height, diameter, and volume over that of the varietal test average at various selection intensities after a decade in a field test.

Keywords: clonal propagation, rooted cuttings, somatic embryogenesis, multi-varietal forestry, genetic gain, weevil resistance

1. Introduction

Forest geneticists have identified the potential incremental gain for a range of traits afforded by alternative, advanced reforestation stock strategies over that provided by traditional seed orchards. These strategies include rooted cutting propagation of tested full-sib families, mass control-pollinated production of full-sib families and propagation of tested varieties capturing additive and non-additive genetic variation. The latter strategy has primarily been employed in reforestation

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with hardwood species such as poplar and eucalyptus species, where vegetative propagation by coppice production of cuttings is biologically feasible (Zobel 1993, Ondro et al. 1995). Varietal production options in conifers have, in general, received less attention due to the relatively higher production costs of clonal plants and the poor success with non-juvenile cuttings.

The development and rationale behind industrial application of advanced reforestation stock technologies is described from the perspective of a forest products company, J.D. Irving, Limited (JDI), in North-Eastern North America. The companyis a privately owned diversified forest products company managing 1.3 million hectares of Freehold land in the Provinces of New Brunswick and Nova Scotia, Canada and the State of Maine, United States. The company land holdings cross a broad diversity of forest types, from high quality tolerant hardwood through to lowland softwood. Products produced include softwood and hardwood pulp, white pine and eastern white cedar lumber, Kraft pulp, tissue, corrugated medium, light-weight coated paper and consumer products (JDI Sustainability Report 2013). The company has maintained a long-standing commitment to land stewardship and as part of that commitment has planted over 620 million conifer seedlings since 1957on its privately owned land, while hardwood forests are managed using natural regeneration. The company initiated a tree improvement program in 1979 and has been extensively involved since then, both independently as well as in collaboration with two regional cooperatives (New Brunswick Tree Improvement Council and Nova Scotia Tree Improvement Working Group). The primary species of interest are white spruce, red spruce, black spruce, Norway spruce, white pine and jack pine. All seedlings produced originate from seed orchards and to dateover 320 million seedlings have been produced with improved orchard seed. The company became interested in vegetative propagation based on its widely understood potential genetic gain (Park et al. 2015, in this volume) and advances that have been made are described below.

The purpose of this chapter is to describe the development of advanced seedling production strategies in an industrial context including gains observed in recent multi-varietal testing (MVF) using somatic embryogenesis.

2. Multiplication of elite families by rooted cuttings

While methods related to rooting juvenile cuttings of spruce were relatively well known at the time when the company's tree improvement program was initiated, successful propagation of rooted cuttings required specialized expertise and resulted in higher production costs than in seed-based production. Seed orchards were coming into production by the late 1980's and progeny testing had not yet provided sufficient genetic data to be used to identify specific combinations of parents that could yield sufficient genetic gain to warrant the additional production costs. By the early to mid-1990's estimates of parental breeding values became available based on open-pollinated or polycrossgenetic test data. This made it possible to produce control-pollinated crosses among high value parents followed by hedging of potted seedlings and multiplication with rooted cuttings (Adams and Tosh 1998). By the end of the 1990's rooted cutting production was semi-operational with annual production in some years in the hundreds of thousands before the program evolved towards the somatic embryogenesis approach.Nevertheless, the rooted cutting approach is often practical when seed orchard seed is not available due to poor seed production or timing (Ahuja and Libby 1993).

