PART 2

THE CONFERENCE

INVITED SYMPOSIA PAPERS

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OVERVIEW OF ADVANCED GENERATION BREEDING AND PRODUCTION

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Abstract

The following paper examines the steps in the advanced generation breeding process, by considering the potential gain achievable and how much is lost at each step of the process. Major areas where improvements could be made include the capture of non-additive genetic variation, the increase of selection differential, the elimination of pollen contamination in seed orchards and concentrating the best genetic material on the best sites.

Introduction

For a thorough review of advanced generation breeding of forest trees one is referred to the proceedings of the Workshop on Advanced Generation Breeding held in 1984 in Baton Rouge (Anon. 1986). In the following, I would like to take a somewhat different approach to reviewing this subject: instead of discussing the steps in detail and looking at the various efficiencies and potential gains obtainable, I will start with the initial genetic potential and see how much we loose or gain in each step of the breeding process.

To illustrate the concept, I'll use *Hypoplatanus obtusa*, vB. as a model species, which is a little known Texas species, allegedly hiding out in the Big Thicket (van Buijtenen, 1975). A very desirable species commercially, it is also an ideal species for the tree breeder: it is a fast growing hardwood with a nice straight stem, it roots readily from stem cuttings, it is a wind pollinated monoecius species and is a prolific seed producer at an early age.

The numbers are presented as absolute values. In other words gains and losses are not expressed in percentages, but in cubic meters per hectare per year. This is critically important from an economic point of view: at harvest wood is sold in terms of absolute units not percentages.

The assumptions of the model are given in Table I. This assumes a very good site and vegetative propagation of the single best clone from a moderately large population (about 275). As a result, the maximum gain obtained with this species is 7 1/2 cubic meters per hectare per year, which is three standard deviations above the average. At this point it is important to make a disclaimer: any resemblance between *Hypoplatanus obtusa* and major southern forest tree species' is purely coincidental.

Table I. Assumptions used in *Hypoplatanus obtusa* model.

Site index		100
Growth rate		15 $m^3/ha/yr$
Maximum gain		50%
Gain from mass selection		8%
Heritability		.10
Phenotypic Variance (V_p)	=	36
Total Genetic Variance (V_g)	=	6.25
Non-additive Genetic Variance (V_D)	=	2.65
Additive Genetic Variance (V_A)	=	3.6

THE ADVANCED GENERATION BREEDING PHASE

Introduction

In this section we will more or less chronologically step through the various operations involved in advanced generation breeding and examine the impact on genetic gain.

Additive Genetic Effects

By adopting a conventional breeding strategy we immediately loose the opportunity to capitalize on the non-additive genetic effects. To reflect this in the graph the additive genetic effects were calculated as three standard deviations above the mean, just as was done for the total gain obtainable from vegetative propagation. On that basis the gain from additive effects would be equal to 5.65 cubic meters per hectare per year, which is still a very substantial amount.

In the remainder of this paper two cases will be considered: one is a typical approach using fairly common breeding procedures, while the other is a worst case scenario (named Murphy's Delight) where everything that could go wrong, did.

Mating Designs

In considering mating designs, one of the first requirements for a sustainable design is that it needs to regenerate a population of the same size as the initial population. In this model we assume a population size of one hundred. Some of the major mating designs available are open pollinated designs, nested designs, factorial mating schemes and partial diallels. The advantages and disadvantages of each are listed in Table II. A partial diallel design is used for the typical case and an open pollinated design for Murphy's Delight.

Table II. Advantages and Disadvantages of Various Mating Designs.

DESIGN	EVALUATION OF PARENTS	ESTIMATING GENETIC PARAMETER	ADVANCED GENERATION SELECTION	DIRECT ESTIMATION OF GAIN
Open Pollinated	Fair	Poor	From Ortets Only	From Orchard Seed Only
Partial Diallel	Good	Good	Good	Fair
Factorial	Good	Good	Potential Inbreeding (4 tester schemes)	Fair
Nested	Poor	Fair	Good (in complementary design)	Poor

As can be seen in Figure 1, there is a substantial drop in gain at this point. Why is this? There are three major causes: one is that there is only a limited selection differential. By selecting only the top 25% of the families and selecting the four best trees from each family, we have a selection intensity of 1.28 for between family selection and selection intensity of 2.41 for within family selection, assuming that the family size is about two hundred members. The second factor is that we are dealing with a low heritability trait with a heritability of approximately .10. This of course makes it possible to realize only a small portion of the genetic potential in any one generation. The third is that the additive genetic variance among family means is only $1/2V_A$. This is a consequence of the fact that each family has 2 parents.

It might be worthwhile to expand a little bit on the concept of genetic potential. The total genetic variance or the additive genetic variance cannot be equated with genetic potential. They are considerably less than the genetic potential. In the initial example of selecting the single best clone, we had effectively exhausted the genetic variance of that population in one step. However we did not really exhaust the genetic potential of that population for two reasons: 1) the selected tree was heterozygous, so by selfing the tree new genetic variability could be generated. This will not be practical since the progeny would suffer from inbreeding depression, however it does illustrate the concept; 2) by selecting the single best clone in the population we probably did not find the best combination of favorable genes available in this population, and therefore did not utilize the full potential.

The above example vividly illustrates that if one selects at a very high selection differential, one can make a large immediate gain, at the expense of not being able to capture an even larger portion of the total genetic potential, achievable by long term breeding at lower selection intensities.

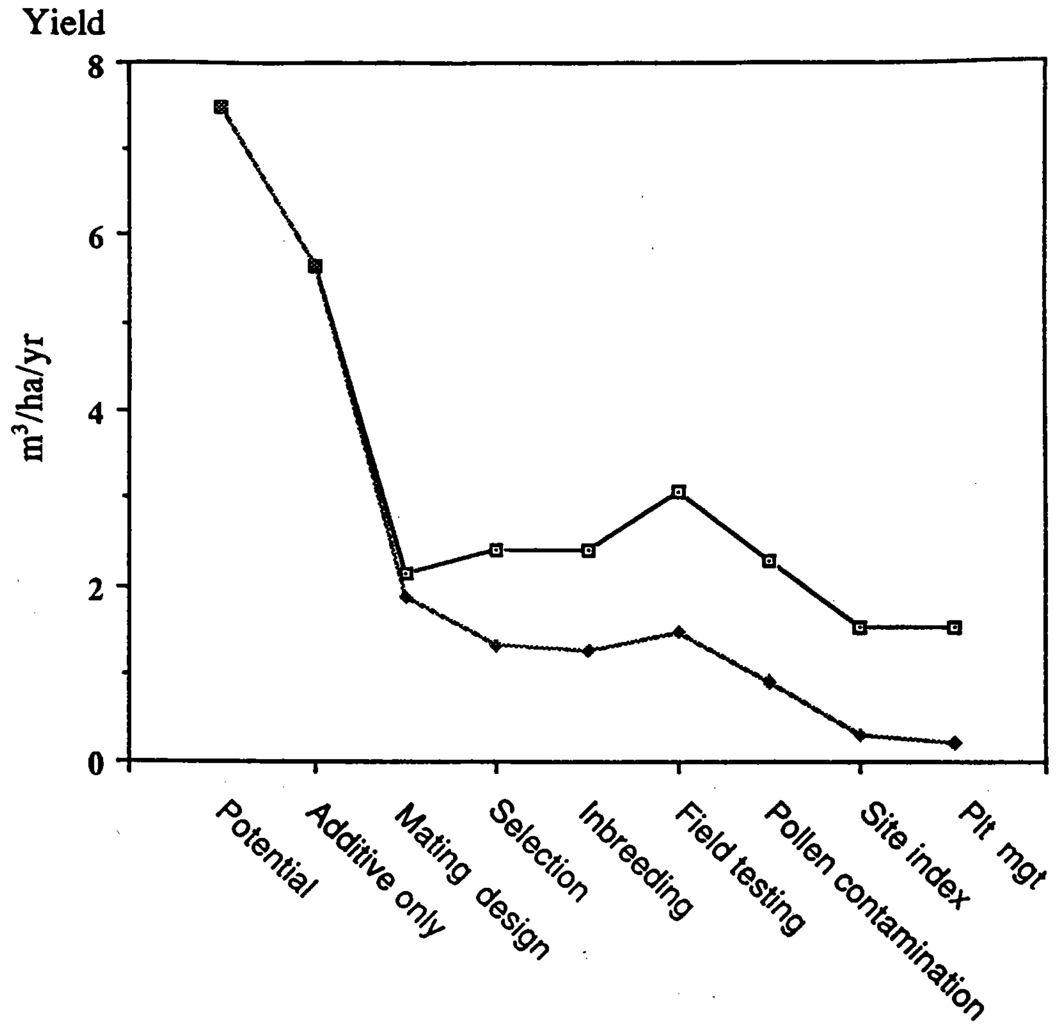

Figure 1. Potential yield of *Hypoplatanus obtusa* and reductions in yield at different steps in the breeding process.

The Selection Process

There are a number of important issues to discuss in connection with selection. 1) The importance of the gain per year versus the gain obtained per breeding cycle. This is the basis for the attractiveness of early selection and accelerated breeding. 2) The potential of marker-aided selection, and 3) the importance of data summarization procedures.

Currently it is not unusual to begin selection at age 5. This is in sharp contrast to the conventional wisdom thirty or forty years ago, when half of the rotation age was considered a suitable selection age. Juvenile-mature correlations are of course by no means perfect, but the losses are more than made up by being able to turn over generations faster.

In the example used here, we are assuming a rotation age of thirty years and all gains are calculated on the basis of thirty years. By selecting at age five it is possible to turn over a generation in sixteen years. Without early selection this could be as much as thirty-six years. As a result, we are actually seeing an increase in gain rather than a loss at this step. For the Murphy's Delight scenario we are assuming that the juvenile-mature correlation is only .4 resulting in a loss of gain.

Marker aided selection promises to become operational within the next five years. Molecular markers associated with genes controlling desirable traits, will be used to improve the efficiency of within family selection. Indications from crop plants are that it could double the efficiency. Since no good figures are available it is not included in the model.

Data summarization procedures are also critical. The BLP system, originally developed for cattle, has been adapted to forest tree breeding by White (1989) and could make the selection process more efficient. Lack of data for trees precluded its use in the model.

Inbreeding

In the early days of tree improvement programs there was considerable concern that continued breeding of the same population would eventually lead to inbreeding and effectively negate the genetic gains that had been made. This no longer is a real problem. By subdividing the population into breeding groups or sublines, inbreeding can be managed quite effectively (van Buijtenen and Lowe, 1979; Lowe and van Buijtenen, 1986). This is done by making crosses only within the breeding groups, and making sure that the clones in the orchards are arranged in such a way that the seed from the orchard is outcrossed. As a result there is no problem with inbreeding in the typical breeding program. In Murphy's Delight we assume a 5% loss due to inbreeding depression.

Field Testing

The statistical design of progeny tests and proper attention to field layouts are a couple of the most critical phases in any tree improvement program. Progeny testing can be done for a number of purposes (McKinley, 1983). The two most important ones are generating information for rogueing orchards and generating a new population of trees to select from and the information needed to make the selections. The field test designs and the genetic parameters assumed are given in Table III. The calculations follow the paper by van Buijtenen and Burdon (1990) page 1652.

Table III. Field Test Designs.

TYPICAL PROGRAM	MURPHY'S DELIGHT
3 locations 12 replications/location 6 trees/plot	2 locations 3 replications/location 100 trees/plot

VARIANCE COMPONENTS

Variance Compart	Typical Program	Murphy's Delight
σ_f^2	12	12
σ_r^2	7	7
σ_{lg}^2	.25	1
σ_{rg}^2	.25	1
σ_{lg}^2	.15	
σ_{rs}^2	.10	
σ^2	10	10

It can be seen in Figure 1, that both field tests actually improved the efficiency of the operation. This is because up to this point field design was ignored and all the environmental variation was lumped together in the error term. In fact, proper field design allows us to effectively eliminate a large portion of the environmental variation by moving it from the error term to the replication term.

SEED PRODUCTION

Introduction

In the seed production phase, proper layout of orchards is important. However this usually presents few problems and minor changes in seed orchard design such as the difference between random designs, interlocking designs, and systematic designs, probably do not have a major affect on genetic gain. There is one major factor though that is of great concern, and that is pollen contamination.

Pollen Contamination

A number of investigations (Friedman, 1985; Wiselogel, 1986) have indicated that 50% contamination in seed orchards is not uncommon. Presuming that the orchard is surrounded by trees only one generation behind that of the orchard, this would lead to a loss of about 25% of the genetic gain obtained in that particular orchard cycle. In the case of the Murphy orchards we assume the situation is particularly bad, having 60% contamination and a consequent loss of 30% gain. The result is shown in Figure 1.

WOOD PRODUCTION

Although the actual production of the trees might not seem to be related to advanced generation breeding, in effect it has a major impact. The impact occurs in two ways: the effect of site index and the effect of plantation management.

Effect of Site Index

The assumptions of the effect of site index on yield are given in Table IV. Since we normally give genetic improvement figures in percent, the impact on the high quality site would be much larger than on the low quality site in terms of absolute numbers. Since profits and losses are measured in dollars and therefore in absolute terms this is critically important. Biologically this is also significant. Actually the effect may be underestimated. The general experience is that genetic differences are more clearly expressed on better sites than on poor sites. The best deployment strategy is therefore to plant the very best genetic material on the best sites first and then on the lesser quality sites if more material is available. Another consideration that needs to be taken into account, particularly for land owned or operated by forest products industries, is the distance from the mill. Because of the transportation costs involved, the best material should be planted closer to the mill other things being equal.

Table IV. Relation Between Site Index and Yield for *Hypoplatanus obtusa*.

	Site Index	Yield $m^3/ha/yr$
Maximum	100	15
Typical	80	10
Murphy	60	5

Effect of Plantation Management

This also can have a major impact. Details are different from site to site, but the goal of plantation management should be to maximize the growth of the trees. Tree improvement and intensive management ought to go hand in hand just like in agriculture, where much of the gains in productivity have been brought about by improving management practices, and by breeding for crops that can take advantage of the intensive management. Good site preparation, competition control, proper spacing, thinning, fertilization where appropriate, and even pruning can be beneficial.

By the same token, bad management practices can more than offset genetic gains. Excessive competition in the early years may completely wipe out any genetic differences if a stand becomes overstocked and stagnant this in effect greatly reduces genetic gain simply by loss of stand productivity.

DISCUSSION

The curve in Figure 1 shows there are four places where major improvements in efficiency could be made. They are the capture of non-additive genetic variation, the increase of selection differential, the elimination of pollen contamination and concentrating the best genetic material on the best sites.

Clonal propagation is obviously the best way to capture the non-additive genetic variation. One problem that must be kept in mind however is that selection differential is a major factor too. In other words the very high gain presented in Figure 1 is in part the result of a very high selection differential. If one for instance were to plant a 20-clone mixture, most of this advantage would be lost.

Trying to increase the selection differential in sexually propagated material would pose a more difficult problem. An obvious method is to simply work with larger populations. This of course would increase the cost in proportion to the population size and the point of diminishing returns is reached quite soon.

Another avenue that perhaps has not been explored as much as it deserves is to work with smaller family sizes and larger numbers of parents. This is a somewhat risky procedure however. It might be feasible to use a two-stage screening procedure where at the first stage a large number of small families is used with a high selection differential among families. This is followed up in the second stage by testing the few selected families very intensively. Such an approach is being used by the CIMMIT group in Mexico in their maize improvement program. One could also approach this, by creating a super-breeding group or elite population composed of the very best individuals from the breeding groups and sublines. This is also referred to as nucleus breeding (Cotterill, 1989).

The reduction of pollen contamination is not an easy task. Occasionally it is possible to locate the orchard in an area where no contaminating pollen is present. Supplemental mass pollination is a good technique and is applied on a limited scale. Including pollinator trees in the orchard has been advocated at various times but as far as I know has not been applied operationally.

Concentrating the best families on the best sites closest to the mill is a sensible approach to increasing the economic returns from improved material. A number of companies are growing their seedlings by families and this gives them an ideal opportunity to accomplish this. It also fits right in with the idea of matching particular families to particular sites.

At the current state of the art good genetic gains are made with many forest tree species. There are still many ways to improve the efficiency of the breeding process and equally many ways to lose much of the gains through lack of attention to important details.

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INCORPORATING CLONES INTO TREE IMPROVEMENT STRATEGIES: TWO CONTRASTING EXAMPLES FROM BRITISH COLUMBIA

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INTRODUCTION

The use of clones in reforestation is becoming increasingly important (Libby et al. 1993). Improved techniques for maintenance of the juvenility of cutting-donors and subsequent rooting, has resulted in the production of quality stecklings available for certain species (Talbert et al. 1993). The production of plants through tissue culture techniques, especially somatic embryogenesis, has also become a reality for a few species (Becwar 1993). Thus, clonal forestry, the testing, selecting, and deploying of clones, is now possible operationally for a number of species worldwide (Ahuja and Libby 1993).

Tree improvement programs are well-established for many commercial species worldwide, with some programs into second and third generations. Strategies are becoming increasingly sophisticated, with the promise (and reality) of substantial gains for many different traits. The incorporation of clones into existing genetic programs can allow for maximizing genetic gain per unit time (Shaw and Hood 1985, Matheson and Lindgren 1985, Shelbourne 1993) and increase the efficiency of genetic tests (Shaw and Hood 1995, Russell and Libby 1987, Russell and Loo-Dinkins 1993). However, too often the development of a clonal strategy is considered separately from the main breeding strategy, quite often by different organizations.

Clonal strategies have been developed for two commercial conifer species in British Columbia, spruce from the interior (*Picea glauca* (Moench) Voss and *P. engelmannii* Parry), and yellow-cedar from the coast (*Chamaecyparis nootkatensis* (D.Don) Spach). The development of these strategies have taken into consideration historical, economic, and biological advantages and constraints specific to each species, and has resulted in two different clonal approaches.

Although in their infancy, both clonal programs are currently being implemented. The clonal strategy for interior spruce, which involves a large existing tree improvement program, features an "add-on" clonal testing strategy. The yellow-cedar genetic program is newer and much smaller, and features "true" clonal forestry, involving the incorporation of clones into the mainstream breeding and testing program.

This paper will outline the above two contrasting clonal programs, with emphasis on the historical and biological factors that have shaped the current strategies. Distribution of testing effort, selection strategies, and expected gains, as compared to the traditional tree improvement approach will also be presented for both strategies.

INTERIOR SPRUCE CLONAL STRATEGY

Current genetic program

The interior spruce reforestation and tree improvement programs are large. Approximately 90 million seedlings are planted annually in British Columbia. Three-thousand wild-stand parent trees are currently being tested in 52 genetic tests with 380,000 seedling progeny. There are 18 seed orchards established with a projected annual output of 68 million seedlings. First-generation orchards have been culled based on open-pollinated progeny test results, and breeding for a second generation has begun. This large program would be difficult to implement on a strictly clonal basis.

The Prince George breeding zone has the largest annual demand for seedlings. There are currently 5 seed orchards which will supply seed for 19 million seedlings to help meet the annual demand of 40 million seedlings. Breeding values are now available based on stem volume and relative density at 15 years (Yanchuk and Kiss 1993). As well, family information on white pine weevil (*Pissodes strobi* (Peck)) susceptibility, an increasing problem with interior spruce forests in British Columbia, is available (Kiss and Yanchuk 1991). There is an opportunity to capitalize on the wealth of genetic information in this breeding zone through an "add-on" clonal strategy by supplementing gains expected from the main-stream breeding program.

Somatic embryogenesis

Protocols for the production of emblings (plants from somatic embryogenesis) have been developed for interior spruce (Sutton et al. 1993). Somatic embryos resultant from excised mature seed embryos, are germinated in test tubes, transferred to containers, acclimatized to ex-vitro conditions, and placed in the nursery (Webster et al. 1990). Early physiological and morphological data indicate emblings are performing similar to seedlings in both the nursery and the field (Grossnickle and Major 1993a, Grossnickle and Major 1993b). As well, techniques for successful cryopreservation have been developed (Kartha et al. 1988), enabling long-term storage of clonal material. Thus, emblings appear to be a reality for practising clonal forestry with interior spruce.

"ADD-ON" CLONAL STRATEGY

The breeding, testing and trait selection for additive gene effects is the main emphasis of genetic improvement for interior spruce. There is an opportunity with somatic embryogenesis technology to not only capture additional gain in volume, but to take advantage of family and clonal differences in weevil susceptibility (Kiss and Yanchuck 1991, Ying 1991). The clonal strategy will involve an "add-on" testing phase to the main breeding program (Figure 1). Clonal testing of progeny from first-generation backward-selected parents will capture additional gain over and above gains from rouged first-generation seed orchards. It is important to recognize that these gains must be evaluated in light of additional time and costs of extra testing. As well, the "add-on" testing phase is a dead-end with respect to further genetic gains. This can be achieved only through additional breeding, testing, and selecting.

Nine parents out of 200 have been selected based on 10-year height and weevil resistance at 15 years, and will be recombined using partial diallels. Approximately 1000 clones will be produced from a maximum of 30 full-sib families using somatic embryogenesis. Clones will be tested over four series with 250 clones per series annually. Four ramets per clone on each site will be tested with a minimum of two sites; one with high growth potential, and the other with potential for a high level of weevil attack.

A total of 30 clones will be selected based on mean height growth and weevil damage at 6 years. Selected clones will be deployed operationally to reforestation sites as mixtures (Libby 1987), with a maximum of one million emblings per year. This will result in the planting of approximately 33,000 emblings from each selected clone each year, which represents 0.037% of the total interior spruce planted annually in British Columbia.

Gains expected from the clonal program will be about 23% in height growth at 6 years, as well as the deployment of clones with minimal susceptibility to weevil attack. This height gain represents an additional 12% over rouged first-generation seed orchards. The selected clones will be deployed for 20 years, at which time a new round of clones can be tested, and selected for production (Figure 1).

YELLOW-CEDAR CLONAL STRATEGY

The reforestation effort for yellow-cedar is much smaller than interior spruce. Although only a minor component of British Columbia's annual timber harvest, the best grade logs have the highest value of any timber produced in the province. Thus, the annual demand of just over one million seedlings can easily justify a genetic program (Russell 1993).