3. Varietal testing and production of rooted cutting with serial propagation

In species where seed orchard production is abundant and predictable, the incremental production cost of rooted cuttings based purely on making crosses among high value parents is a significant obstacle, especially when making use of such advances as flower induction using gibberellic acids (Greenwood et al. 1991) and supplemental mass pollination or controlled mass pollination. It was well understood that there is a substantial amount of non-additive genetic variation in spruce species (Mullin and Park 1992, 1994). Utilizing this variation to increase incremental gain is complicated by the poor rooting and plagiotrophic growth of non-juvenile spruce cuttings,i.e., typically around age 4 juvenility wanes (Bentzer 1993). By the time varietal field testing is accomplished, the donor plants are no longer viable for cutting production. Partial circumvention of this issue was proposed by Kleinschmitt (1993) who described a technique whereby juvenility could be maintained through serial propagation. By this method, hedge plants are re-propagated on a four-year cycle. In order to test the potential gain through this approach, JDI produced 32 control pollinated full-sib families among 34 selected black spruce parents. From each family, eight varieties were developed and hedged for a total of 256 varieties. In 1996, varietal tests from rooted cuttings were established at two sites in New Brunswick with 10 replicates at each, consisting of a single tree plot for each variety. Tests were evaluated for height, breast height diameter and individual calculated tree volume (Horner et al. 1983) at ages 5, 10 and 15. Based on varietal means and selection of the top 20% (51 of 256 varieties),gains of 8.5% and 29.2% for height and volume growth respectively were observed compared to the height and volume growth of all varieties at age 15 (6.41 m height, 0.027 m^3 volume). This test series also illustrated important genetic diversity management implications of varietal selection. For instance, the top 20% of varieties selected, based on height, were distributed across 19 of 32 families and 28 of 34 parents. If selection had been based on family means alone, selection at the same intensity would have included only 6 of 32 families and 10 of 34 parents.

At the same time, gain based on family selection would have been lower at 6.1% for height and 18% for volume. While advantages of varietal selection with rooted cutting compared to multiplication of elite families was demonstrated to be significant, there is little information in the literature regarding serial propagation of the species that JDI is concerned with. Furthermore, serial propagation in a nursery setting during the lengthy field testing phase involves significant logistical management and costs.

4. Integration of somatic embryogenesis into advanced seedling production

The potential for advanced seedling production was improved with the discovery of somatic embryogenesis (SE) in conifers in the mid-1980's. The use of cryo-storage of SE cell lines presented a solution to the problem of not being able to root cuttings from trees which were no longer juvenile. Storing cell lines in liquid nitrogen during the lengthy field testing phase to identify superior varieties has become essential to progress in this area. Immediate recognition of the enabling potential of cryogenic storage drove initial efforts by the company to evaluate somatic embryogenesis. Tree improvement programs at the time were most advanced for black spruce and on this basis, this species was chosen for initial focus (Adams et al., 1994). Initial efforts demonstrated the responsiveness of spruce species to all phases of somatic embryogenesis, including initiation of SE callus from zygotic embryos using modified Litvay (mLV) (Park et al.1993), maturation of somatic embryos, germination of mature embryos and transplanting of germinantsinto *ex-vitro* conditions in the greenhouse (Park et al. 1993; Adams et al. 1994.).

Currently, JDI's SE-based advanced seedling production system, a.k.a. Multi-Varietal Forestry (MVF), is following the process presented by Park et al. 2015 (in this book, Figure 3). Briefly, it involves: Selected parents from a long-term breeding program are controlled crossed and the resulting seeds are subjected to somatic embryogenesis for development of clonal lines;embryogenic lines are cryopreserved and then a portion of each line is thawed and propagated to produce plants for varietal field testing; once field testing shows which are the best lines, the corresponding embryogenic lines are retrieved from cryopreservation, mass propagated, and deployed in the plantations.

White spruce is most suited for planting on highly productive sites. Yields fromsilvicultural investments will be greater than on lower productivity sites, in contrast to species such as black spruce which are ecologically adapted to poorerlocal conditions. By the mid-1990's, early progeny testing results in white spruce enabled identification of first-generation white spruce selections with high breeding values, which were then used as the parents of controlled crosses. The seeds from these crosses were subjected to SE for the development of candidate varietal lines. As a result of the company's changing focus on higher value species, white spruce varietal tests were initiated beginning in 1999 using candidate varieties derivedfrom the full-sib crosses. From 1999-2004, 1367varieties of white spruce from 82families and 58parents were established at multiple sites across the region. Varietal testing was also initiated with full-sib crosses among Norway spruce parents with demonstrated resistance to white pine weevil (Lavallee et al. 1999; Figure 1). This species has been widely planted in North-Eastern North America for over 100 years and in many cases it out-performs local spruce species (Nova Scotia DNR report). However, Norway spruce is highly susceptible to white pine weevil damage, as are a number of native spruce species such as Sitka spruce and interior spruce in British Columbia (Alfaro and Ying 1990). White pine weevil kills current year leaders of trees and does not result in mortality but seriously impacts quality of stems. While work continued on improving SE productivity and greenhouse culture, emphasis was mainly on producing a small number of somatic seedlings from as many varieties as possible for establishment of field tests across the region. Varietal tests are typically established at 3-4 sites with the test design being ten replications with single-tree plots for each variety randomly planted in each replication.