Steckling production

Cone crops of yellow-cedar are infrequent and small, and seed that is available has poor and erratic germination. Thus, in order to meet the demand for planting stock, an operational system for producing yellow-cedar stecklings (rooted cuttings) has been developed (Karlsson 1981, Russell et al. 1990). Currently, most of the annual demand is met by stecklings. Cuttings are obtained from hedged donors varying in age from 2 to over 15 years. Cutting-donors are mostly seedlings from either operational seedlots or phenotypically-selected trees.

Through a combination of hedging and serial propagation of cutting-donors, it is possible to delay maturation of tissue for at least 15 years such that rooting percentages exceed 75% (Russell et al. 1990), and subsequent stecklings are comparable to seedlings both physiologically and morphologically (Karlsson and Russell 1990, Russell et al. 1990, Grossnickle and Russell 1993, Folk et al. 1993). Thus, in conjunction with early selection, the favourable production of stecklings makes clonal forestry a viable strategy for the genetic improvement of yellow-cedar.

CLONAL FORESTRY STRATEGY

The genetic improvement strategy for yellow-cedar involves 1) breeding population advancement through the exploitation of additive genetic variation by repeated selection and breeding, and 2) deployment of genetic gain through propagation of selected clones. In order to implement an efficient program and to maximize genetic gain per unit time, the above two objectives will be combined into one test by clonally replicating seedlings from controlled matings (Figure 2). This will allow for a dual purpose test: selection of an advanced generation breeding population based on additive gene effects, and selection of clones for the production population. This strategy has the potential to maximize total genetic gain (Shaw and Hood 1985, Shelbourne 1993), as well as genetic gain per unit time (Matheson and Lindgren 1985).

Preliminary results from a genetic architecture study (Russell 1993) indicates the feasibility of using one breeding zone which encompasses 90% of future areas to be reforested with yellow-cedar. The breeding population for this zone currently consists of 192 phenotypic selections from wild stands established in a breeding orchard. A mating design involving 8-clone disconnected circular partial diallels (Lowe and van Buijtenen 1981) will be used to produce full-sib progeny. Each diallel will result in 16 full-sib families for a total of 384 full-sib families. Four series of breeding, testing, and selection will be done, with six diallels per series.

Progeny testing for ranking parents based on GCA and clonal testing for selecting superior clones based on genotypic values (clonal mean) will be combined as mentioned above (Figure 2). A proposed design involves the cloning of 20 1-year-old seedlings per full-sib family and deploying two ramets per clone on each of three sites. This would result in 3,840 trees planted on approximately 2 hectares per site with $4m^2$ spacing. Clones will be deployed using sets within replications with full-sib families balanced across all replications within a site (Foster and Shaw 1987). This strategy will result in the testing of 1,920 clones per series for a total of 7,680 clones over four series.

The above design is a compromise between numbers of test sites, families, clones, and ramets tested with respect to gain in both the production and breeding population. The optimal distribution of effort assuming a fixed resources scenario, depends upon many factors including genetic and phenotypic variances, genotype x environment interactions, selection intensity, and selection strategy. Based on previous and ongoing theoretical studies, the above strategy seems reasonable with respect to maximizing gain in both populations (Shaw and Hood 1985, Russell and Libby 1987, Russell and Loo-Dinkins 1993).

Selections for the production population will be deployed operationally using rooted cuttings (Figure 2). The top 25 clones per series will be selected at age 12 based on growth and wood quality. A restriction of three clones per full-sib family and six clones per half-sib will be imposed to minimize relatedness among selected clones. Combined-index selection (family and clone-within-family) may be used for clonal selection, however, since coancestry will be controlled, simple mass clonal selection may yield similar results. This has yet to be fully explored and will depend upon levels of coancestry control, proportional amount of additive and nonadditive genetic variance, and selection intensities.

At the time of progeny planting, a cutting-orchard will be established with one ramet per clone transplanted into four-litre pots and pruned annually for the maintenance of juvenility. A 50% rouging of the progeny-tested clones will be done in the cutting-orchard based on four year data. Each of the remaining selected clones will be repropagated to further maintain juvenility and to multiply each clone to two ramets. A similar rouging will be done at age eight with another 50% reduction in clones in the cutting-orchard and a repropagating of selected clones and a doubling of ramets to four per clone. At age 12, the best 25 clones per series will be selected, repropagated, and vegetatively multiplied, and within two years of selection the cutting-orchard will be in full production (10,000 stecklings per clone x 25 clones/series x 4 series = 1 million stecklings). Successive rougings at four year intervals serves two purposes:
1) maintenance of juvenility through a combination of serial propagation and pruning, and;
2) producing a rouged orchard at production-scale levels soon after final selection age.

Selections for an advanced generation breeding population will be based on the same traits, and at the same age as the production selections. Again, given coancestral control, optimal gains with clonal replicates may not be achieved with index selection but with 3-stage tandem selection (Shaw and Hood 1985). Eight clones per diallel for a total of 192 clones will be selected for the second generation breeding population.

The use of cloned genetic tests as compared to seedling progeny is expected to increase gains by 1.26 for the breeding population, and 1.21 for the second-generation clonal seed orchard. The use of clones in reforestation is expected to result in 7 to 14% additional gain over seed orchards derived from seedling progeny tests, and 4 to 11% over seed orchards from clonal progeny tests, depending on the selection intensity and proportion of additive to nonadditive genetic gain.

SUMMARY

The incorporation of clones into existing tree improvement programs must consider historical, economic, and biological factors pertinent to the appropriate species. Interior spruce tree improvement is a large program which is into the second generation. Breeding values are available for economically important traits, and techniques for embling production and cryopreservation have been developed. Thus, an "add-on" clonal strategy in this zone meshes well with the existing production population effort. Future genetic gains will be realized through additional breeding, testing, and selecting.

The yellow-cedar program is newer, smaller, and more manageable clonally than the spruce program. Thus, in conjunction with the ability to delay juvenility and produce acceptable stecklings operationally, cloning genetic tests in the mainstream breeding program seems reasonable. The resulting strategy will be capable of delivering increased genetic gains more efficiently as compared to the traditional seedling progeny and seed orchard approach.

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Figure 1. "Add-on" clonal strategy for interior spruce.

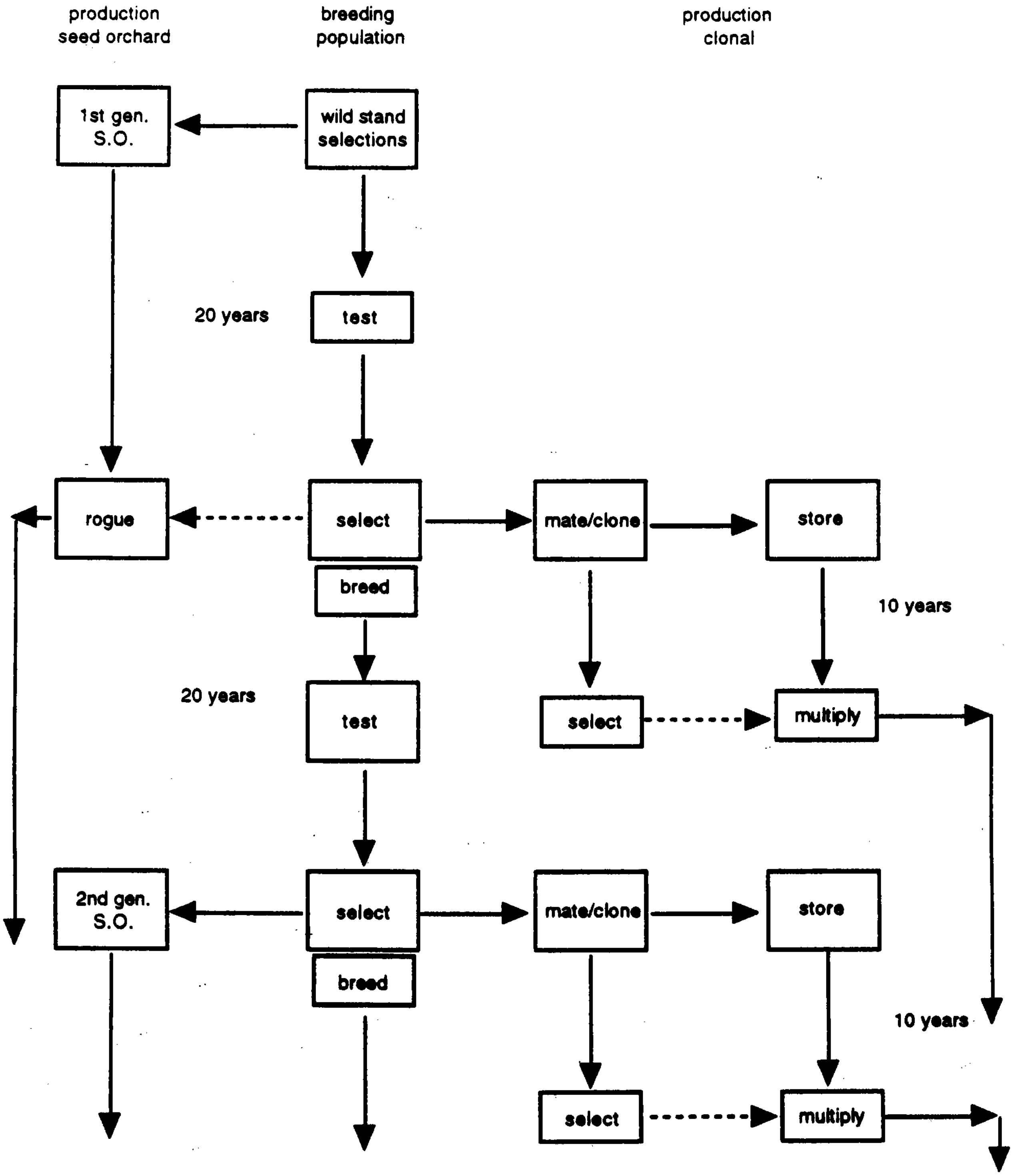
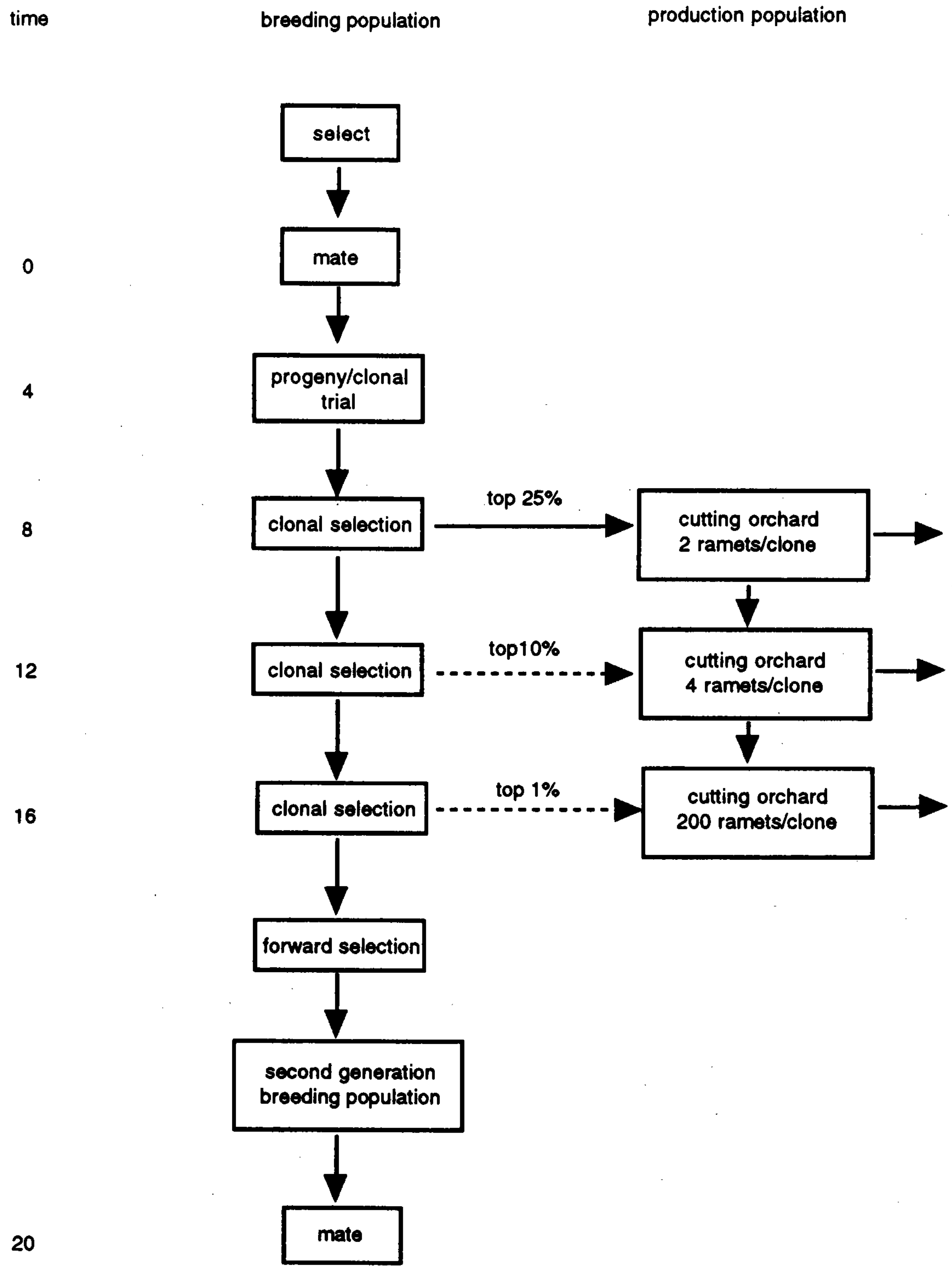


Figure 2. Yellow-cedar clonal forestry strategy.



USING RECENT ADVANCES IN THE CONTROL OF CONIFER REPRODUCTIVE DEVELOPMENT TO INCREASE GENETIC GAIN

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ABSTRACT

The effects of $GA_{4/7}$ applications by foliar spray and stem injection on flowering are compared in black, white and red spruce. In all cases, a single stem injection is as or more effective than foliar spray for the promotion of both female and male flowering, and requires less labor and GA. In jack pine, stem injection also effectively promoted female flowering. After the GA is injected into the stem, it appears to move rapidly via the transpiration stream to the upper part of the tree, where it persists long enough to promote flowering. Potential applications of the more cost-effective stem injections to increasing seed production, genetic gain, and inclusion of poor flowering clones in breeding programs are discussed.

INTRODUCTION

Operational application of GAs to induce flowering in conifers via foliar spray requires at least several applications of $GA_{4/7}$ at a concentration of 200 mg/L or more (e.g. Greenwood et al. 1991, Ho 1991). In contrast, one or two stem injections of GA can effectively promote flowering. For example, Philipson (1985) reports that two stem injections of $GA_{4/7}$ effectively promoted flowering in Sitka spruce, while Ross and Hollefreund (1991) report that single stem injections of $GA_{4/7}$ were as effective as three successive weekly foliar sprays in promoting female flowering in white spruce, but were more effective in promoting male flowering. Nielsen (1993) has recently reported that stem injection and foliar sprays both promote flowering in Norway spruce. Not only does a single stem injection require far less time to apply than several foliar sprays, but much less GA may be required. However, these observations also raise the question as to why a single GA application, via stem injection , is as effective as three to eight weekly foliar sprays. Both treatments are applied shortly after the elongation of the current year's shoot has begun, and potentially reproductive primordia have been initiated. The apparent requirement for the presence of GA for several weeks if applied by foliar spray suggests that elevated GA levels are required for an extended period prior to the differentiation of reproductive primordia. Does GA applied via stem injection persist within the plant longer than that applied via foliar spray?

Herein we report on the results of a number of trials of stem injections on a number of species, as well as observations of the movement of $GA_{4/7}$ through the xylem of a conifer stem segment. The results will be discussed in terms of what is known about GA metabolism and how GAs influence the development of reproductive buds in conifers. In addition, potential applications of improved flower stimulation techniques to increasing seed production and genetic gain in conventional and new types of seed orchards, such as meadow orchards, are discussed.

MATERIALS AND METHODS

GA application

Foliar sprays were applied as described in Greenwood et al. (1991) using $GA_{4/7}$ at 250 to 400 mg/L in 5% ethanol plus 0.2% (active ingredient) Aromox C-12W (Armax Chemical Corp. Ltd., Saskatoon). Stem injections were applied by dissolving the total dose (5 to 50 mg/tree) of $GA_{4/7}$ in 1 mL of absolute ethanol, and equal amounts were injected into each of two 45° downwards sloping holes 1.5 cm long. The holes were drilled with a 6.4 mm (1/4") bit using a cordless electric drill on opposite sides of the main stem about 25 cm above the root collar of the tree. The holes were then sealed with liquid grafting wax. Time of treatment application and GA dose are shown in the captions for the tables. Overall, foliar sprays were applied throughout the elongation period of long shoots, while stem injections were performed towards the end of long shoot elongation.

Plant material

Stem injections were applied to plantation-grown trees of both jack pine and black spruce about 4 m tall (about 14 y old). Ten trees were randomly selected for each treatment within a 0.5 ha section of the plantation. Foliar sprays and stem injections were compared in white spruce using 15 grafted clones 2-3 m tall (6-10 y old from grafting for outdoor-grown trees, 4-6 y old for indoor grown trees), growing in pots in the breeding hall at J.D. Irving's Sussex, NB nursery, and in the ground at the Parkindale seed orchard. Both foliar sprays and stem injections were applied to 16 clones of grafted red spruce 2-3 m tall (6-7 y old from grafting, located at Bowater-Mersey's Melvern Square seed orchard near Kingston, NS). Foliar sprays were applied only to the upper 1/3 to 1/2 of the live crown, since most female flowering occurs there. In order to wet all the foliage, about 50 mL of solution was used for each application to the tops of trees. All treatments were replicated within each clone.

Data analysis

Flower counts were performed the spring after the treatments were applied, after the strobili had emerged from the cone scales. In some cases, all male and female strobili were counted, while only females were counted in the black spruce and jack pine plantations. Flower counts were subjected to analysis of variance using treatment and clone (when grafted clonal material was used) as main effects. Mean separations were carried out using Scheffe's method or Duncan's test. All statistical tests were carried out using SAS or SYSTAT.

$GA_{4/7}$ movement in stems

The passage of GA through a 13 x 1 cm piece of larch stem was monitored by allowing water to continuously flow through the stem(which was connected to a water reservoir consisting of a 500 mL separatory funnel connected to the stem by Tygon tubing, so that the water dripped onto the upper end of the stem). $GA_{4/7}$ solution (0.25 mL of a 50 mg/mL stock solution in absolute ethanol) was injected onto the upper cut surface of the stem. The presence of GA in the effluent was detected using a spectrophotometer. GA in water absorbs strongly at 200 nm, and can be detected if the stem is thoroughly flushed with distilled water (which removes interfering compounds, probably phenolics, which also absorb strongly at this wavelength) prior to addition of the GA. Using stem effluent collected prior to adding the GA as a blank, GA could be detected in the effluent.

RESULTS

Stem injections on large trees

Stem injections significantly promoted female flowering in plantation-grown jack pine trees which were almost 5 m tall ($p<0.013$), but single applications done in mid-July were most effective, and were significantly different from the control (see table 1). The split application of 25 mg in June and July was least effective treatment, and also resulted in significant kill of the top two whorls of foliage. There was no significant treatment effect on height and diameter growth.

Table 1. Jack Pine. Effects of 1 vs. 2 stem injections at 25 mg $GA_{4/7}$ per tree, and single injections of 25 mg and 50 mg per tree at 2 different dates on plantation-grown trees. Ten randomly selected trees received each treatment. The means for ht., dia., # female strobili, and the number of terminal flushes killed are shown for each treatment. Different letters indicate treatment means are different at $p<0.05$ (Duncan's test).

Total Dose	# Injections	Ht. 1990 (cm)	Dia. 1990 (mm)	♀ Strobili/ Tree	Damage
25 mg	2^A	448	101	45 b c	1.5 a
25 mg	1^B	478	112	78 a b	0.2 b c
50 mg	1^B	468	99	98 a	0.6 b
50 mg	1^C	481	94	64 a b c	0.0 c
Control	0	487	101	18 c	0.0 c

[A] Applied on 6/13/89 and 7/12/89

[B] Applied 7/12/89

[C] Applied 8/3/89

Stem injection of 25 to 50 mg GA/tree significantly promoted female flowering in plantation-growth black spruce ($p<0.01$) that were similar in age and size to the jack pine (see table 2). There was no significant difference between one injection in late June and two applications done in late June and mid-July. No foliar damage was noted for either treatment.

Table 2. Black Spruce. Effect of one vs. two stem injections on successive dates of 50 mg $GA_{4/7}$ per tree on flowering by plantation-grown trees. Ten randomly selected trees received each treatment. The means for ht., dia., and # ♀ strobili per tree are shown for each treatment.

# Injections	Ht. 1990 (cm)	Dia. 1990 (mm)	♀ Strobili/Tree
1^A	495	95	128 a
2^B	493	93	113 a
Control	497	91	19 b

[A] Applied on 6/27/89, 25 mg

[B] Applied on 6/27/89 and 7/12/89, 25 mg on each date.

Foliar sprays vs stem injections

Both stem injection and foliar spray significantly promoted female flowering by red spruce ($p<0.0001$). but surprisingly the lower rate of GA via stem injection yielded the best results (see table 3). There was no significant treatment effect on male flowering which was abundant on all treatments including the control ($p<0.45$). There were also significant clonal differences in flowering ($p<0.022$), but no interaction between clone and treatment could be calculated because there was no replication by clone within each treatment.

Table 3. Red Spruce. Comparison of the effects of stem injection and foliar spray of $GA_{4/7}$ on ♀, ♂ flowering. Foliar sprays began on 6/8/89 and stem injection done on 6/12/89. Different letters indicate that treatment means differ at -$p<0.05$ (Scheffe's Test).