Figure 1.*Norway spruce varietal test at age 15. Embryogenic varietal lines are developed from controlled crosses among weevil resistant parents.*

5. Observations from varietal tests

Of the many series of SE-based varietal tests, we present data from two tests; however, we observed similar trends from other series. Evaluation of varietal tests began after 5 growing seasons with subsequent remeasurement at ages 10 and 15. Individual tree volume is calculated using metric volume equations (Honer et al. 1983) when height and diameter measurements are available. At an early age, e.g., age 10, the calculated individual volume may not have practical use but it can be used as an index value combining height and diameter. Significant variation in average varietal height and diameter is observed and results are summarized in Tables 1 and 2 at age 10 for two separate varietal test series planted in 2000 and 2002 with 224 and 315 varietal lines included,respectively. From a quality perspective, the ratio of branch and stem diameter at breast height is also measured.

In Table 1, the 10 year performances of series 1 test of the varietal mixture consisting of 10, 20 , .., 100 top ranking varieties based on the volume indexis tabulated and compared to the overall test mean. Incidentally, the overall test mean represents expected seed orchard output, without inefficiencies of the seed orchard, because the parents used in producing embryogenic lines are the same asthose used for the seed orchard clones. The deployment of the top 10 varieties in the test would result in a realized gain of 18 and 27% for height and diameter, respectively, over the seed orchard gain at age 10. In general, at approximately 20% selection intensity, results are very similar across the two test series at 10-12% gain over the varietal means for height and approximately 17% for diameter (Table 2).

Table 1*.Varietal test of white spruce (Series #1) at age 10. The test was established in 2003at 3 locations in New Brunswick, Canada using plants produced by somatic embryogenesis while maintaining corresponding embryogenic tissue in cryo-storage.*

No. of top ranking varieties	Proportion selected	Height (cm)		Diameter (mm)		Volume (m^3)		Diversity	
		Mean	% of overall mean	Mean	% of overall mean	Mean	% of overall mean	No. of Families	No. of parents
10	4.4%	452.9	18%	70.8	27%	0.009724	78%	7	10
20	8.9%	441.7	15%	69.0	23%	0.008950	63%	11	14
30	13.4%	435.9	14%	67.5	21%	0.008476	55%	13	17
40	17.9%	432.6	13%	66.3	19%	0.008139	49%	16	19
50	22.3%	429.6	12%	65.4	17%	0.007889	44%	17	19
60	26.8%	425.4	11%	64.8	16%	0.007688	40%	18	20
70	31.2%	422.3	10%	64.3	15%	0.007509	37%	18	20
80	35.7%	419.0	9%	63.7	14%	0.007324	34%	19	23
90	40.2%	416.2	9%	63.1	13%	0.007167	31%	21	23
100	44.6%	413.5	8%	62.6	12%	0.007014	28%	21	23
Varietal test summary									
Number of varieties tested		224							
Number of parents		27							
Number of Families		27							
Mean Height		383.1							
Mean Diameter		55.91							
Volume		0.0054							

Table 2.*Varietal test of white spruce (Series #2) at age 10. The test was established in 2002at 2 locations in New Brunswick, Canada using plants produced by somatic embryogenesis while maintaining corresponding embryogenic tissue in cryo-storage.*