Treatment	Female	Male
Control	44 a	120 a
Foliar spray (8 weekly applications, 250 mg/L)	160 a b	195 a
Stem injection, 5 mg/tree	415 c	224 a
Stem injection, 50 mg/tree	200 b	179 a

Both stem injection and foliar sprays were also compared on white spruce clonal material grown both indoors in pots and outdoors in the ground (see table 4). The larger number of strobili observed per tree outdoors is in part due to the greater size and age of these trees. In the outdoor study, even though the sample size was small (6 trees per treatment) both treatments significantly increased female flower production over the control ($p<0.048$) but not male production ($p<0.201$). The apparent increase in male flowering observed due to foliar spray outdoors was due to the positive response of a single ramet within the heaviest flowering clone. There was a significant clonal effect on flowering across all treatments ($p<0.007$) but no clone by treatment interaction could be calculated because there was no replication within each clone-treatment combination. The indoor trial did not include an untreated control since it has been established that foliar sprays consistently stimulate female flowering indoors; therefore the effects of foliar spray versus stem injection were compared. The apparent greater response in female flowering to stem injection was significant at $p<0.023$, and there were significant differences among clones across treatments ($p<0.000$), but the clone by treatment interaction barely missed significance ($p<0.063$). Despite the apparent greater male flowering response to stem injection, this effect was not statistically significant ($p<0.534$) because of a highly significant clone by treatment interaction ($p<0.000$) which was used as the error term.

Table 4. White Spruce. The effects of stem injection (10 mg $GA_{4/7}$/tree, done on June 20, 1990) and foliar sprays (8 weekly sprays of $GA_{4/7}$ at 400 mg/mL, begun on May 3) applied to grafted ramets of clones grown in pots indoors, or in the ground outdoors. Outdoors, one ramet per clone received either no treatment, foliar spray or stem injection (a total of 6 clones was treated). Indoors, 1-3 ramets from each of 15 clones received each treatment. Different letters indicate means are different at $p<0.05$.

	Indoors		Outdoors	
Treatment	♀	♂	♀	♂
Stem Injection	107 a	43 a	596 a	275 a
Foliar spray	70 b	6 a	514 a	1,104 a
Control	–	–	191 b	139 a

GA movement

We observed that $GA_{4/7}$ (50 mg/mL in absolute enthanol) forms a fine precipitate when it comes in contact with water, which, however, does not clog the xylem and obstruct the flow of water. Subsequently, a saturated aqueous solution of GA moves readily through the stem, but not as a discrete front. Although GA can be detected in the effluent minutes after its addition to the top of the stem, its presence is detectable for several hours. The concentration of GA was highest after the first hour, and declined steadily, reaching 0 about 6 h after the GA was added. Most of the GA was eluted from the stem in a total volume of 150 mL.

DISCUSSION

Both stem injections and foliar sprays effectively promoted female flowering in grafted ramets (2-3 m tall) of red, black and white spruce (tables 3-4). But a single injection of 5 to 50 mg/tree of $GA_{4/7}$ was as or more effective than about eight applications of foliar spray. The latter will result in a total application of about 160 mg/tree (assuming a total application volume of 400 mL of solution at 400 mg/L of GA). Halving the number of foliar sprays in an attempt to save time and GA decreased the response in both white and black spruce (Greenwood et al. 1990). At J.D. Irving operational trials of both methods have shown that the GA used for eight foliar sprays cost about $2.70 per tree, while that used for a single stem injection costs only $0.40 per tree. This savings is clearly a function of reduced labor costs as well as use of less GA. Furthermore, GA applied via stem injection has no effect on the number of seeds per cone, nor on the number of cones per bushel, but more than doubled the number of cones produced in a black spruce seedling seed orchard (results not shown).

The observation that stem injections of 5 mg/tree were more effective than 50 mg/tree in red spruce is surprising, although neither Philipson (1985) nor Ross and Hollefreund (1991) noted a proportional increase in flowering when the amount of GA injected into the tree was doubled. Stem injections applied to larger, field-grown trees were also very effective, and doubling the dose applied to jack pine on 7/12/89 resulted in only a slight but insignificant increase in flowering (see table 1). More trials to determine the optimum dose for stem-injected GA as a function of species and tree size would be advisable.

Time of application of stem injection was confined to the later stages of shoot elongation, and in all the experiments reported here injections were applied probably well after reproductive primordia had initiated, but well before they began to differentiate into reproductive buds. According to Harrison and Owens (1983), differentiation of reproductive buds in spruce has begun when bud scale formation is complete and the apex has begun to enlarge, with bract buttress or sporophyll primordia just visible at its flanks. Sporophyll primordium initiation continues for several more weeks before the buds begin to enlarge. Both Ho (1991) and Marquard and Hanover (1985) have shown that foliar sprays are ineffective if begun after shoot elongation has ceased in black and white spruce, respectively. At this time differentiation is probably just starting, but detectable only by microscopic examination. Harrison and Owens (1992) carefully examined the response of male and female flowering to the timing of two GA foliar sprays, while at the same time following reproductive bud developmental stages by microscopic examination in western hemlock. They clearly showed that the two sprays were most effective when applied just before the process of reproductive bud differentiation became visibly detectable. They also noted that a single stem injection of GA at this time also effectively promoted both female and male flowering. Although we have not performed anatomical examinations of developing reproductive buds on our study trees, our results for the spruces also appear to show that stem injection or foliar spray just prior to the beginning sexual differentiation is optimally effective.

The timing of sexual bud differentiation in pines is later than the spruces. In jack pine, initiation of floral primordia begins in late June or early July, while differentiation probably occurs at least a month later (Curtis and Popham 1972). This would explain why a single stem injection applied in mid-July appeared to be the most effective treatment to promote female flowering (see table 1). In mid-August, differentiation of some female buds has already begun, so some of the buds have probably been treated too late. On the other hand, the split application in June and mid-July was probably in part too early. Furthermore, the early application of GA caused significant kill of the top two whorls of the tree in this experiment, which was also observed in a similar experiment performed the year before (results not shown).

The kill of the tops of the jack pine clearly shows that the GA moves to the top of the tree, and appears to be phytotoxic to expanding foliage in this species. Fully mature foliage does not appear to be affected, nor is damage observed in the spruces. Although the control trees were not injected with alcohol only, the phyto-toxicity observed is probably due to the GA itself rather than the alcohol carrier, because in other conifers where alcohol controls were used damage was only observed on GA-treated trees (Joe Weber, personal communication, B.C. Ministry of Forests, Victoria, B.C., Canada V8Z 5J3). Presumably, the GA moves to the top of the tree in the transpiration stream, and appears to concentrate in the upper part of the crown, where most female cones form naturally. We have also observed that although $GA_{4/7}$ dissolved in ethanol forms a fine precipitate when it comes in contact with water, this suspension moves readily through the xylem, becoming solubilized as it becomes diluted by diffusion. The reason that a single stem injection is as effective as a number of foliar sprays is probably because a much smaller proportion of foliarly applied GA is taken up by the target buds. In addition, when an excess of plant hormones such as auxin or gibberellins is applied to plant tissue, some of the hormone is conjugated intact with either sugars (to form glucoside esters) or with amino acids (to form amides) which no longer have hormone activity (e.g. Zhu et al. 1991 and Moritz et al. 1989 and 1990). These conjugates can be hydrolysed later to release the hormone in its active form. Thus the excess GA applied via stem injection could be stored as conjugates after reaching the buds and surrounding tissue, and hydrolysed slowly thereafter.

Tree improvement programs for a number of northern conifers are advancing into the second generation. Genetic transformation is becoming available to provide the knowledge required to apply more sophisticated seed production and stock deployment techniques than traditional seed orchard establishment and seed harvesting. The application of flower induction techniques has had a large impact on tree improvement programs. Until now, flower induction using $GA_{4/7}$ has been used mainly to expedite tree breeding programs because of the cost of the chemical and labor involved in repeated foliar sprays. Carson et al. (1992) state that although conventional seed orchards have been successful in producing large quantities of improved seed, potential genetic gains have not been realized. One of the reasons for this is non-panmictic mating to which differences in reproductive capacity among clones is a contributing factor. The use of $GA_{4/7}$ could certainly reduce this problem and also improve the genetic diversity of orchard seed.

In our opinion the considerable savings in both time and money associated with stem injection makes GA application to increase seed production more attractive than ever. This treatment can result in consistently large (over 100 fold for some clones) increases in female flowering, and is effective on poor flowering clones in poor flowering years (Ron Smith, personal communication, 1993). Furthermore, in combination with more rapid growth of grafted scions due to growth acceleration procedures described by Greenwood et al. (1991), and intense culture of grafted selections which is possible with indoor breeding halls or outdoor meadow orchards, genetic gain can be effectively increased over a relatively short period of time. The application of either controlled or supplemental mass pollination to GA-induced female strobili on closely spaced trees in a meadow orchard can result in the early production of specific crosses (e.g. Sweet et al. 1992). Vegetative propagation via rooted cuttings of controlled cross seed after early selection of genetically superior families (e.g. Sulzer et al. 1992) can result in operationally plantable numbers of a specific cross within less than 7 years after the parents have been selected.

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IS GENETIC ENGINEERING A VIABLE OPTION FOR TREE IMPROVEMENT?

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ABSTRACT

Genetic engineering can be considered an extension of tree breeding. In its wider meaning biotechnology includes both disciplines. Tremendous progress has been achieved in animal and crop plant molecular biology during the last 10 years and the first transgenic crop will be commercialized this year. Similar work is now being done with trees. The first report of transgenic trees (poplar) was published in 1987 and the first transgenic conifer report (white spruce) in 1992. Very little is known of tree gene structure and function and most of the work has been on photosynthesis and lignin biosynthesis-related genes. However, information gathered with crop plants can be used for genetic engineering of tree species. Numerous traits can be modified through genetic engineering that would have commercial benefits but some obstacles have to be overcome. At PNFI we are concentrating our efforts on the study and use of conifer genes involved in tree development and differentiation that could have potential in engineering flower sterility and help induce somatic embryogenesis from mature conifer tissues. Genetic engineering is a viable option for tree improvement but the knowledge base, appropriate tools, and mechanism to integrate this technology in the tree breeding cycle have to be developed.

INTRODUCTION

The human race has a long history of manipulating genes in plants mainly for agricultural use. Recently, a new biotechnology called genetic engineering has permitted modification of single genes and the crossing of species-specific barriers. Genetic engineering can be seen as a refinement and extension of breeding and conventional tree improvement can be considered a biotechnology because it consists of manipulating genes through the natural sexual crossing of selected individuals.

Biotechnologies for tree breeding can be separated into two categories; analytical and manipulative (Adapted from Hughes and Batty 1992, Fig. 1). Analytical biotechnologies include molecular tools for characterizing tree genomes based on the study of DNA sequence variations within a group of trees of the same species (family, population, etc.) or between different species. These tools include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), and quantitative trait loci (QTL). Their use will allow the association of qualitative or quantitative traits with a specific marker that can be followed through the breeding cycle. Manipulative biotechnologies are directed toward the modification and introduction of genes to affect the genotype and phenotype of a tree. Again, tree breeding can be considered a manipulative biotechnology. Genetic engineering is also manipulative, but a process that possesses several advantages over tree breeding. It allows the manipulation of single trait genes and the crossing of species- and kingdom-specific barriers. For instance, genes isolated from bacteria have been transferred to crop plants and genes from one species have often been transferred to another species (Table 1). Genetic engineering used in this way is a tool to increase the genetic diversity of a species by introducing foreign genetic material. Additionally, genetic engineering could be used to rescue genes from extinct or endangered species. Molecular biology methods using the polymerase chain reaction (PCR) have allowed the rescue of DNA sequences from mummified plant tissue (Pyle and Adams 1989, Rollo et al. 1991, Paabo et al. 1989). The PCR method permits amplification of minute amounts of DNA in case where only a few bases are known. Once these genes from extinct species are isolated, they can be reintroduced into related species for observation of phenotypic effects.

One key to successful engineering of tree species is the availability of genes coding for important traits. Such genes have been characterized in crop plants and in tree species (see next section) via molecular biology. In animal molecular biology, the characterization of mutant organisms has permitted rapid progress by allowing the identification of genes responsible for abnormal phenotypes by comparing mutant and normal individuals . In crop plants, chemical mutagenesis of a weed called *Arabidopsis thaliana,* which has a very small genome (8×10^7 base pairs/ haploid genome), has produced similar breakthroughs in isolating and characterizing plant genes. With tree species, this avenue has not yet been exploited. There are a number of natural mutant trees that have been identified and are stored in plantations across the world. Such a collection exists at the Petawawa National Forestry Institute and could serve as a basis in pursuing the isolation of the genes responsible for a mutant phenotype. Mutants for tree form, disease resistance, and chlorophyll content have been characterized (Figure 2). The use of these mutants could lead to the manipulation of the genes involved in tree improvement.

Table 1. Examples of genes transferred from one species to another, unrelated, species.

Engineered trait	Species of origin	Target species
Herbicide resistance (chlorsulfuron)	*Arabidopsis thaliana* (weed)	tobacco, tomato, flax, rapeseed
Herbicide resistance (glyphosate, 2,4-D)	*Salmonella typhimurium*, *Alcaligenes eutrophus* (bacteria)	tobacco, tomato, petunia, rapeseed, poplar
Insect resistance (B.t. toxin gene)	*Bacillus thuringiensis* (bacteria), Synthetic gene	cotton, soybean, tobacco, tomato, poplar, white spruce
Heavy metal resistance	chinese hamster	tobacco
Bacterial disease resistance	*Pseudomonas syringae* (bacteria)	tobacco
Growth regulator ratios	*Agrobacterium tumefaciens* or *A. rhizogenes* (bacteria)	tobacco, rapeseed, poplar
Flower colour	corn	petunia
Antibodies	mouse hybridoma	tobacco
Biodegradable plastic	*Alcaligenes eutrophus* (bacteria)	*Arabidopsis thaliana*
Enkephalins	synthetic gene	rapeseed

It is important to keep in perspective the fact that plant genetic engineering is relatively recent. The first account of genetic engineering was published in 1983 for transgenic tobacco (Fraley et al. 1983, Herrera-Estrella et al. 1983). Since then, rapid progress has been made but, even now, there are no cultivars of transgenic crops commercially available. The first one will be the FLAVSVR tomato (engineered for long shelf life) of Calgene (California, USA) which is to be commercialized this year (Leemans 1993). Given this fact, we must keep our expectations of genetic engineering of tree species at a realistic level. The first report of tree genetic engineering of trees was published in 1987 and dealt with hybrid poplar (Fillatti et al. 1987); the first transgenic conifer (white spruce) was produced in 1992 (Ellis et al. 1993). Our laboratory at PNFI succeeded in producing transgenic black spruce in 1992 (Charest et al. 1993). It is certain that this area of research will have an effect on tree breeding but specific applications of the technology are dependent on the knowledge available on tree gene structure and function. Very few genes have been characterized in deciduous or coniferous trees (Table 2) and most of these are involved in photosynthesis or lignin biosynthesis. This constitutes a very limited knowledge of the large range of physiological and developmental processes in trees. However, this does not mean that nothing can be done with tree species and we should take advantage of the information that 10 years of research have generated with crop plants.

Table 2. Tree genes characterized to date.

Type of trees	Deciduous	Conifers
# of genes characterized	15	23
main genera studied	*Alnus, Betula, Hevea, Malus, Populus*	*Larix, Picea, Pinus, Pseudotsuga, Thuja*
examples of genes	-small subunit of ribulose bisphosphate carboxylase -bark storage protein -calmodulin -O-methyltransferase -phenylalanine ammonia lyase	-small subunit of ribulose bisphosphate carboxylase -seed storage protein -CuZn superoxide dismutase -phenylalanine ammonia lyase chalcone synthase -dihydropinosylvin synthase -large subunit of ribulose bisphosphate carboxylase -chlorophyll a/b binding protein -photochlorophyllide reductase
main phenomena under investigation	-photosynthesis -lignin biosynthesis	-photosynthesis -lignin biosythesis

Adapted from Charest et al. 1993

POTENTIAL TRAITS FOR GENETIC ENGINEERING OF TREES

Before discussing opportunities, we need to review the state-of-the-art of tree genetic engineering. Presently, 13 species of angiosperm trees and three species of conifers have been genetically engineered (Table 3). In most cases, only marker genes, which permit detection if successfully transferred into the host genome, have been introduced into trees, such as the b-glucuronidase gene and antibiotic resistance genes. In poplar, genes for herbicide resistance, insect resistance, and phenolic detoxification have been inserted. Other traits that have been genetically engineered into crop plants could be used with tree species to impact productivity, tree breeding, and the economic use of the trees. Table 4 summarizes the traits that can be modified and their potential impact.

METHODS OF GENETIC ENGINEERING

Numerous methods of gene transfer have been developed for plants (Charest and Michel 1991). The method that has been used most extensively with crop plants is *Agrobacterium*-mediated transformation. This method takes advantage of the natural ability of the bacterium *Agrobacterium* to transfer some of its DNA to the plant, which is then integrated into the host genome. The process includes engineering of the bacterium first and its use to transfer the engineered DNA into plants. This method works well with some species and hybrids of poplars and with certain species of fruit trees but it has proven unsuccessful with conifers. For such species, microprojectile DNA delivery has yielded transgenic white and black spruce (Ellis et al. 1993, Charest et al. 1993).

Microprojectile DNA delivery consists of coating microprojectiles (1-4 micrometers in diameter) with DNA containing the genes of interest and bombarding the target tissues with microprojectiles accelerated by gas flow, electrical discharge, or gun powder. Other methods are available but they are rarely used to regenerate transgenic plants.

To successfully regenerate transgenic trees, the following components have to be available:

1. a tissue culture method to regenerate trees
2. a gene delivery method effective for the species to be engineered, and
3. a gene that will function in the host genome

These three conditions are very hard to fulfill. For instance, some species such as pine and oak are not easily propagated through somatic embryogenesis. The two gene delivery methods mentioned above are far from routine in several species and only a few transgenic trees can be produced per experiment. The genes isolated to date are mostly from crop plants and there is always the possibility that they will not function properly in trees. The methodologies are available but there is still quite a bit of work to make them routine techniques.

Table 3. Tree species genetically engineered.

Species	Traits	Reference
Allocasuarina verticillata	hairy roots	Phelep et al. 1991
Citrus aurantifolia	antibiotic resistance β-glucuronidase	Moore et al. 1992
Citrus jambhiri (rough lemon)	antibiotic resistance	Vardi et al. 1990
Juglans regia (walnut)	antibiotic resistance	McGrananhan et al. 1988
Liriodendron tulipifera (yellow poplar)	antibiotic resistance β-glucuronidase	Wilde et al. 1992
Malus pumila (apple)	nopaline synthase antibiotic resistance	James et al. 1989
Populus alba x *P. grandidenta*	antibiotic resistance insect resistance wound inducible antibiotic resistance	Fillatti et al. 1987 McCown et al. 1991 Klopfenstein et al. 1991
Populus alba x *P. tremula*	herbicide resistance antibiotic resistance	De Block 1990
Populus trichocarpa x *P. deltoides*	herbicide resistance antibiotic resistance	De Block 1990
Populus tremula x P. alba (aspen)	herbicide resistance antibiotic resistance	Miranda Brasileiro et al. 1992
Prunus domestica (plum)	antibiotic resistance β-glucuronidase	Mante et al. 1991
Vaccinium macrocarpon (cranberry)	antibiotic resistance insect resistance	Serres et al. 1992
Vitis rupestris (grapevine)	antibiotic resistance β-glucuronidase	Mullins et al. 1990
Picea glauca (white spruce)	insect resistance antibiotic resistance β-glucuronidase	Ellis et al. 1993
Picea mariana (black spruce)	antibiotic resistance β-glucuronidase	Charest et al. 1993
Larix decidua (European larch)	hairy roots	Huang et al. 1991

Note: antibiotic resistance is used to select for genetically transformed cells by selecting for resistance encoded by the introduced gene. β-glucuronidase is a marker gene that allows detection through colourimetry, fluorometry, or histology. Hairy root is the phenotype caused by introducing hormone genes.

Table 4. Traits for tree improvement through genetic engineering.

Trait	Mechanism	Impacts
Flower sterility	killer gene in specific tissue	-male or/and female sterility for field release or tree breeding -increased yield
Pathogen resistance	resistance genes, plantibodies, change in cell structure	-increased yield
Photosynthesis efficiency	more efficient photosynthetic apparatus	-shade tolerance -increased yield
Lignin biosynthesis	hormone genes, biosynthetic pathway	-designer trees with specific types of fibers
Frost resistance	vegetative storage protein, antifreeze protein	-colder environment -increased yield
Drought resistance	increase in sugar synthesis	-dry environment -increased yield
Tree form	homeotic genes, hormone genes	-ornamental -different environments
Pollution resistance	heavy metal binding genes, superoxide dismutase gene	-polluted environment and urban areas -increased yield
Nitrogen fixation	nitrogen fixation in chloroplasts, nitroplasts	-increased yield

OBSTACLES TO GENETIC ENGINEERING IN TREE SPECIES

What are the main obstacles to genetic engineering of tree species? Some obstacles have been already highlighted: the lack of knowledge of tree genes, the difficulties in genetically manipulating these and the absence of good tissue culture methods for some species. Other factors are also important for the field application of this technology, such as a proper mechanism to integrate biotechnologies into the tree breeding cycle (Fig. 3). For instance, somatic embryogenesis is an ideal tool to integrate genetic engineering into conifer tree improvement but it is still not ready for large scale operation. Also, tree breeding itself is a limitation because of the length of time required to produce improved material. Additionally, there are some environmental issues currently being addressed (Table 5) that need appropriate mitigation. Last, there is a limited number of research groups directing their efforts towards tree biotechnology.

Table 5. Environmental issues related to the introdution of transgenic trees in the environment.

Issues related to the engineered tree species	**Issues related to the environment in which the engineered tree is located**
-Genetic pollution of wild individuals -Emergence of new pest to the species -Reduction in genetic biodiversity	-Impact on ecosystem components -Potential for weediness

THE PNFI APPROACH

At the Petawawa National Forestry Institute, our core work on tree biotechnology is dealing with the development of basic knowledge of tree gene structure and function related to development and differentiation that could have potential field application (Figure 4). Two main thrusts are being explored in conifers; somatic embryogenesis and flower formation. The genes related to the phenomena studied are oncogenes and homeotic genes, first identified in mammals as important for animal development. Later, they were isolated in plants such as corn and *Arabidopsis thaliana*. These genes were associated with flower development and organ differentiation. Our group was able to isolate similar genes in black spruce which had the potential to manipulate tree form, to increase growth rate by preventing flowering, and to induce somatic embryogenesis in mature conifer tissues. Furthermore, these genes could serve as molecular markers (RFLPs=Restriction length polymorphisms) for tree improvement. Figure 5 illustrates the PNFI approach, which emphasizes that differentiation of various organs is linked to the expression of genes in the meristems of origin.

CONCLUSION

So, is genetic engineering a viable option for tree improvement? Yes. There are numerous examples of scientific successes with crop plants during the 10 years of active genetic engineering, even though the first commercial product will be out this year. With tree species, we should expect the same lag phase in the application of genetic engineering. After all, we have to build up a knowledge base, develop appropriate tools and work out mechanism to integrate this technology into the breeding cycle. However, the potential of genetic engineering is greater with tree species because this biotechnology could accelerate the tree improvement process by allowing rapid transfer of genes coding for useful characteristics such as disease resistance and increased yield. Additionally, genetic engineering could provide tools such as selectable marker genes for producing artificial hybrid species through the somatic hybridization. This technique has been successful with crop plants and has permitted the fusion of otherwise sexually incompatible species. Genetic engineering will certainly complement other biotechnologies such as tissue culture and molecular marker-aided breeding.

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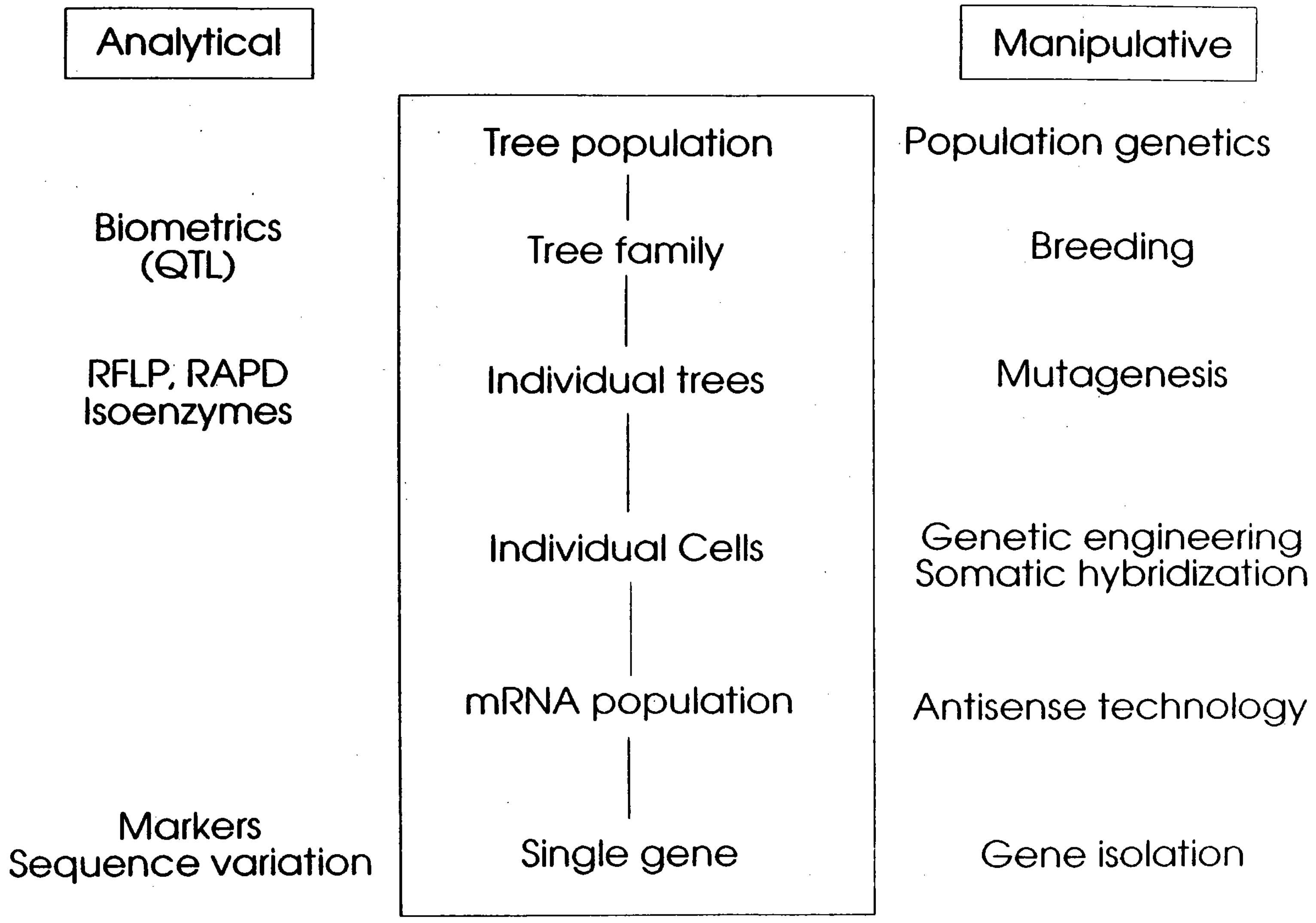


Figure 1. Analytical and manipulative Biotechnologies in tree improvement

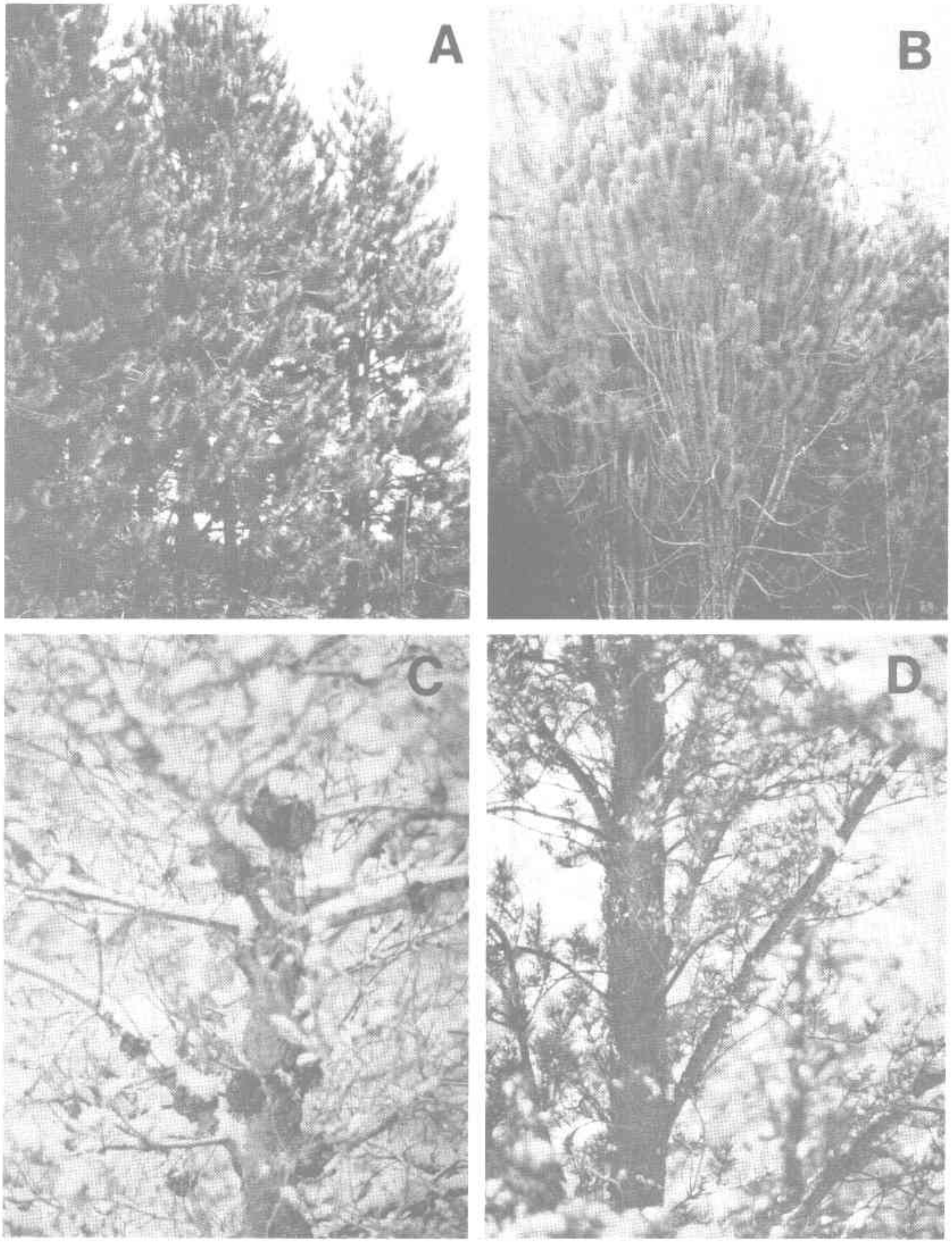


Figure 2. Examples of tree mutants in red pine and Scotts pine. A) normal red pine, B) branch angle mutant red pine, C) normal Scotts pine infected by Eastern gall rust and D) mutant Scotts pine resistant to Eastern gall rust. All trees are located at PNFI in the Pine Graft Arboretum.

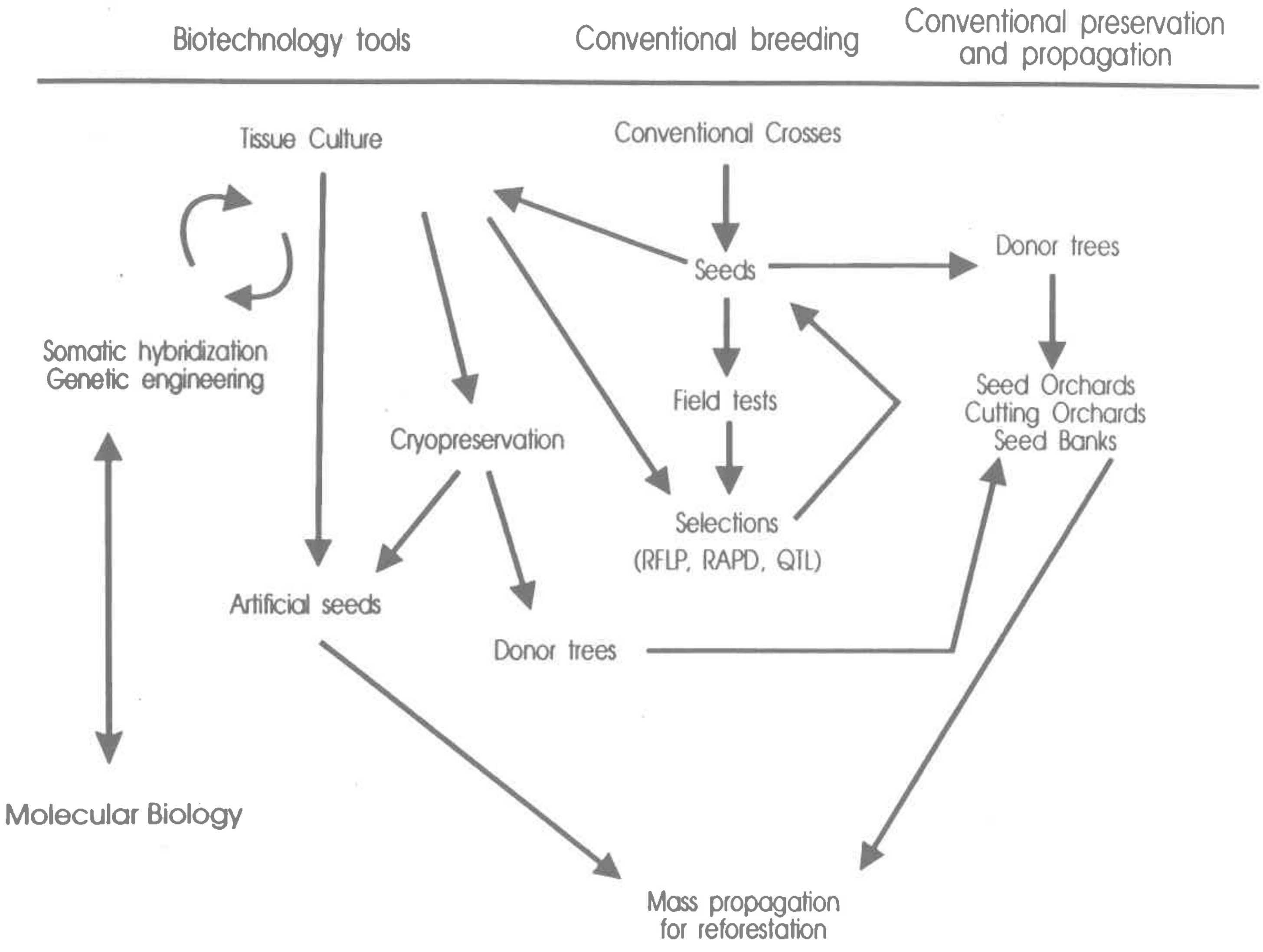
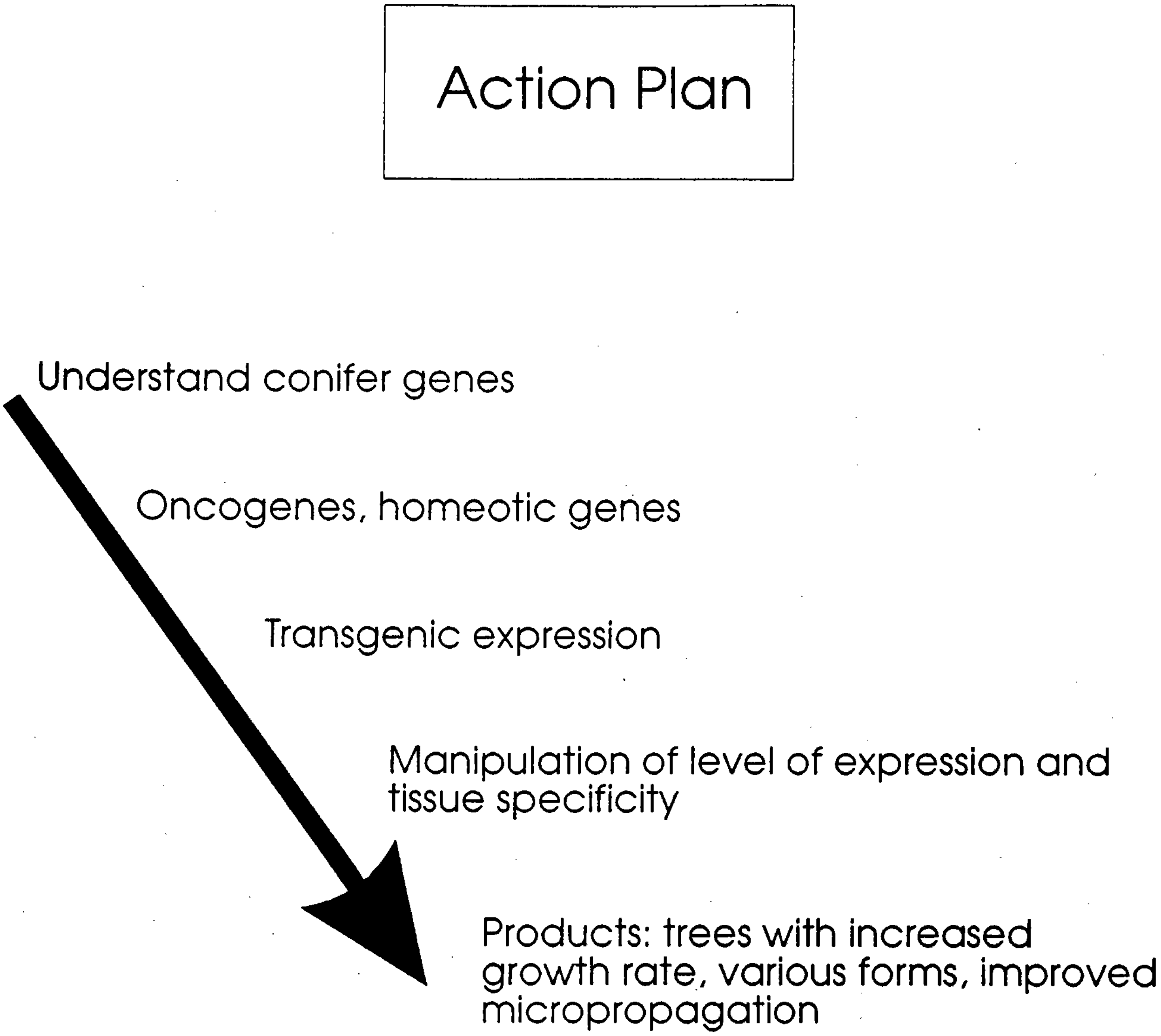


Figure 3. Integration of analytical and manipulative biotechnologies into the tree breeding cycle



Action Plan

Understand conifer genes

Oncogenes, homeotic genes

Transgenic expression

Manipulation of level of expression and tissue specificity

Products: trees with increased growth rate, various forms, improved micropropagation

Figure 4. Approach taken at the Petawawa National Forestry Institute in regard to genetic engineering and molecular biology

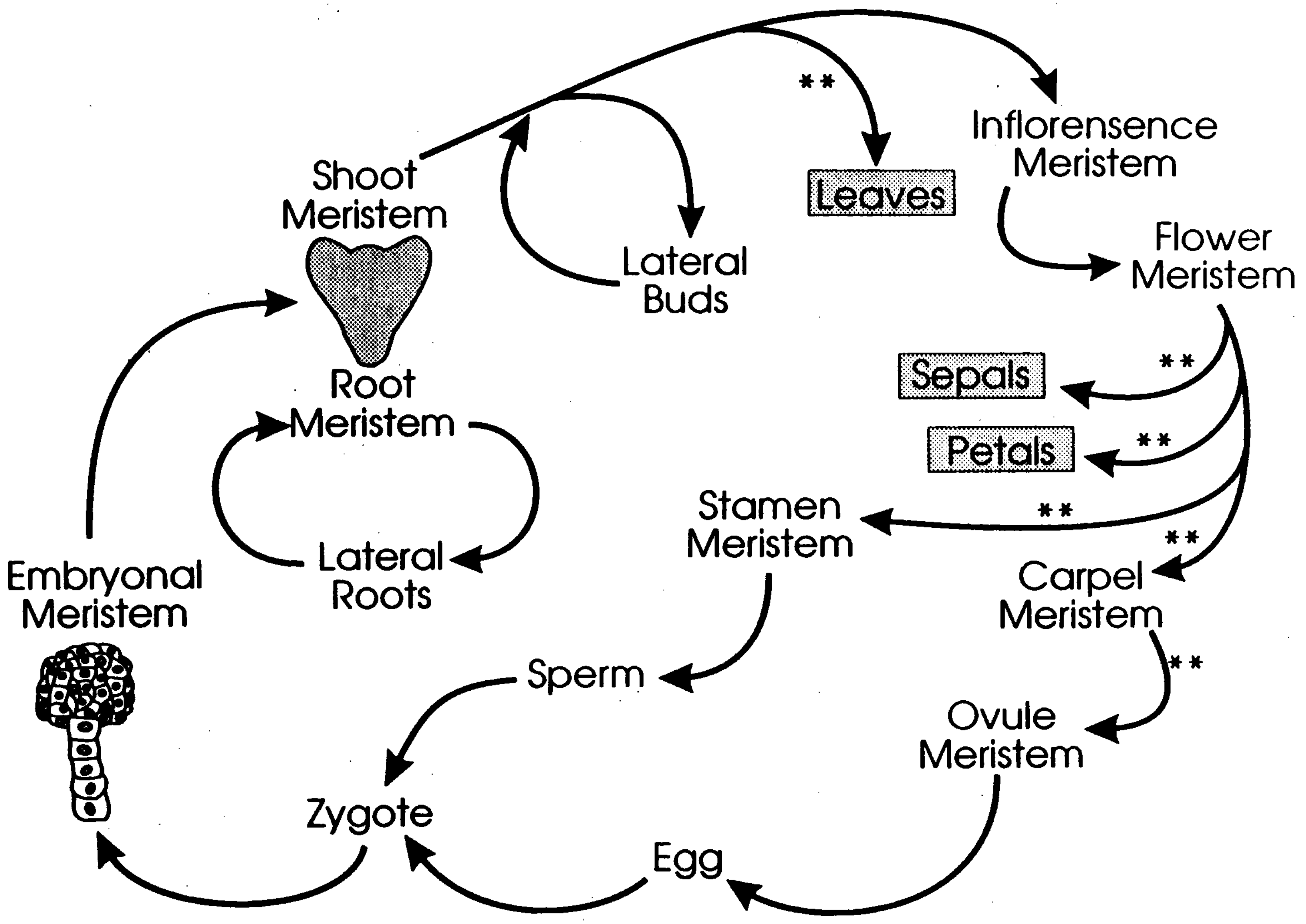


Figure 5. Schematic of develomental pathways in plants.
** indicates phenomenom where homeotic genes have been identified.

THIRD-CYCLE BREEDING STRATEGY: A DESCRIPTION AND ECONOMIC APPRAISAL FOR THE NORTH CAROLINA STATE UNIVERSITY - INDUSTRY COOPERATIVE TREE IMPROVEMENT PROGRAM

Robert J. Weir and David Todd [1] /*

ABSTRACT

A third-cycle breeding strategy for loblolly pine (*Pinus taeda* L.) has been developed to provide maximum and accelerated genetic gain in the short-term and to maintain genetic diversity for long-term improvement. A hierarchy of three populations will be managed with differing levels of intensity. The **mainline population** will consist of 160 selections that are available to each member operating a tree improvement center in a given geographic region. These populations will be managed as subdivided breeding populations (40 sublines of 4 trees each) primarily to provide for long-term genetic gain. The most intensively selected and managed hierarchy will be **elite populations**. An intensively selected group of approximately 40 trees will be managed to provide maximum and accelerated short-term genetic gain for each member's program. A third hierarchy will be the **genetic diversity archives** managed to preserve a broad range of genotypes, including those with extreme breeding values for individual traits. These diversity populations represent insurance for environmental or selection criteria changes in an uncertain future.