No. of top ranking varieties	Proportion selected	Height (cm)		Diameter (mm)		Volume (m^3)		Diversity	
		Mean	% of overall mean	Mean	% of overall mean	Mean	% of overall mean	No. of Families	No. of parents
10	3.2%	494.1	16%	83.76	27%	0.014176	75%	6	11
20	6.3%	483.9	14%	81.49	24%	0.013228	63%	7	13
30	9.5%	479.5	13%	79.87	21%	0.012652	56%	12	17
40	12.7%	473.5	11%	79.09	20%	0.012272	51%	14	18
50	15.9%	472.0	11%	78.18	19%	0.011956	47%	15	18
60	19.0%	470.8	11%	77.40	17%	0.011701	44%	15	18
70	22.2%	469.0	10%	76.83	16%	0.011485	41%	15	18
80	25.4%	467.9	10%	76.21	16%	0.011286	39%	16	20
90	28.6%	465.2	9%	75.75	15%	0.011128	37%	16	20
100	31.7%	462.9	9%	75.26	14%	0.010944	35%	17	21

Varietal test summary	
Number of varieties tested	315
Number of parents	23
Number of Families	21
Mean Height	425.4
Mean Diameter	66.0
Volume	0.0081

Genotype by environment interactions are observed but the varietal rank changes were small at the upper and lower ranges of overall performance. From a selection standpoint, varietal rank shifts across sites are evaluated based on consistency across the environmental gradient tested.

6. Scaling up production of somatic embryos

While incremental gain added through varietal production has clearly been demonstrated to be substantial, benefits of using SE and realizing return for the investment in technology development and varietal testing relies on producing SE seedlings for operational reforestation. The key factors are the cost of production compared to the value of additional volume produced, sawlog versus pulp production ratios and/or reduction in rotation length. Since 2008, JDI has focusedon improving all aspects of producing high quality SE seedlings. Important indicators include number of mature embryos per gram of embryogenic suspensor mass (ESM)matured, conversion of mature embryos to acceptable germinants and successful transition through transplanting into the greenhouse. Current laboratoryprotocols include proliferation of thawed embryogenic lines in suspension culture (Figure 2 A) and/or on semi-solid media,maturation of embryos on filter paper over semi-solid growth media in petri dishes, separating mature embryos through several cleaning steps (Figure 2 B), drying embryos and dispensing them onto semi-solid germination media in petri dishes or trays (Figure 2 C). An optimized timing has been developed for the germination period prior to greenhouse transplanting. Germinants are transplanted manually into pre-slit miniplugscontaining polymerized peatmoss(e.g., Jiffy Preforma mixture manufactured by Jiffy Products of America Inc., or Grow-Tech FlexiPlugs manufactured by Grow-Tech LLC). Greenhouse culturehas also been optimized and after several months, miniplugs are transplanted into Multipot 67 cavities (currently done manually,Figure 2 D). Success rate through to transplanting is typically 80-90% for white spruce and 70-80% for Norway spruce. Total production targets have been in the 300,000 – 400,000 range annually in the last several years with production cost and greenhouse recovery being the determining factors for future production increases.

Figure 2. *A) Proliferation of thawed embryogenic lines in liquid culture;* ***B****) Mature somatic embryos ready for germination;* ***C****) in vitro germination of somatic embryos;* ***D****) Operational production of somatic seedlings at J.D. Irving's greenhouse.*

7. Challenges for cost effective production of SE seedlings

Significant challenges remain to be addressed before the cost of SE seedlings will allow for step changes in production. In the laboratory production phase, the greatest costs occur after the maturation stage. Multiple steps are required to separate mature embryos from ESMmaterial and increased handling and drying introduces the potential to damage embryos. Uniform distribution of embryos on germination containers has an important impact on germinated to transplanted conversion rates as well as on transplanting productivity. Technology development is currently underway to automate these steps which are intended to improve both productivity and quality.