This new breeding strategy has improved efficiency and lower costs resulting from reduced population sizes. The increased selection intensity used to reduce the population sizes and the increased rate of breeding made possible with fewer trees will substantially increase genetic gains and value returns in the next cycles of improvement. An economic appraisal of elite populations contrasted with mainline populations demonstrates that there is a large economic advantage and little economic risk encountered with a very aggressive elite population management strategy. At the same time, the long-term well-being of the genetic resource will be maintained by judicious management of all three hierarchies.

BREEDING STRATEGY BACKGROUND

The breeding, testing, and selection component of any tree improvement program is a costly and critical part and the genetic gain achieved is a direct result of the strategy employed. The North Carolina State University-Industry Cooperative Tree Improvement Program plans for third cycle breeding (the foundation for a fourth cycle of improvement) were developed to provide an efficient, cost effective breeding strategy to ensure both short- and long-

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* Breeding Strategy Architects were: Dr. Steve McKeand, Geneticist and Assoc.Professor, Cooperative Tree Improvement Program, (same address as Weir), and Dr. Floyd Bridgwater, Research Geneticist and Professor, USDAForest Service, (same address as Weir). They were assisted by a Task Force drawn from Cooperative Program member organizations.

term gains for participating Cooperative members. A primary criterion for the breeding strategy was that it be flexible for current and future breeders. Decisions made in the third-cycle breeding program will influence all future loblolly pine breeding within the Cooperative's working area, and options available to future breeders must not be seriously constrained by methods chosen now. The strategy must also be flexible in accommodating the diverse objectives of the membership. While the members share in the support of the mission and objectives of the Cooperative, there is diversity in product goals, and aggressiveness of program investment levels. Additionally, as new information is derived from research, the plan should be flexible enough to incorporate new opportunities for genetic improvement.

Third-cycle breeding will begin around 1997 when 30-40% of the third-cycle selections are available. A brief summary of the breeding strategy adopted by the Cooperative is given in this paper. More details are available in the paper by McKeand and Bridgwater, 1992, and in a report prepared for Cooperative members (Anonymous 1992).

MAINLINE POPULATIONS

The size of the mainline breeding population is a critical factor in the design of the overall breeding strategy. The appropriate breeding population size depends upon the trade-offs of short-term versus long-term gain. Short-term gain is maximized by intensive selection and, hence, small effective population size. Long-term gains are maximized by milder selection and larger population sizes. Population size affects the risk of losing favorable alleles through genetic drift, and this is a more serious problem for those alleles that are of low frequency. Lost favorable alleles constrain the improvement that can be made from breeding programs in the long-term. The problem of choosing population size "reduces to one of deciding on the minimum effective population size that will avoid the major effects of drift, that is, that will retain most of the genetic potential in the population" (Rawlings 1970).

Many simulation studies as well as long-term selection experiments indicate that effective population sizes ($N_e \geq 50$) are sufficient for maintaining low frequency favorable alleles in a breeding population. Long-term gain should be possible for many generations provided that $N_e \geq 50$ is maintained (Namkoong et al. 1980). Namkoong et al. (1988) also emphasized the benefits of maintaining a relatively large population size in the first generations of breeding. Low frequency alleles will be selected and will move into the intermediate frequencies where future selection will be more effective in smaller populations. Fortunately, the Cooperative managed very large populations in the first two cycles. Each cooperator had access to an average of 600+ selections for a given geographic area - over 4000 selections throughout the Cooperative.

Given the long-term nature of the Cooperative's breeding program and uncertainties about future selection pressures (Kang and Nienstaedt 1987), a third-cycle breeding population with an initial effective population size of Ne = 160 selections available to each cooperator was adopted. While this population size is much smaller than the 600+ selections currently available to each cooperator, the reduction to 160 selections is warranted. Our reasoning is that the mainline breeding populations will be composed of local selections only. The option of combining selections from adjacent or even distant geographic regions may be exercised in the future. The decision to combine trees from different regions will depend on the results of a large geographic variation study of select tree populations derived from unimproved plantations. These plantation selections that are being source tested make up a large proportion (85+%) of the foundation population for the third-cycle breeding. We also argue that all of the 600+ selections in the foundation population will have been tested and estimates of breeding values for each tree will be available. If our mainline population were comprised of untested selections having poorly estimated breeding values, a population size of 160 would be a much greater risk, and possibly unacceptable.

A sublining or subdivided population breeding and testing system will be used for all mainline populations. This system is effective for managing inbreeding, but also is very effective for managing a population of trees that spans a continuous environmental gradient. There are no discrete "breeding regions" in the N.C. State Cooperative, but a continuous series of smaller populations ordered along a cline, or a continuous environmental gradient. Since environmental factors which affect genotypic performance are continuous, different selection criteria can be utilized in a continuous fashion in managing sublines. For example, the importance of fusiform rust resistance gradually changes both north-south and east-west. The economic weight for rust resistance can be changed in a selection index for sublines occurring along rust gradients. Sublining also simplifies breeding logistics, since each cooperator will exercise complete management control over a given set of sublines. The practical problems of exchanging selections, pollen, seeds, and seedlings for breeding, testing, and selection will be minimized.

The number of sublines and the number of entries per subline are directly related to the size of the mainline population and number of unrelated selections needed to establish a seed orchard. The Cooperatives 160 tree mainline population will be subdivided into 40 sublines of size 4. There are several advantages for small sublines: 1) Inbreeding within a line will increase rapidly, thus increasing the total genetic variance from which to make gain (Falconer 1989); 2) The 40 sublines allow for exploitation of the increased genetic variance among lines, since selections from only the best lines would be used for orchard establishment each generation; 3) Favorable alleles can be fixed faster in small populations (Kang 1991); 4) Breeding with small discrete units (4 tree sublines) will progress rapidly; 5) If it is determined to be advantageous, in the future small sublines can be combined into fewer larger sublines. Restructuring in the reverse direction is difficult; 6) A minimum effective population size of 40 can be maintained after many generations of breeding; and 7) If selection criteria change over time, selection in a small subline system can provide a more rapid response (Namkoong et al. 1988).

Disadvantages of a small subline system may include: 1) Inbreeding builds up so rapidly that alleles (both favorable and unfavorable) may be fixed before selection can be effective; 2) Inbreeding may adversely impact seed production for breeding and operational needs; 3) recombination is restricted to within lines in the breeding population; and 4) Sublines will be eliminated due to selection and/or drift.

The mating design for the sublining system will be a complementary design that combines pollen-mix mating to estimate general combining ability (GCA) and control-pollinated matings for within family selection as discussed by van Buijtenen and Burdon (1990) and Burdon and van Buijtenen (1990). The GCA pollen-mix tests will be planted in a randomized complete block design. Seedlings derived from control-pollinations will be planted in nonreplicated full-sib family blocks that will only be used for within-family selection. We will select trees from the full-sib family blocks having the highest mid-parent values as determined from the GCA tests.

ELITE POPULATIONS

Our breeding strategy includes an elite population hierarchy. The concept, as outlined by Cotterill et al. 1988, advocates investing most heavily in the trees with the highest breeding value. Our elite population will be comprised of the best 40 tree subset of any given 160 tree mainline population. The trees will be very intensively selected and breeding will be aggressive. Genetic gain benefits are derived from a one time selection intensity boost and over time from more rapid turnover of the small aggressively managed population (Figure 1).

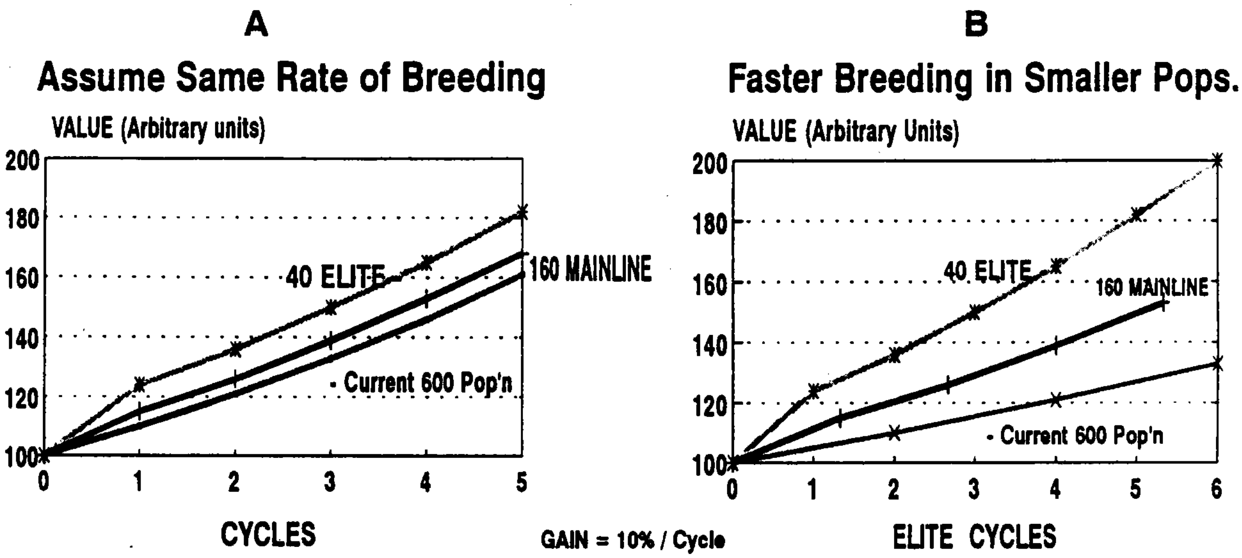


Figure 1. A comparison of genetic gains from elite, mainline, and foundation populations derived from increased selection intensity (A) and from increased selection intensity and more rapid breeding of smaller populations (B).

Rather than developing one elite population breeding strategy for all program members, elite populations will be customized for individual cooperators or small groups of cooperators working together. Populations can be structured using local or non-local selections to utilize only general combining ability effects or both general and specific combining ability effects. Since one of the most attractive benefits of small elite populations is rapid accumulation of genetic gain, any strategy adopted must have fast generation cycling as a feature.

One option we have under evaluation is the use of inbreeding to rapidly increase homozygosity, the frequency of favorable alleles, and to increase additive genetic variance. Another advantage of rapid inbreeding (selfing) is increased among family intensity of selection. With selfing, the best parent based on GCA performance is mated with itself and can be selected without reducing the breeding value by crossing it with another lower value parent. While selfing has potential to give the most rapid gain, there are concerns. For loblolly the pine we expect selfing to be feasible with only about 50% of the selections. Selfing may also increase homozygosity so rapidly that selection may not have a chance to operate. Slower inbreeding such as full-sib mating or half-sib mating could be more effective in the long run.

Elite populations need not be considered as closed populations. There will be overlap in breeding values between trees in the mainline and elite populations (i.e. heritabilities are relatively low and selection is far from perfect). Each cycle, the best genotypes from the mainline population in equivalent geographic areas can be infused into the elite population if the breeding values meet selection criteria. Infusion of new genotypes will also enhance the genetic variation in the elite populations. Flexibility for future options must be a characteristic of any elite population management scheme that is adopted.

GENETIC DIVERSITY ARCHIVES

We have made no attempt to elaborate on the economic, biological, or ethical needs for gene conservation, yet we recognize the need to manage the extremely diverse and rich gene pool of loblolly pine. For long-term preservation of genetic diversity, an additional management strategy (apart from the mainline breeding program) will be the judicious use of Genetic Diversity Archives to preserve all existing selections bred currently and in the past, as well as many future selections. Approximately 4000 trees have been selected from natural stands, unimproved plantations, and first generation progeny tests from 1957 through the late 1980's. The vast majority of these selections still exist in seed orchards, clone banks, and breeding orchards. A concerted effort will made to preserve these selections in genetic diversity archives.

In future breeding cycles, rather than lowering the selection intensity in the mainline population to ensure the maintenance of rare alleles or rare allelic combinations, up to 150 "unique" genotypes (in addition to the 160 in the mainline) will be selected from each recruitment population for inclusion in the archives. These trees will have unique and/or extreme values for different traits (e.g. very large volume but relatively poor straightness or very high wood specific gravity and poor rust resistance). If environments change or selection criteria are dramatically altered in the future, these archives will be a rich source variation to be used. If future breeders find that collection of open-pollinated seeds or additional crossing among selections in the archives is warranted, all trees will be available for this effort. We have elected to add the genetic diversity archives as a low cost insurance for diversity in an uncertain future.

FINANCIAL COMPARISON OF ELITE AND MAINLINE BREEDING STRATEGIES

Given the hierarchical breeding system adopted for our Cooperative, it was of interest to quantify the financial advantage the elite population strategy would provide. Clearly, a small population that is selected more intensively and bred more rapidly should provide greater economic return. Our focus in the following analyses was to determine the magnitude of this advantage and to assess the nature of any additional risk that might be encountered.

For the purposes of the economic analyses several financial and related assumptions were made. These assumptions are:

1) A typical southern mill uses 500,000 cunits of wood per year.

2) A cunit = 100 cubic feet (approximately 2.9 cu. meters) of solid wood.

3) The typical mill manages as fee land 200,000 acres (81,000 hectares) of land in pine plantations.

4) Approximately 1/3 of the annual mill furnish is harvested on fee land and 2/3 of the furnish is purchased from other land owners.

5) Annual planting with seedlings derived from seed orchards = 8000 acres.

6) Genetic gains produced from breeding strategies are fully realized.

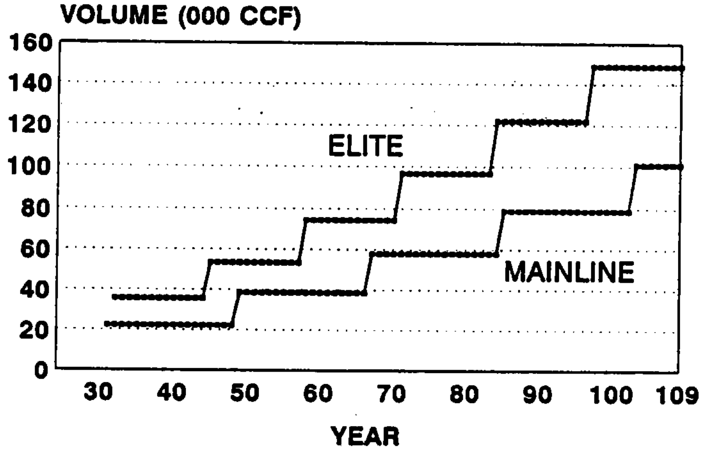
7) All cash flows are before taxes, since taxes vary so much by organization.

8) The analyses spans 6 elite cycles, and 4+ mainline cycles or 110 years.

9) Wood values are escalated at 1% per year which is a conservative estimate.

10) We assumed that annual breeding costs are equal for elite and mainline strategies, and that more progress can be made with the smaller elite populations. This is a simplifying and very conservative assumption since our experience suggests that for elite populations not only can more progress be made, but annual costs may be lower.

The gains depicted in Figure 1 for elite and mainline populations are translated into wood volume production over cycles and shown in Figure 2. Substantially more wood is projected to be produced from the elite population breeding strategy for the period of the analyses. The stair step appearance of the wood volume gains over the firm's land base reflects the life expectancy of each seed orchard developed from successive cycles of breeding, testing and selection. We have been conservative in these estimates since the increments of gain to be realized from seed orchard roguing have been ignored. In reality the profile of wood volume would be expected to have several smaller steps within each cycle that would be realized following each orchard roguing.



CCF = CUNIT =100 CU.FT. OF SOLID WOOD

Figure 2. Wood volume production for elite and mainline Breeding Strategies

A second step in estimating the benefits of elite and/or mainline breeding investments is to assign an economic value to the extra wood produced. To do this we introduce the concept of marginal or replacement wood value. Traditionally, forest economic analyses have used market stumpage prices as the measure of wood value or cost. However, the cost structure of wood delivered to a converting plant is a distribution of many levels of cost (Figure 3). The real value of improving forest productivity is the ability to replace the most expensive wood the mill must buy with generally cheaper wood produced on company owned and managed fee land. Conceptually, this is the real value to Southern forest products company which depends on outside purchases for a substantial portion the annual raw material supply. The value of increasing productivity through investment in tree breeding is not the difference between fee wood and average delivered wood cost, rather it is the difference between the cost of delivered fee wood and the delivered price of the most costly wood purchased in a given year. Delivered fee wood cost in our region has been estimated by Timber Mart South to be $52.00 a cunit. The average cost of all delivered wood is $83.00 a cunit. The average cost of the most expensive 55,000 cunits purchased annually is $98.00 per cunit. Thus, we estimate the marginal or replacement wood value to be $98 - $52 = $46. If alternatively, the average cost of all delivered wood is used as a base, then productivity enhancing silviculture (eg. tree improvement) will be valued less than its true value and may not be adequately funded.

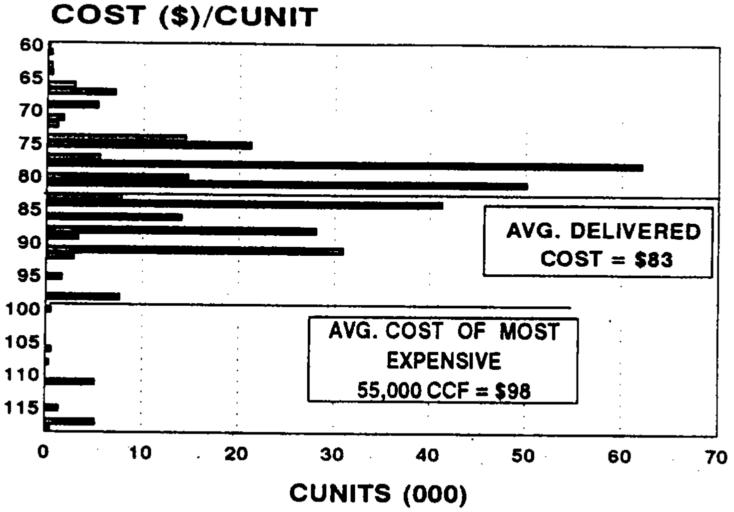


Figure 3. Cost distribution of purchased wood delivered to the mill.

Given the gains in wood volume and value, a second part of any useful economic appraisal includes estimates of the costs associated with achieving the projected improvement. Break-even wood values per cunit are depicted for elite and mainline breeding strategies in Figure 4. These break-even wood values are the values at which net present values approximate zero. Across several categories of site quality (site index at base age 25), the mainline breeding strategy has break-even wood values that are essentially double the values for elite breeding. Relative to the elite breeding, the mainline strategy requires nearly twice the wood value to be profitable. Since marginal wood value for both breeding strategies is $46.00 and the break-even (no profit net present value) are in both cases equal to or less than half of $46.00, it is clear that both the mainline and elite breeding strategies are worthwhile investments. The elite breeding strategy appears to be an exceptionally good investment.

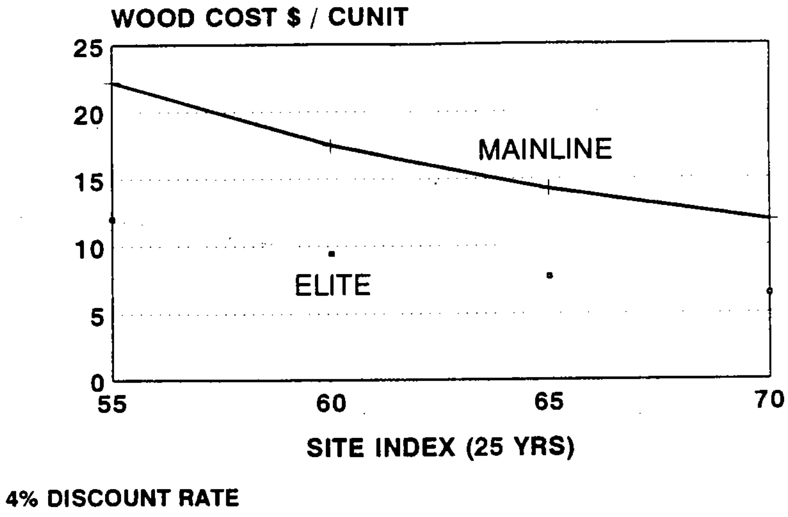


Figure 4. Break-even wood values for elite and mainline breeding at various site index levels (base age 25).

In the next analyses we compared the net present value (NPV) for elite and mainline breeding strategies over a range of site index values and discount rates. NPV seems to be an appropriate measure for this comparison since it incorporates investment costs and benefits measured as cashflows over time. The objective of a corporation is to increase net worth of shareholder or corporate owners. NPV is the only tool that integrates both timing and magnitude of cash flows. The best investment to increase owner's net worth are those investments that give the highest net present value at a given or acceptable level of risk. In Figure 5 we show the NPV estimates for elite breeding and mainline breeding over a range of site index values (base age 25) and for several discount rates. NPV estimates are very sensitive to both site index, inherent land productivity potential, and to discount rates. NPV estimates show a large drop as discount rates increase, they are more sensitive to variation in discount rates than to site index.

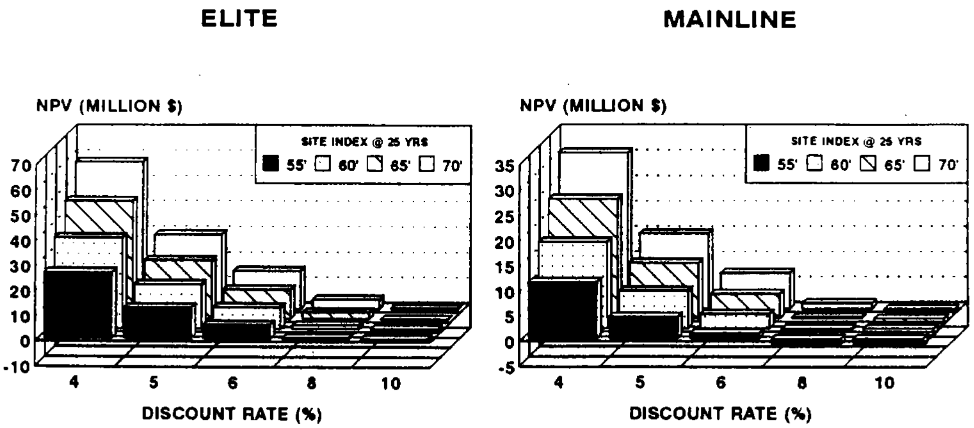


Figure 5. The effect of site index and discount rate on the net present value (NPV) for the elite and mainline breeding strategies.

Risk or uncertainty analyses can be complex and tedious. Risk implies that all possible outcomes are known with associated probabilities of occurrence. Uncertainty on the other hand implies that all outcomes are not known. With the elite breeding strategy we are most likely dealing with uncertainty. Will inbreeding become a serious problem with these extremely small populations? If inbreeding becomes a problem will infusion from the mainline population solve the problem and at what cost and over what time scale? We have used uncertainty and risk interchangeably.

For this paper we were not able to do a comprehensive risk-uncertainty analyses of the numerous possible outcomes. However, a crude estimate of the risk associated with elite breeding was made as follows. Normally, risky investments demand higher return on principal invested. Long-term U.S. Treasury Bill rates have historically been about 4%. Because silvicultural investments are inherently low risk, the discount rate used should be something close to the Treasury Bill rate. With uncertainty, as in the case of elite breeding, about the only way to quantify the effect is to change discount rates. More uncertain investments should be discounted at higher rates. The question still remains how much higher? If the discount rate of the more uncertain investment is changed until the NPV is equal to the lower risk investment (mainline), then the difference in the discount rate is the risk-premium rate of the risky investment. In Figure 6 we have extracted the site index 55 NPV's depicted previously along with others in Figure 5. Comparing the elite and mainline NPV's it is apparent that we would have to discount the elite population a full percentage point to make it an equivalent NPV to the mainline. The one percent (1%) risk rate translates into over 10 million dollars (NPV). However, this is much less than the difference between Treasury Bill rates (4%) and the Standard and Poors 500 average for the past 30 years (8%). Is it a good idea to pay 1% more in interest to obtain the additional wood value produced from the elite breeding strategy? — YES!

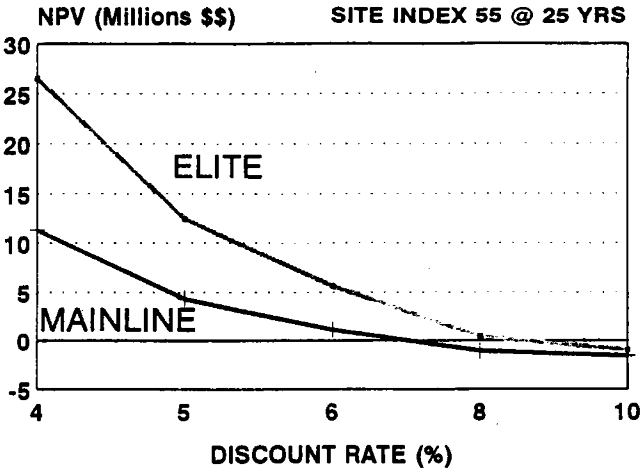


Figure 6. Net present Value (NPV) of elite and mainline breeding at various discount rates for site index 55 at base age 25.

The final chart developed from our analyses shows the cumulative cash flows for elite and mainline breeding over a 110 year period (Figure 7). This is the accumulated net effect of all costs and benefits over time. We see higher and diverging cash accumulation for the elite population compared to the mainline. The elite breeding system will make more money faster than the mainline alternative. It is a relatively low risk investment that has high value. The divergence of cash flows is due to gain from a one time selection intensity increase and more rapid turn-over of breeding, testing and selection cycles.

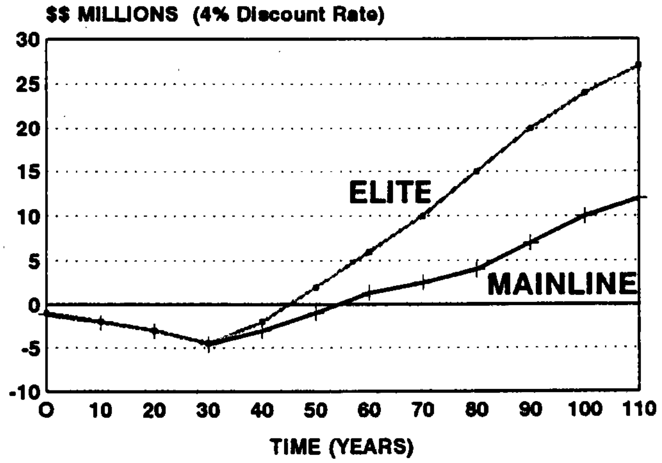


Figure 7. Cumulative discounted cashflows for both elite and mainline breeding strategies.

The N.C. State University - Industry Cooperative Tree Improvement Program is satisfied with the economic appraisal of our new breeding plan. We anticipate elite population strategies will be aggressively pursued by members of the program and that the results of more gain realized at a faster rate than systems used in the past will provide for continued support of tree improvement investments for several additional cycles of improvement of loblolly pine.

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DECISION PROCESSES FOR EFFECTIVE ALLOCATION AND MANAGEMENT OF GENETICALLY IMPROVED PLANTING STOCK: A CASE STUDY FROM AN INDUSTRIAL BREEDING PROGRAM IN DOUGLAS-FIR

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Keywords: *Pseudotsuga menziesii*, realized gain, allocation, regeneration, seed source movement, genetic diversity, inventory, family blocks.

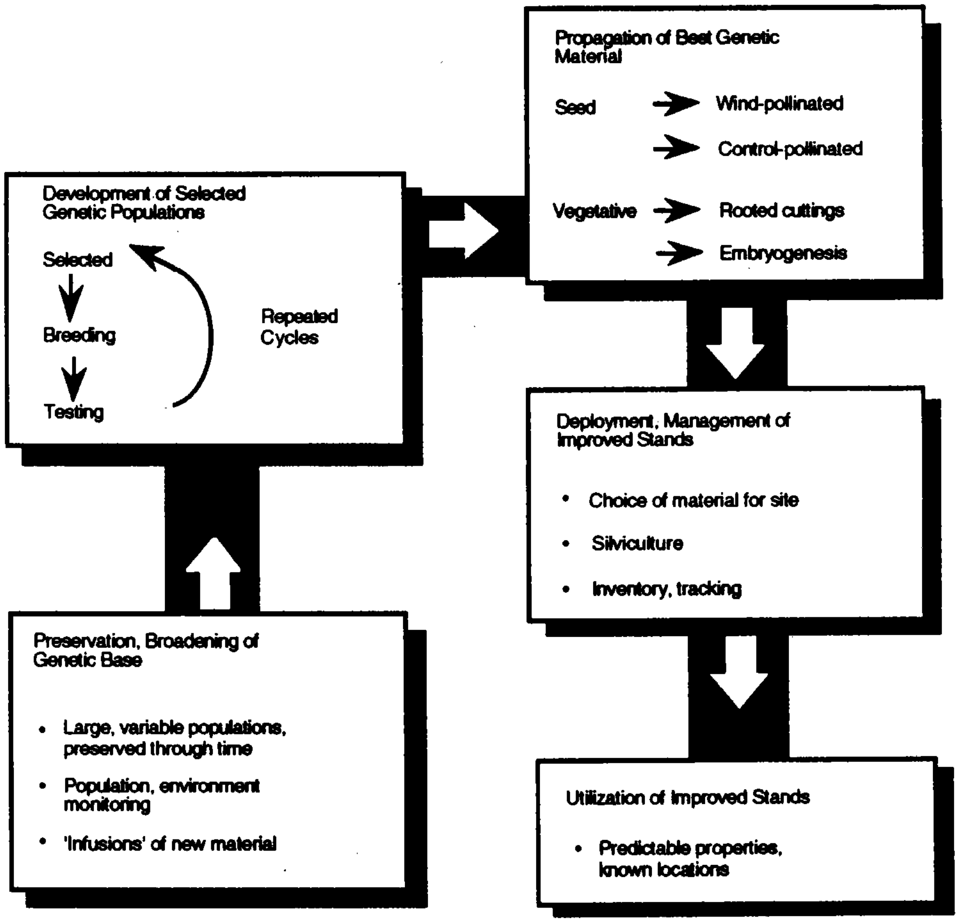
ABSTRACT

Tree Improvement programs worldwide have devoted significant time and resources, quite successfully, to the development of varieties of commercial trees with substantially improved value potential. However, successful realization of the value of tree improvement requires that the appropriate genetic materials is chosen for the site and management objectives, consistently; that silviculture and genetic material are aligned with one another to meet the management objectives; and that information flow and tracking systems are set up so that the values created through silviculture and genetics are realized in harvest, processing and marketing. These elements will not come together unless focused attention and resources are given to the so-called 'technology transfer' phase - in this case, the development of planning, decision making and information management processes for operational implementation of tree improvement. Weyerhaeuser Company plants over 15,000 ha. annually to genetically improved seedlings of coastal Douglas-fir on its western timberlands. Beginning in 1991, a comprehensive set of processes were developed and implemented for allocation and management decision making for genetically improved stock. From this experience, many insights have been gained in terms of the factors and activities most important to successful implementation of genetic improvement in field forestry. These insights will be discussed, and supported by specific examples.

INTRODUCTION

The term 'allocation' refers to the decision making processes involved in choosing which genetic material will be planted on what sites, in producing the planting stock, and in getting that stock successfully established in the field. The 'management' of stands of that genetic material then encompasses another set of decision processes including the choice of silviculture, the collection and application of stand data, and the planting of harvests and processing. These processes have the potential to add considerably to the value of any set of genetic material, and conversely, lack of attention to these steps can negate much or all of the value potential created through selection, breeding, testing and propagation. This will become even more true as more organizations begin to plant clonal varieties (Talbert et al., 1993). They must be viewed as an integral part of the overall tree improvement process (Figure 1). However, for a variety of reasons many tree improvement programs have been slow to take on the development and refinement of allocation and management processes. Product and market strategies are often weakly defined in the very long-term timeframe associated with tree improvement, so trait requirements are uncertain. Data relating genetics and silviculture to product yields, recovery and value are often either entirely lacking or strongly limited in scope. The skills mix and experience of the people involved in tree improvement often do not encompass even field forestry or growth and yield modeling, let alone management planning and economics, milling or product marketing. The structure of many organizations into separate 'functions' often limits the frequency and effectiveness of communication among the functions and the availability of resources for 'cross-disciplinary' research and process development.

Figure 1. A conceptual model of the tree improvement process.



However, some organizations have begun to tackle the task of coupling more traditional selection, breeding, testing and propagation programs with silviculture, manufacturing and product marketing. Families or clones known to produce timber with particular characteristics for specific site types or markets are being identified and planted in proximity to mills designed to be highly efficient in processing that timber, and managed using silvicultural prescriptions which complement the genetic potential of the stands. Many more organizations are taking on various parts of this continuum.

Certain general features characterize programs which successfully integrate functions from tree improvement through silviculture and manufacturing to the end-use product:

1. Forest management strategies and objectives are clearly defined and based on a well-articulated end-use market strategy.

2. Linkages among tree improvement, propagation, silviculture, harvesting and processing, and their impacts on value for the target end-use markets, are understood and built into forest planning and inventory systems so that synergies among them can be identified and captured.

3. Explicit criteria or rules have been developed, and are well-understood, to guide day-to-day decision making in each of these activities or processes, based on the strategies and objectives.

4. Success against the objectives is measured, and processes continually refined based on this feedback.

5. People all along the chain of functions understand the strategies and objectives and their role in carrying them out; they exchange ideas and information effectively and frequently; and they are actively engaged in the improvement of their function relative to the objectives.

6. Flexibility and risk are explicitly considered in the development of tree improvement and forestry prescriptions.

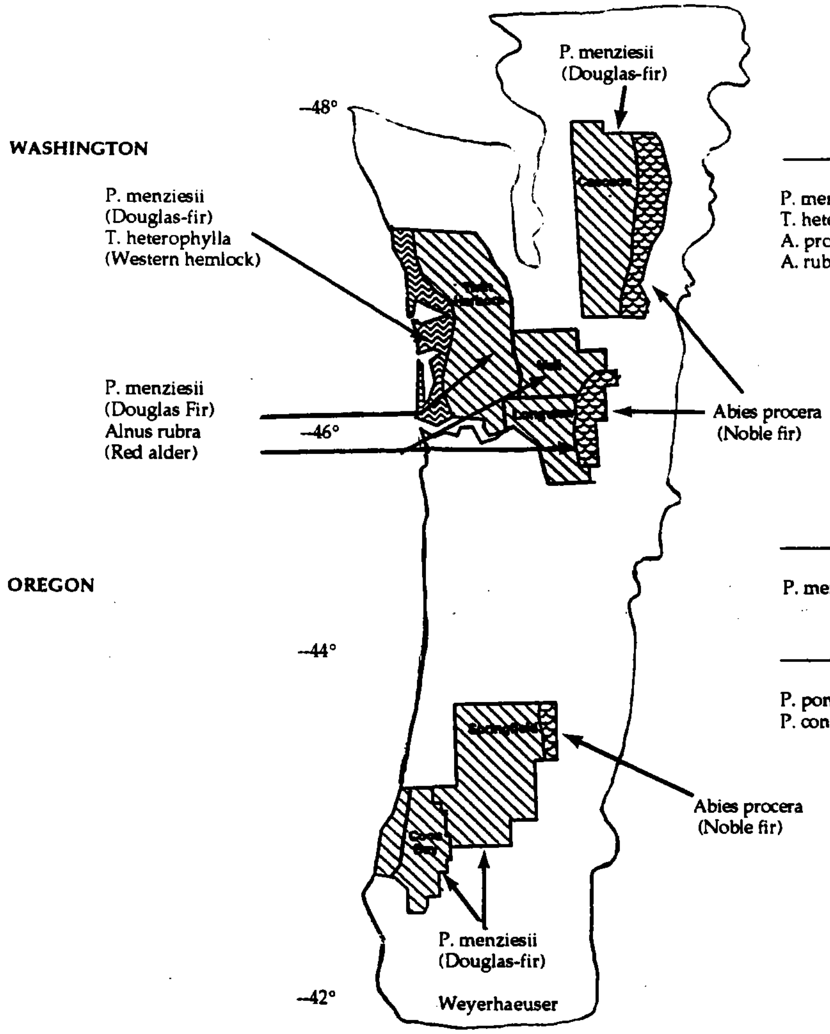
7. Costs and efficiency in use of people and resources are considered in the context of the total, strategic value created, and resources are allocated based on long-term return on investment.

The greatest challenge is in the translation of these generic features of a successfully integrated tree improvement program into a specific set of strategies, decision processes and activities which suit the circumstances of a particular organization. In the next section, one specific example will be described based on Weyerhaeuser Company's experience in design and implementation of a comprehensive set of allocation and management strategies and procedures for improved coastal Douglas-fir planting stock on their land holdings in the state of Washington.

DECISION PROCESSES FOR ALLOCATION AND MANAGEMENT OF GENETICALLY IMPROVED STOCK: A CASE STUDY

Weyerhaeuser Company is a major owner of forest land in the southeastern and northwestern United States, and is also a major international manufacturer distributor of solid wood, pulp and paper products. The company's northwest U.S. landholdings and distribution of primary species is outlined in Figure 2. Company lands in this region are generally mild in climate and highly productive (average site index 35-40 m in 50 years), but are associated with significant local variation in temperature, soil moisture, productivity, animal damage, vegetation competition and other factors.

Figure 2. Weyerhaeuser Company ownership and primary species managed on its northwest U.S. timberlands.



Washington	HA	%
P. menziesii	445,000	40
T. heterophylla	60,000	5
A. procera	80,000	9
A. rubra	60,000	5

Western Oregon	HA	%
P. menziesii	245,000	22

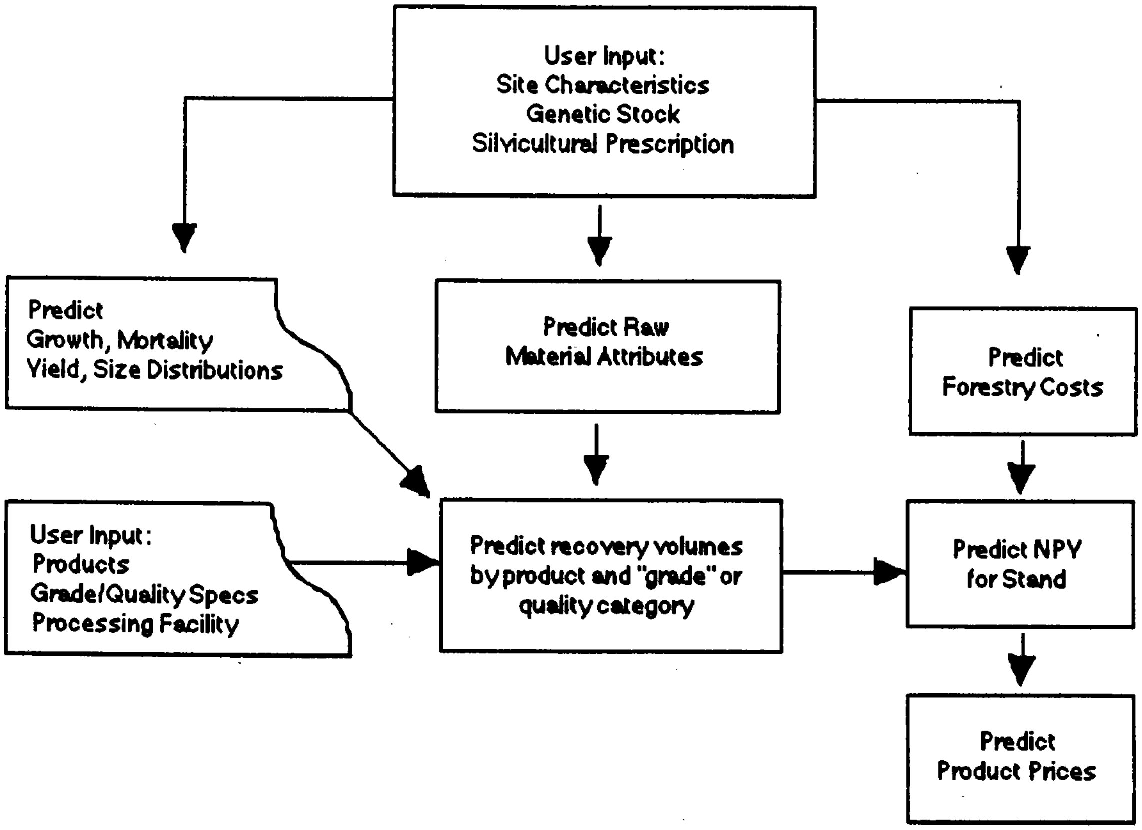
Eastern Oregon	HA	%
P. ponderosa	60,000	5
P. contorta	41,000	4

Sites also vary in operability, ease of management and regulatory restrictions. Certain site types are poorer than others in expression of stem defects and other quality characteristics.

The Company's timberlands in western Washington and Oregon are divided into 5 administrative regions, three in Washington and two in Oregon. The primary species managed on these lands is coastal Douglas-fir (*Pseudotsuga menziesii* v. *menziesii*), because of the location and nature of Company lands, and because of its target markets. Douglas-fir is a species which is particularly valued for product applications where strength, stiffness and dimensional stability are required. Weyerhaeuser manages its Douglas-fir stands with an emphasis on production of high-quality sawlogs and solidsawn products for domestic and export markets, although residual fiber for pulping is an important by-product. Different geographies within the Company's western ownership emphasize different product mixes and markets which maximize return given each local situation. The strategy in terms of raw material requirements to support target products and markets is becoming increasingly refined.

On most sites, forest management is very intensive, including careful site preparation, planting, control of competing vegetation, thinning and multiple applications of urea fertilizer. Forestry prescriptions are recommended based on in-depth analysis of the growth, yield and product value implications of available or potential treatment combinations, given the target market(s). A system of simulation models has been developed to facilitate this analysis based on decades of observations from thousands of growth and yield plots as well as many mill and product studies relating silviculture to raw material properties and product value (Figure 3). These models are continually expanded and refined as market specifications change and more data are collected about the growth, yield and product implications of various site conditions and silvicultural options. Within the general guidelines provided by the simulation, the treatments applied to any site are highly prescriptive - that is, the forester may vary the timing and intensity of treatments depending on his/her diagnosis of site and stand conditions.

Figure 3. Structure of simulation system used to evaluate and recommend forest management prescriptions for Weyerhaeuser Company's timberlands.



Since the mid 1980s, Weyerhaeuser has been meeting its 15,000 ha/year annual 'low-elevation'[1] Douglas-fir planting requirement in western Oregon and Washington with

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[1] This discussion will focus exclusively on the Company's planting and tree improvement programs for 'low-elevation' lands, that is, lands below approximately 650 m elevation (2000 feet).

seedlings from first-generation seed orchards. Information on the growth, stem form and adaptive characteristics of the parents in these orchards has been collected from genetic tests, and used to complete orchard roguing[2]. Until recently, Douglas-fir orchard seed was collected and distributed in family mixtures of 15-30 parents grouped for genetic test volume-growth performance within 6 geographic breeding zones. In the early 1980s, a series of research trials were established across the Company's ownership to evaluate the stability of its first-generation families across a wide range of environments both within and across breeding zone boundaries in a state. A variety of hardiness and growth rhythm traits are regularly assessed in these trials, along with growth performance, survival, stem form and wood density. A comprehensive discussion of these trials, their philosophy and results was given by Stonecypher (1990) and Wheeler et al. (1990). In summary, these trials to date show stable superiority of most half-sib and full-sib families across the range of environmental gradients characteristic of Weyerhaeuser's ownership within a state, from the Cascade west slope to the coast, whether the family originated from the 'local' breeding zone or not. Variation associated with sites and families within zones was much larger than zonal variation, for growth performance, growth rhythm and frost hardiness traits, likely reflecting the mild selective environments and the 'fine-grained' pattern of site variation within this part of the Company's ownership, as well as the lack of data which was available to properly define the original 'zones' in terms of adaptationally significant environmental gradients.