The most critical step from a cost standpoint is transplanting of germinated SE seedlings from sterile germination media to miniplugs in the greenhouse. This step typically involves individual handling of the fragile germinants with forceps and placing them in the peatmoss plug, whichis a significant hurdle from an economic production perspective. This remains the largest challenge to JDI implementing varietal production on a larger scale.Transplanting of miniplugs following greenhouse growth to larger seedling containers is currently done manually,however, horticultural automation systems are well developed to handle the transfer of miniplugs to larger containers which should reduce a significant portion of the overall cost of SE production.

7. Long-term opportunities for advanced seedling production

Traditional tree improvement by field testing and more recently, varietal testing, have demonstrated the broad genetic variability of conifer species which is important worldwidefor providing traits related to economic value and adaptiveness. Plantation establishment is a long-term investment in our region with rotations in the 35-45 year time window. Traditional seed orchard approaches, while effective, are not very flexible to respond to changing values and conditions. As well as providing incremental gains to growth rate through accessing additive and non-additive genetic variance, varietal production through SE offers significant gain through trait stacking. An important example is selection for white pine weevil resistance in Norway spruce. Increased resistance could be achieved through traditional seed orchards; however, the timeframe for having resistant seed would be approximately 15 years because of the long time period required to establish and wait for a seed orchard with resistant parents to come into production. In contrast, varietal testing and evaluation has made the production of weevil resistant seedlings possible in a much shorter timeframe, while at the same time increasing the number of resistant individuals deployed to plantations by selecting and propagating highly resistant individuals. Genetic gain for individual traits is often compromised by addition of other traits due to negative or even neutral genetic correlationsamong traits (Novaes et al. 2010). Varietal selection often offers the potential to mitigate this issue (Park et al. 2012). While growth rate is always important, value of plantations is also influenced by branching, stem form and wood quality. Varietal selection is a more efficient method for incorporating these traits and this helps to produce seedlings with greater value. Another long-term opportunity is related to integrating new technologies such as genomic selection. Progress in genomic selection may make it feasible to evaluate individual varieties for a range of traits much earlier than can be quantitatively measured in the field. Traits that can thus be tested include growth rate and wood properties such as density and microfibril angle (Park et al. 2012). Once this technology is proven, it could be incorporated much earlier in the selection cycle than is currently possible (one year versus 10-20 years).

Adaptation to changing climatic conditions is a concern thathas been looming for over a decade. Tree improvement field testing, as well as varietal testing has been conducted across a climate gradient that exceeds projected climate changes over the next 50 years. While breeding zones were established based on adaptive potential within the region, this aspect will need to be re-examined constantly in response to projected climate change. While forest tree genetics testing will provide intelligence regarding the adaptive potential of parents within regional breeding programs, varietal production strategies will provide the best means to respond from the standpoint of providing the best adapted genotypes for operational reforestation stock in a changing environment.

8. Deployment strategies

Most discussion around deployment strategies of varietal production has focused on pure versus mixed varietal planting (Park et al. 1998). These discussions weigh factors such as risk and advantages based on uniformity. The JDI approach has been mainly one of varietal mixture deployment. To determine the number of varieties in a mixture, an approach called"desired genetic gain and diversity" is used. In this approach, plantation diversity is managed dynamically based on the most up-to-date varietal test data. For instance, when the test is young we can include more varieties in the mixture with a reduced genetic gain. When the test is mature and varietal lines are well characterized, we can use a smaller number of varieties in the mixture while optimally increasing genetic gain (Park et al 2015, in this volume).

9. Conclusions

Advanced Reforestation Stock Technologies (ARST) of J.D. Irving, Limited is based on a long-term tree improvement program and incorporates the latest technological advances. Multi-varietal forestry based on somatic embryogenesis at JDI has demonstrated a substantially higher range of genetic gain than can be obtained with conventional seed orchard breeding. The main challenge for implementing industrial MVF, however, is the relatively high cost of SE seedling production due to manual handling of embryos, both pre- and post-germination. Thus, the development of an automated embryo handling system is required. J.D. Irving's ARST program will be ideally suited to incorporate genomic selection with vegetative deployment as outlined in Park et al. (2015, in this volume).

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