In 1991, an effort was initiated to review, update and document standard rules and procedures to guide the operational allocation and subsequent management of genetically improved Douglas-fir planting stock on Company lands, within the context of the Company's market and forest management strategies and established genetic resource management goals (Table 1), and integrating the best available science and data. The task of development of these procedures was carried out by a team representing the Company's forestry operations, nurseries and orchards, tree improvement and research, and implementation is underway. The approach was structured around the features of successfully integrated tree improvement and forest management processes which were described in the first section of this paper. The approach can be described as a series of steps, each associated with a logic and set of guidelines for effective decision making given the best available information.

Table 1. Weyerhaeuser Company genetic resource management goals for commercial tree species on its northwest U.S. timberlands.

1. Improve genetic potential for stand growth and end-use value for target markets as quickly and cost-effectively as possible relative to 'local' unimproved stands.
2. Maintain or improve genetic potential for survival and adaptive performance relative to 'local' unimproved stands.
3. Maintain a level of genetic diversity and adaptiveness within each species, across Canada ownership, which controls the risk of economic loss at or below the level of 'local' unimproved stands.
4. Maintain/manage the gene pools of the Company's primary commercial tree species in a manner which preserves their ability to adapt to changing conditions in perpetuity.
5. Set quantifiable, trackable standards for each of these goals, and track performance against those standards through time.
6. Base all genetics resource management decisions on the best available science and data.

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[2] Weyerhaeuser is actively engaged in an advanced-generation tree improvement program in coastal Douglas-fir. An overview of this program will be given in the review of Douglas-fir tree improvement programs by Jack Woods later in this symposium.

ALLOCATION AND MANAGEMENT OF IMPROVED STOCK: PROCESS STEPS

Step 1. Describe market objectives and desired raw material attributes for a stand or geography - the *trait requirements*

The most important criterion by which tree improvement and forestry activities should be evaluated is their impact on the ultimate management objectives. If these objectives are not clearly stated and their sensitivity to tree improvement and forestry decisions understood, there is a high probability that resources will be wasted on activities which do not further the objectives, or that no management regime will be pursued aggressively or effectively for fear that it is not the 'right' one. This in turn can create a situation in which results are never sufficiently dramatic to maintain long-term support for the tree improvement or silvicultural enterprise.

On some sites, Weyerhaeuser's principal management objectives are expressed in terms of 'non-timber' values such as water quality, fish or wildlife habitat, or endangered species protection. On sites which will be dedicated primarily to timber production, the management objectives are increasingly expressed in terms of specific target markets, which in turn have certain combinations of raw material characteristics which are important to value. These target markets and raw material specifications are established by the management team for a geography, in close cooperation with wood products, marketing and forecasting specialists, and sometimes with the assistance of specific customers or groups of customers. Sometimes the same market and trait strategy will apply to all stands in a geographic area; in many cases, however, the operability of a site, 'non-timber' objectives, regulatory restrictions, distance from a market center, or environmental conditions on a particular site will make that site better suited to a different objective. It is necessary for foresters ordering improved stock or prescribing silvicultural regimes to have in hand clear rules for what conditions should dictate what market and strait strategy.

Obviously, the definition of target markets for a stand prior to planting must be fairly general, and flexibility must be maintained to allow for changes in markets and raw material specifications through the management rotation. However, even general market specifications (for example, fine paper vs. newsprint vs. market pulp; appearance wood vs. high-performance structural wood vs. standard dimension lumber) can be extremely helpful in focusing forest management decision making. At this level there is also considerable information available in the literature and from current customers about the types of raw material characteristics which will be desired for those markets. Mill trials, customer interviews, market surveys and in-use testing are useful methods to expand this information base. Characteristics influencing both the value of the final product and the efficiency of processing should be considered, because both will impact the future value of a stand of trees.

Step 2. Describe available genetic materials in terms of their value with respect to the above raw material specifications

At the present time, Company foresters may choose to plant first-generation wind-pollinated family mixtures, individual first-generation families, or first-generation control-cross families 'bulked up' using rooted cuttings. Within five years, untested and tested second-generation families will become widely available. A wide variety of combinations of volume growth, growth phenology[3] , cold hardiness, stem form and wood specific gravity are available

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[3] Timing of budburst and budset, tendence toward free growth

among these materials, and additional traits are being assessed each year. Three steps have been carried out to describe these genetic materials:

A) Prediction of breeding values. Breeding values are predicted for first-generation and second-generation parents using a form of the standard Smith-Hazel selection index in which sites are treated as separate traits in the index (Burdon, 1979). First-generation parental breeding values are predicted from progeny means, and breeding values for second-generation orchard parents (untested, forward selections from first-generation tests) from a combined index weighting their phenotype and their family mean. Breeding values are currently predicted using a separate index for each trait, although the method would accommodate multiple trait predictions. The prediction of breeding values for Weyerhaeuser's Douglas-fir program is complicated by the fact that the nearly 750 parents in first-generation orchards were tested in largely nonoverlapping groups or 'series' over a period of 23 years, using a variety of mating designs and field layouts, on sites which varied widely in scale and precision. To allow comparisons across test series and sites, common unimproved check lots were well replicated in all tests. The treatment of sites as separate index entries enables different genetic and phenotypic parameter estimates and family sizes to be entered for different sites, accounting for differences in test design and precision. The resulting breeding value predictions are scaled to a common variance and expressed as a percent difference from the mean of the common check to allow comparability across test series and test types.

B) Prediction of family genetic values or 'orchard lift values'. The genetic potential of a wind-pollinated family or mixture of wind-pollinated families is predicted by averaging of the breeding values of the female and male parents involved in the seedlot for a given trait. These values are termed 'orchard lift values'. Likewise, the genetic value of a control-cross seedlot is predicted as the average of the breeding values of the two parents involved in the cross. Where tested-cross information is available from a genetic test, the actual full-sib family's genetic value is predicted using the method described in step A, but bringing in the specific combining ability of the cross. A side benefit of this approach has become apparent: with this simple method, and breeding values in hand for all production parents, orchard managers are able to evaluate the impacts of various roguing, pollen management and flower-stimulation scenarios, enabling them to leverage genetic gain upward still more each year.

C) Projection of genetic potentials to harvest age. Because of the general lack of long-term unit-area test data for families in the program, this is an area with marked room for improvement. Currently, for example, breeding values and 'orchard lift' values for tree volume are on a per-tree basis and come from age-8 genetic test conditions[4]. Conservation assumptions are used to modify growth, mortality and distribution functions in the Company's growth and yield models, and the modified models are then used to run simulation analyses with a particular genetic material or to project the age-8 volume 'lift values' for that material to harvest age. Changes through time in the magnitude of family differences in stem form, wood specific gravity and adaptive traits are even less well understood, and research is ongoing to improve understanding and modeling of time trends in these traits. Currently, the genetic test differences are used as an index of relative family values but are not used in a predictive sense.

Updated orchard lift values for all traits are provided to foresters, orchards and nurseries each year in the form of a Family Catalog. In the future this Catalog will also

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[4] Trees average 5-8 m in height at age 8

incorporate information feeding back from operational nurseries and stands regarding any unique production characteristics of the material.

Step 3. Develop and document a clear decision logic to choose genetic material for any new planting site, and train foresters to use that logic

A three-step process has been developed and implemented to guide this decision.

A) Classification of planting unit value potential, special trait requirements. A worksheet was developed into which scores are entered for any new planting unit (i.e. a newly harvested tract ready to be planted the next spring), reflecting the basic productivity of the site, biotic limiting factors such as vegetation competition or animal browse intensity, and management constraints such as operability, regulatory restrictions and distance from a market center. These separate components of 'value potential' are weighted (weighting factors are currently 'best judgment', subjectively derived weights) and summed to a single planting unit score. Also recorded for any unit are special adaptive issues on the site (frost pocket, high drought/heat area) or the existence of a high environmental predisposition for defect. The worksheet is filled in by the field foresters as they survey their planting units - the survey has always been a standard practice, but now the survey follows a standard structure across foresters and geographies, and data are now recorded and archived. Unit scores and the 'special issue' notes for all planting sites are entered into a common, regionwide Company database to be used in allocation decision making. Also on the summary worksheet, the forester records the desired number of trees to be planted per acre on that unit and the type of seedling which is desired, both determined based on the market objectives and growing environment on the site.

B) Assignment of genetic material to planting units. In the current process, available low-elevation Douglas-fir genetic materials are ranked across breeding zones within the state according to their 'orchard lift values' for volume, planting unit scores are ranked across administrative regions in the state, and the best families are given a preliminary assignment to the top-scoring planting units. Then, adjustments to this preliminary assignment are made to match unique family and/or site conditions to improve value and control risk of loss. For example, families with a genetic potential for low defect are assigned preferentially to planting units with a high environmental potential for defect or a unit where a particularly defect-sensitive market is the target. The converse would apply to families with a genetic predisposition to high defect. Families which are frost-hardy or frost-susceptible are allocated to units which are particularly high or particularly low in risk for unseasonal frost. As more families are characterized for wood specific gravity, high-density families might be allocated to sites where high-end structural performance markets are targeted. The examples could go on but the concept is obvious. As the relative economic impacts of different genetic traits and site characteristics are better understood for target markets and the planning of markets and raw material specifications at the unit level becomes better refined, it will be possible to make this assignment based on various weighted combinations of family characteristics appropriate to the particular market objective.

Certain types of nursery stock are more expensive to produce than others, and therefore it is necessary to have a means of evaluating a more costly stock type is appropriate. This question is particularly relevant when considering whether to plant a seedling of a wind-pollinated family or a rooted cutting from a control-pollinated family. A 'break-even analysis' approach has been applied to this question for Douglas-fir and seems to provide a workable solution. This approach begins with a calculation of the incremental percentage gain which is required from the control-cross families, over the wind-pollinated alternative, in order to provide the minimum acceptable return given the projected production cost of the rooted cutting stock - the so-called 'break-even gain'. This analysis is done using the simulation system described in Figure 3. The cost which can be borne at an acceptable return is higher for more productive sites and lower for less productive sites, because the absolute value of a percentage gain is less on a poorer site. The wind-pollinated families which would be planted in the absence of control crossing are next ranked for the target measure(s) of gain, and compared to gains predicted for control-cross families which could be produced. Control-pollinated seedlings are assumed to replace wind-pollinated seedlings from the bottom of the list of families which would have been used. If the incremental gain offered by a control-cross family is above the break-even point then the decision would be to generate and use that controlled cross. Limits are set on the maximum production allowed from any one control-cross family (see 3C below).

Another important allocation decision is the number of families to plant on any unit. The approach chosen for Weyerhaeuser's low-elevation, Douglas-fir planting program emphasizes planting of individual wind-pollinated or control-pollinated families on individual planting units, unless a site is extremely harsh and the risk of unpredictable stand failure is assumed to be high. Controls are imposed over the size of any contiguous area planted to one family or related families (see 3C). Single-family management is reviewed as an essential tool to allow maximum value to be derived from particular sites and silvicultural prescriptions for target markets. In addition, single-family management enables direct tracking of family growth and adaptive performance in the field, so that any unforeseen positive or negative response of a family to a set of operational conditions can be used to reduce future risk or enhance future gain. For example, a family showing unexpected problems in the nursery or the field could be removed from production, or the allocation and management of the family altered to address those problems. Unique, valuable responses of families to operational conditions can be identified and capitalized upon, greatly broadening the scope of traits which can be used beyond just those measurable in genetic tests. It should also be noted that it is not necessary to compromise genetic diversity at the landscape level to do this - in fact, the same number of families would be harvested and used whether they were planted in individual family units or mixtures. By scattering families across the landscape, in blocks of controlled size, interspersed with other ages, species and management regimes, a high degree of diversity and buffering against unexpected events is possible.

C) Application of additional constraints for management of risk and flexibility. Certain rules are applied uniformly regardless of the characteristics of families or sites in order to provide added control over risk and ensure management flexibility:

** *Controls over the maximum proportion of any one family, or related families, which can be planted in any year or over time.*

** *Controls over the maximum contiguous area which can be planted to any one wind-pollinated or control-pollinated family or their relatives.*

** *Controls over the maximum 'environmental distance' across which stock can be moved, based on origin stand/site characteristics and planting unit characteristics (particularly latitude, elevation, mesic/xeric conditions).*

Adherence to these rules is tracked through a report generated annually from the Company's forest inventory.

The subject of seed transfer and associated concerns about maladaptation deserve special mention here due to the likely movement of material which will occur across traditional 'breeding zones'. As mentioned in the background, the results of stability trials have indicated strongly that most Washington families (about 85%), regardless of their zone of origin, are stable in their growth superiority across the range of environments typical to Company ownership across western Washington, and families which are unstable, or which show poor frost or drought hardiness, tend to exhibit those problem responses on harsh sites whether those sites are in the 'local' zone or not. Observations of field growth, survival, phenology and frost damage through age 8-13 have been or are being supplemented by nursery and laboratory studies of growth rhythm and cold hardening/dehardening patterns, and both types of data support the same conclusion. Allocation based on 'breeding zone' does NOT provide sufficient control over the risk of maladaptation, and unnecessarily restricts selection intensity available from the use of proven performers with appropriate adaptive and quality characteristics from other breeding zones. Far more effective management of value and adaptive risk is achieved through direct assessment and selection of families based on the suite of traits important to value and adaptation under a particular set of planting site conditions. Additional control over risk can be obtained through the kinds of rules described above which limit transfers beyond certain environmental distances, or which limit the extent to which any one family or group of families is used across the landscape. The latter limitation also ensures a desired level of genetic diversity within the species across the landscape.

Step 4. Track the operational effectiveness of the decision logic in achieving the management objectives, and use the tracking results to regularly improve the process

The tracking, feedback and improvement mechanisms built into Weyerhaeuser's allocation process involve three elements:

A) Tracking of adherence to family-usage, block size and movement rules. This tracking takes place via the Company inventory system. The family identity of any planting unit becomes part of the basic regeneration data entered into the inventory at the end of each planting season, and a report is produced from the inventory each year summarizing historical family usage, maximum contiguous area planted to a family, and the movement distance.

B) Monitoring of operational performance of the improved stands. Traditional land-exam or stand survey processes are being modified to encompass sampling of a subset of operational stands of each major production family. Particular attention will be given to survival, vigor and damage, and growth during the establishment period, where stresses are highest. The monitoring data will be archived and summarized annually, and exceptional negative or positive responses of particular families will be used the next year to modify the allocation of those families.

C) Effective information flow and improvement planning among groups involved in the forest management 'chain'. A cross-functional team involving region foresters, nurseries and orchards, tree improvement and forestry research was set up to develop and implement the allocation and management rules and processes described above. It became clear during that implementation process that this team could play another, even more important role. Each year, one representative from each administrative region and one each from nurseries, orchards, tree improvement and research gather feedback from their work groups on issues and ideas for improvement of the allocation process. These representatives then meet to review the tracking and monitoring results, to discuss the feedback, and to plan process improvements. The focus and enthusiasm for the goals of the allocation process is effectively maintained by this regular review and interaction, and all the groups involved in implementation have a direct role in shaping and improving the process. The involvement of representatives from each administrative region has been very useful in establishing and maintaining common procedures and standards across regions, and the interchange among region foresters and others has stimulated improvement activities in a number of related and unrelated processes. The varied perspectives brought to the team steepens the learning curve about families, site requirements and other aspects of the allocation process.

KEY LEARNINGS FROM THE ALLOCATION AND MANAGEMENT PROCESS

1. Clear specification of planting stock requirements and family attributes is extremely powerful. Not only are limited supplies of exceptional genetic material used most effectively, but also, no one is forced to trade off volume for quality or adaptive characteristics unless their market strategy or site conditions dictate as such. Decisions are increasingly made based on science and data rather than on opinions and personalities. The entire organization is able to focus on a common set of goals, and look for synergies among functions. There has been a direct feedback of trait priorities back into the breeding program which has been very useful, and foresters have become enthusiastic about, and highly involved in, the tree improvement program.

2. Management of single-family lots creates a very rapid learning curve, and much greater knowledge than mixed-family management allows. Even though single-family allocation and management has only been going on for 3-4 years in the Douglas-fir program, there is already information feeding back from the nurseries about differences in family responses to operational nursery conditions. This information is being used by foresters and nursery managers to refine their growing regimes. As more operational stands are planted to families this information flow will extend rapidly to site and silvicultural responses, quality and adaptive responses. It must be noted that an inventory structure must be present to track family identity, and a database structure put in place to manage and report on tracking and monitoring data from each operation along the production process.

3. Stand management planning now begins before planting, with a direct tie to end-use objectives. A commitment has been made to a set of objectives, and future prescriptions can be set to build synergy with what has gone before.

4. It has been extremely valuable to get family information out in the hands of orchards, nurseries and foresters in a form which facilitates tactical decision making. As mentioned above, the availability of breeding values for production orchard families has enabled orchard managers to make better tactical decisions to maximize realized gain. The more, different people who have this information in hand, the more separate fronts on which improvement and better decision making will proceed. This information has also created a significant 'demand-pull' for certain families and for information on other traits, and this is very exciting and healthy.

5. Focused attention and leadership must be given to the implementation process itself, so that new procedures and rules become an integral part of doing business. We have found that this requires a substantial investment of time, energy, persistence and salesmanship, even when the organization is generally supportive. Change is difficult, people have many demands on their time, and many or most people will tend to fall back to old habits and procedures unless the entire system has been set up to support and facilitate the change, and unless adherence to the new procedures is continually tracked and reinforced.

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VARIETAL DEPLOYMENT STRATEGIES AND GENETIC DIVERSITY: BALANCING PRODUCTIVITY AND STABILITY OF FOREST STANDS

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ABSTRACT

Large-scale reforestation programs with genetically improved varieties create an urgency to utilize deployment strategies to insure their optimum use. Development of deployment strategies depends on a clear understanding of the relationships among genetic diversity, intergenotypic competition, stability of varietal performance, and stand-level productivity. There is a clear lack of information in the forestry literature regarding these relationships. The available research results are reviewed, and important areas of future research are discussed. Forest stands contain a large amount of genetic diversity, and this diversity is maintained even through regeneration cuts with natural seeding or through artificial regeneration with genetically improved varieties. A great deal is known about the general patterns of genotype x environment interaction with tree species, yet, the published studies invariably used small varietal plots which opens the question of validity on the stand-level. Establishment of appropriate experiments to gain stand-level information in forest genetics studies is emphasized.

INTRODUCTION

Forest genetics as a science has evolved beyond its early responsibility to demonstrate genetic variation and heritability of traits and has produced the applied field of tree improvement. As an integral part of many reforestation programs, tree improvement programs are producing large quantities of genetically improved planting stock; yet research into optimum use or deployment of that planting stock remains in its infancy.

Several tree improvement programs throughout the world are in their second or even third generation of improvement, and in several regions of the world, all reforestation employs genetically improved stock. In the southeastern U.S. for example, approximately 1.3 billion seedlings are planted annually. The vast majority of the seedlings are either loblolly (*Pinus taeda* L.) or slash pine (*Pinus elliottii* Engelm.), virtually all of which are genetically improved. Tree improvement has come of age!

Forest geneticists and tree breeders find themselves in the next phase of the growth of our science, which is the large-scale implementation phase. How do we most efficiently use this genetically improved stock? Much effort has been devoted to development of the improved genotypes, i.e., selection, testing, and orchard development, yet, very little research has been conducted regarding deployment of the genetically improved stock. In this context, *deployment* is defined as *the strategic use of existing varieties of tree species in both time and space*. We can learn a lot from agronomic crop breeders who have conducted a considerable amount of research into mixtures versus pure varieties (Trenbath, 1974). However, with the exception of grasses and a few other crops, most of their research has been with annual crops which commonly have a life span of only a few months. In contrast, the situation with forest tree species is vastly different. Our species live for decades, at least, and we plan on competition-induced mortality or thinning to remove most of the trees in the stand prior to final harvest. Most of the economic value in a forest stand is concentrated on a few trees per hectare.

We find ourselves with the dilemma of having forest management and forest industry (mills and other converting facilities) guiding tree breeders toward within-stand and product uniformity while ecologists, the general public, and uncertainty of environmental stresses are guiding us toward maintaining high levels of genetic diversity. Is it possible to breed varieties of forest tree species which are uniform for production traits and yet variable for adaptation traits? Theoretically this seems possible but as yet, remains untested.

I question whether we, as forest geneticists, have carefully analyzed these opposing forces and designed and installed experiments to address the issues. In general, I think that the answer is no! This paper will examine the major issues involved in balancing productivity, genetic diversity, and stability of forest stands and then suggest some experiments which will address those issues.

PROGENY TESTS VERSUS STAND LEVEL GENETIC STUDIES

Traditional progeny test designs have proven useful for estimating breeding value but are not useful for estimating genetic gain in volume per hectare. Foster (1989) showed that the ranking of families is similar despite differing plot designs (e.g., non-contiguous, row, or block plots). However, the level of genetic variance among families increased dramatically from block to row plots and again from row to non-contiguous plots. Hence, estimates of genetic gain, will differ among the progeny tests using the various plot designs. An additional concern is that different levels of intergenotypic competition between progeny tests and operational plantings with genetically superior stock will cause a difference between predicted and realized genetic gain (Foster, 1992). Progeny tests include varieties (families or clones) with a wide range of genetic variability while operational stands of genetically superior stock will include only a select subset of the best varieties. These varieties may be deployed as single variety blocks or mixtures of two or more varieties. If the varieties differ in their competitive ideotypes, the tree breeder should anticipate that the difference in mean performance among varieties, hence variance among varieties, could differ between the progeny test and the operational planting (Cannell, 1978). This is especially true when row plots are used in the progeny test.

Stand level responses to genetic effects and intergenotypic competition can only be assessed with large plot sizes with large trees, i.e., using juvenile trees to simulate mature stand response has proven to be inconclusive. For at least the past 15 years, many research studies have been published which examine the value of early genetic testing (often in growth chambers or greenhouses) as a substitute or addition to more traditional field-based progeny tests. The value of these early genetic tests, especially for predicting volume growth, remains largely unproven.

There was a flurry of short-lived research activity in the early to mid-1980's regarding genetics and growth and yield modeling. Such studies are large and generally must be at least 1/3 rotation length in age to become informative. Such studies must allow natural forest stand development to occur. Competition among the trees in the stand then forces stand structure to develop. If unchecked by thinning, stand development will lead to some mortality. These processes are well known to foresters, and growth and yield modelers have built models which can predict the result of the processes. From the few published studies in the area of growth and yield and genetics, it is obvious that the growth pattern of different varieties in pure plots can be characterized (Nance and Wells, 1981a, 1981b; Buford and Burkhart, 1987; Sprinz, 1987), and their growth and yield projected (Knowe and Foster, 1989). These projections can be used to predict genetic gain at the stand level. Theoretical modeling results were developed for mixed family stands of loblolly pine (Nance, 1981), but no empirical results were available to validate the model. Predicting genetic gain in mixed variety stands via growth and yield modeling remains largely untested, or at least the available results have not been published.

PREDICTING THE EFFECTS OF INTERGENOTYPIC COMPETITION IN MIXED VARIETY STANDS

To date, there is little firm guidance in the literature which can be used to predict the results of intergenotypic competition, within mixed variety stands, on stand-level growth and yield. The "replacement series" is a common experimental design used in the agronomic crop literature to test the effect of intergenotypic competition on yield. In a replacement series, the varieties are grown in a series of treatments with a fixed number of plants per plot and one or more, often two, varieties in different proportions (Harper, 1977). For the purposes of this paper, the commonly used term "mixing study" will be used synonymously with replacement series.

The majority of mixing studies in forestry have used seedlings or young (one- or two-year-old) rooted cuttings. These studies have shown all possible types of competitive responses among families or clones: complementary (mixture yield equals the weighted mean of the pure varieties), overyielding (mixture yield exceeds the weighted mean of the pure varieties), or underyielding (mixture yield falls below the weighted mean of the pure varieties) (Adams et al., 1973; Tauer, 1975; Adams, 1980; Tuskan and van Buijtenen, 1986; von Euler et al., 1993). Such studies are very useful for exposing the range of responses that can be anticipated. However, with the exception of the study by von Euler et al. (1993) [15-year-old Scots pine (*Pinus sylvestris* L.)], the studies are with young, small trees which limits their usefulness for construction of growth and yield models to predict forest stand-level response.

Results from one replacement series study with seven eastern cottonwood (*Populus deltoides* Bartr.) clones planted at two sites is in the process of being published by Knowe et al. (in review) and Foster et al. (in review). The study design is given in Table 1. Although the data were from young trees (ages 2, 3, and 4), they were quite tall (13.23 m average) by age 4. One of the purposes of the study was to provide information which would enable modification of a stand level growth and yield model to incorporate the effects of mixing. Knowe et al. (in review) modeled diameter distributions with the goal of predicting the diameter distribution of mixed clone stands based on monoclonal distributions. They were able to gain a good fit with their model and found that the general shape of the distribution was the same for monoclonal versus two-clone mixtures, however, the distribution shifted (Figure 1) depending on the specific clone or mixture. Foster et al. (in review) found that mixture yields for some of the two-clone groups (a group includes the two monoclonal plots plus the two mixtures with those two clones) increased linearly with an increase in the proportion of the highest yielding clone. For other two-clone groups, the trend was non-linear (either overyielding or underyielding), and in some cases, different trends were found at the two sites (see Figure 1 for results of two of the seven groups).

Another major type of mixing study is one in which there is a simple comparison between the mean of single variety plots and a single mixed plot of all varieties in equal proportions. Unfortunately, these studies provide little or no predictive value but do serve to demonstrate the general effect of single variety (family or clone) stands versus a mixed variety stand. Most field tests of mixing studies follow this experimental design. However, researchers have failed to find a common result, and a significant difference may (Williams et al., 1983; Lundkvist et al., 1992) or may not occur (Markovic and Herpka, 1986; von Euler, 1993) between the mean of the single variety plots and the mean of the mixed plot.

Table 1. Treatments in the replacement series with seven eastern cottonwood clones [from Foster et al. (in review)]. Treatments on the diagonal represent monoclonal plots while off-diagonals represent two-clone mixtures.

	Clone						
Clone	ST244	S7C1	S7C4	S7C8	ST75	ST238	ST66
ST244	X			X[a]	X		
S7C1		X			X	X	
S7C4			X			X	X
S7C8				X			X
ST75					X		
ST238						X	
ST66							X

[a] Each two-clone mixture is represented by two treatments: 75% clone A:25% clone B; and 25% clone A:75% clone B.

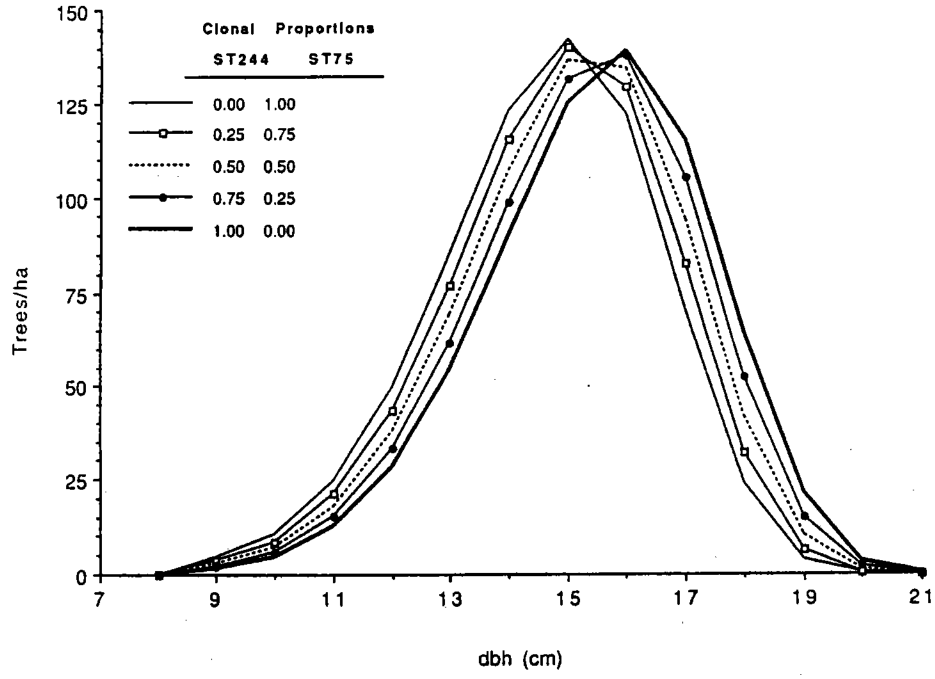

Figure 1. Predicted diameter distributions for monocultures and two-clone mixtures of eastern cottonwood clones ST244 and ST75 at age 4 in a replacement series study in Mississippi (adapted from Knowe et al. in review.

To date, these mixing studies have failed to detect a single pattern of growth performance when forest tree varieties are mixed. That the results of intergenotypic competition on stand growth and yield depend on the specific varieties involved and even on the site should not be surprising given the range in tree ideotypes with their resulting classes of predicted interaction (Cannell, 1978 and 1982). Field mixing studies must be established which allow morphological, physiological, or phenological components of intergenotypic competition to be examined with the results being integrated into growth and yield models.

SELECTION AND POPULATION SIZE EFFECTS ON GENETIC DIVERSITY

The few empirical studies published in forestry, to date, have failed to demonstrate a general reduction in genetic diversity in a population as a result of phenotypic selection. In addition, there has been no demonstration of a strong relationship between number of trees in a population and genetic diversity of the population. Theoretically, there must be a diminution of genetic diversity with very few trees in a population or when few trees serve as parents for an offspring generation; yet this point has not been found in the few studies published to date (i.e., sample size in the tens or low hundreds).

Selection

Most of the published studies have compared genetic diversity of forest stands with that of the stand following a natural regeneration cut such as a shelterwood (Neale, 1985), seed-tree (Yazdani et al., 1985), and two-story and group selection plots (Shimizu and Adams, 1993). These studies have shown little or no changes in genetic diversity due to regeneration cuts (see Table 2 for comparisons of expected heterozygosities).

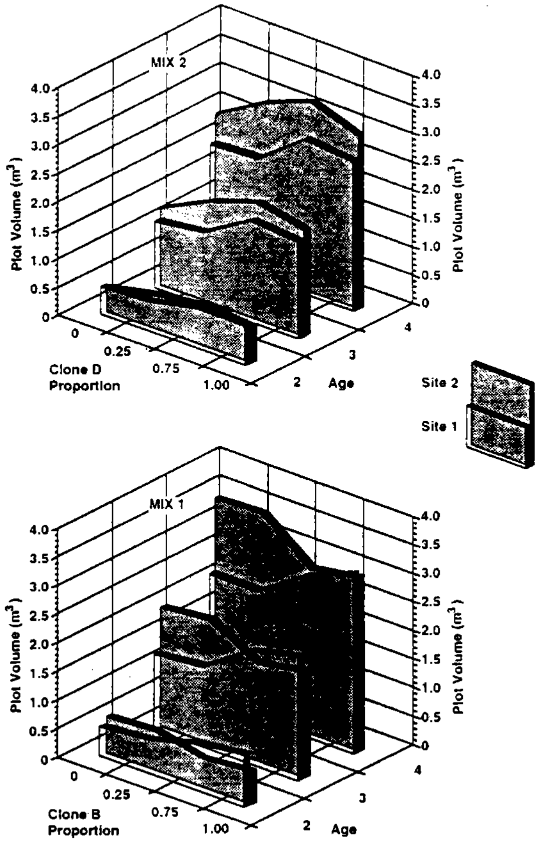

Figure 2. Plot volume production of eastern cottonwood clones at ages 2, 3, and 4 in a replacement series study with two-clone mixes at 2 planting sites (adapted from Foster, et al., 1993). Mix 1 has clones A and B, and Mix 2 has clones C and D. The ratios sum to 100% (i.e., 0% A:100% B) at each of the four sample proportions.

Table 2. Expected heterozygosities at isozyme loci in natural stands, regeneration cuts, and in seed orchards (or among select trees).

Species	Natural	Regen. cut	Seed orch.	Reference
Pinus sylvestris	0.28		0.27	Muona and Harju, 1989
Pinus sylvestris		0.35		Yazdani et al., 1985
Pseudotsuga menziesii	0.21		0.23	Shaw and Allard, 1982
Pseudotsuga menziesii	0.22	0.23		Neale, 1985
Picea sitchensis	0.15-0.22		0.24	El-Kassaby, 1992
Thuja plicata	0.06		0.06	El-Kassaby, 1992
Pinus ponderosa	0.12		0.13 (Select trees)	Yow et al., 1992

A few studies have tried to assess the impact of a tree improvement program on genetic diversity in a population. The researchers have compared genetic diversity between phenotypically select versus non-select trees in natural stands (Yow et al., 1992; Fins, 1993) and between seed orchard populations and natural stands (El-Kassaby, 1992; Savolainen and Kärkkäinen, 1992). Again, these studies have shown little or no detectable differences in genetic diversity between the two groups (see Table 2 for comparison of expected heterozygosities). In fact, scenarios can be envisioned in which the seed orchard population may be more-or-less genetically diverse than the natural population, depending on effective population size or level of inbreeding in either case.

Many reasons may account for the maintenance of genetic diversity from natural stands to regenerated populations (either via natural seeding or tree improvement program). First, forest trees, especially conifers, appear to be among the most genetically diverse plant species (Hamrick et al., 1992; Moran, 1992). Second, much of this genetic diversity seems to be displayed as within population variability, with significant yet lower levels of variation among populations (El-Kassaby, 1991; Hamrick et al., 1992; Moran, 1992). Third, tree seeds and pollen are widely dispersed via wind, animals, and insects leading to large level of gene flow for most species. Fourth, forest sites are highly variable, characterized by fine-grained variability, hence environmental effects encourage survival and growth of a wide array of genotypes as opposed to large patches of uniform site selecting for a single genotype (Campbell, 1979). Finally, microsite variation masks genetic differences among trees (Cannell, 1984) which inhibits directional selection and may serve to maintain variation in populations.

Population Size

Although genetic diversity theoretically declines with effective population size, the relationship probably is a threshold function rather than a smooth function, based on empirical studies in forestry. Actually, few studies in forestry have addressed the issue directly, most examine its practical results in the form of genotype x environment interaction. Moran (1992) did state that for Australian tree species there was "no discernible relationship between current population sizes and genetic diversity." In the isozyme-based studies listed above, sample sizes were in the tens or low hundreds, and a similar sample size was set for both the natural stands and regeneration cut stands or seed orchard trees. Yet, even if the sample size was different between the two populations, there was little or no difference in genetic diversity. For example in the *Pinus ponderosa* (Dougl.) study by Yow et al., (1992), 18 select trees and 50 non-select trees were sampled. No significant difference in allozyme frequencies between the select and neighboring non-select trees was found. The high within-tree and within-population heterozygosity apparently lead to this result. In addition, most of the adaptive or growth and yield traits usually emphasized in tree improvement are polygenic and may be conditioned by hundreds of loci, each with multiple alleles. Through genetic recombination, a small number (in the tens) of select trees in a seed orchard or "leave-trees" in a regeneration cut can produce an enormous number of genotypes in the offspring generation.

Selection in either a tree improvement program or regeneration cut is based on quantitative traits. However, most of the recent studies of genetic diversity utilize isozymes which are simply inherited and appear to be uncorrelated with the common adaptation and growth traits (El-Kassaby, 1991). Whether genetic diversity for growth and yield traits has been diminished significantly by tree improvement is generally untested. However, Buford and Burkhart (1987) detected no significant difference in variability of dbh between open-pollinated families of select parents and an unselected checklot.

DEPLOYMENT OF IMPROVED VARIETIES

Tree improvement has provided the forest manager with a choice of not only different species but also different varieties of a given species to use in reforesting a site. An organization should have a deployment strategy for use of improved varieties across reforestation sites (over space) each year and as well as over the years (over time).

Improved Varieties Defined

An improved variety can take many forms in the context of modern tree improvement. Hence, a variety may include a seed source, open-pollinated family, controlled-cross (full-sib) family, clone, or a mixture of any of the above. The families may originate from wind-pollination, be derived through supplemental mass pollination, arise via bulking-up (rooted cutting or tissue culture plantlet multiplication) seedlings in a seedlot, or be created through controlled-crossing. Clones may be produced by either rooted cuttings or tissue culture plantlets. Strategies exist for the creation of all of these forms of varieties (e.g., see Shelbourne et al., 1989 for examples). The question remains as to how to use them for reforestation once they are available.

Aspects of Deployment

The temporal and spatial deployment of varieties must be evaluated at both the individual site and the geographic level. Many organizations own or control large areas of forest land of which they may be reforesting hundreds or thousands of hectares annually. Over time most, if not all, of a watershed may be reforested with improved varieties, and care must be taken to consciously manage the genetic diversity over the entire area.

Several general options are available in deploying varieties to one site. Varieties may be combined in four basic ways on a single site: (1) a bulk mixture of all varieties in the production population, (2) two or more mixtures, each of which contains a subset of the varieties in the production population, (3) a mosaic of single variety stands, or (4) a single variety (Libby, 1987; Foster, 1992).

The bulk mixture is probably the most common deployment strategy [termed "widespread intimately mixed plantations" for clones (Libby, 1987)] for most organizations. All seeds are collected from a seed orchard and mixed regardless of maternal parent. The seedlings from the mixture are planted at each site. In the case of clones, the rooted cuttings or tissue culture plantlets are combined into a single mixture. The proportion of planting stock arising from a single parent or clone is related to the number of parental trees in the seed orchard and their fecundity, or to the number of ramets per clone in the cutting orchard (in the case of rooted cuttings) and their propagation ability. Lindgren et al. (1989) have suggested that a larger number of clones can be included in a mixture by including higher proportions of the better clones and lower proportions of the lesser quality clones. They gave an algorithm to optimize genetic gain via choosing the best proportion of each clone based on its genetic value. Their procedure should enhance genetic diversity without sacrificing much, if any, genetic gain.

The use of several mixtures of different subsets of varieties from the production population is less common. But such mixtures may be used to group varieties for use on specific sites to take advantage of genotype x environment interaction or for genetic diversity issues [e.g., specifically related to pest resistance (Gould, 1991)]. In this case, a single mixture deployed at a site would contain a subset of the available varieties.

Several varieties may be deployed to a site, but each is planted in a single-variety stand in a mosaic covering the entire site. This is becoming increasingly common around the world. For example in the U.S., several of the large timber companies are planting mosaics of single open-pollinated families per site (e.g., Duzan and Williams, 1988; Talbert, 1993). A single family may occupy 10 to 20 hectares, while the entire 150 hectare site may contain 10 families. These families may be combined purposefully or at random on the site. With clones, this deployment arrangement is termed "mosaics of monoclonal stands" (Libby, 1987). Based on theoretical considerations, 10 to 30 clones per site seems to optimize the balance between risk of forest stand failure versus the desire for stand uniformity (Libby, 1982; Hühn, 1985). With *Eucalyptus* spp. clones, the Industrial Afforestation Unit of the Congo (UAIC) operationally plants 50 hectare monoclonal stands around Pointe-Noire, Congo (Leakey, 1987). Aracruz Florestal S.A. uses at least four different clones for each 150 hectares in mosaics of monoclonal stands (Foster and Bertolucci, 1993).

Finally, a single variety may be deployed to a site. This practice has been used operationally with clones of *Populus* spp. in Europe for many years. It may represent a very large risk, though, due to the drastic reduction of genetic resistance to pest attack or to climatic maladaptation.

Less emphasis has been placed on developing strategies regarding deployment of varieties over geographic space (landscapes) as well as over time. This issue will become more important in the future (Talbert, 1993). Some strategic development for tree improvement programs in general has led to rules-of-thumb for size of a breeding population (e.g., 100 to 300 genotypes) or production population (10 to 50 genotypes). In the same sense, efforts should be made to enhance genetic diversity at the landscape level by using different varieties or mixtures of varieties in adjacent stands. Emphasis should be placed on maintaining an aggressive tree breeding program with regular release of new varieties (Heybroek, 1978). In this way, new genetic variability is created and deployed with each generation of the tree improvement program. Most managed forests have a wide range of stand ages, even between adjacent stands. Therefore, over time a changing array of genetic diversity will be deployed at the landscape level.

Factors to Consider in Deploying Varieties

Site Variability: Procedures to determine breeding zones are well known in forest genetics (Wright, 1976; Zobel and Talbert, 1984). Despite the type of forest tree variety being developed, genetic tests are established with the candidate varieties at a range of field locations, each of which represents a major site type. The trees in the tests then serve as bioassays with their phenology, growth, and response to various stresses providing data which is used to stratify the site types into more-or-less homogeneous sites (termed a "breeding zone"). Varieties with consistent growth and stress response are assigned to their optimum breeding zones. Soil type, rainfall pattern, elevation, latitude, length of frost-free growing season, soil moisture, and frost-free date in the Spring are common factors leading to the delineation of breeding zones.

A significant interaction of varieties with sites is termed a "genotype x environment interaction." Breeding zones and varieties are usually matched to minimize genotype x environment interaction. Generally, the level of genotype x environment interaction (site sensitivity) increases with type of variety, beginning with seed sources (most stable), mixtures of families, individual open-pollinated families, individual full-sib families, and individual clones. In a Norway spruce [*Picea abies* (L.) Karst.] study in the southern third of Sweden, Bentzer et al. (1988) found mixtures of 56 clones to be least interactive with sites, followed by seed sources, and individual clones (least stable). In an analysis of part of the same study at a later age, Bentzer et al. (1990) found that the number of clones in the mixtures could be reduced from 56 to 20 with no increase in the clone x site interaction. They found little difference in the results despite trying three different clone elimination processes: (1) random deletion, (2) deletion based on inferior height growth, and (3) deletion based on lack of growth stability (Eberhart and Russell, 1966).

Further testing, selection, and breeding is often confined to a particular breeding zone, hence, the breeding and production populations are developed within this zone. Usually, deployment of improved varieties is restricted to a single breeding zone. One exception to this rule involves deployment of highly superior varieties to specific sites (very good sites, high disease hazard sites, drought-prone sites) even though the specific varieties may be unstable generally across sites. This is one method to take advantage of genotype x environment interaction. A separate breeding population is warranted for these specific sites only if economically justified by an adequately large landbase.

Probability of Biotic and Abiotic Stresses: Fear of forest stand failure due to biotic (diseases or insects) or abiotic (climate or soil nutrient factors) stresses is probably the most important single factor leading to the goal of large genetic diversity in the population. In the case of diseases or insects, a forest manager may be concerned with adopting a deployment strategy which spreads the risk of loss and also takes advantage of positive features of host-parasite interactions and tree-to-tree interactions (Heybroek, 1982).

Theoretical considerations regarding spreading the risk of loss of a genotype have been addressed (Heybroek, 1982; Libby, 1982). Libby (1982) found that using 7 to 30 clones seemed to optimize the risk of plantation failure from too few clones with the expense and lack of uniformity resulting from too many clones. A similar rationale can be used for families rather than clones. The natural process of stand development entails loss of many trees within the stand, through mortality or thinning. As trees get bigger, they need more space, hence, they can compensate for losses of trees within the stand, up to a limit. A forest manager is interested in how many trees per hectare can be lost, due to biotic or abiotic stress, without imposing a financial loss. One way to maintain genetic diversity on the site without totally sacrificing both product and stand uniformity is to use mosaics of single variety stands on a site (Heybroek, 1982).

The disease process has two phases: (1) the parasite must reach the individual tree and (2) the parasite must multiply until the damage threshold is reached (Heybroek, 1982). The rate of spread of some diseases may be slowed by mixing resistant and susceptible varieties (Marshall, 1977; Heybroek, 1982) or several partially resistant varieties (Gould, 1991), although the success of this deployment strategy depends on the disease. In some cases, mixing resistant varieties with susceptible ones caused greater losses in the resistant trees than if they were planted in a stand with all resistant trees (Heybroek, 1982). Strategies to reduce build-up of the disease, once the host is infected, which work with some agronomic crops (Browning and Frey, 1969) may not work with forest trees. Trees are so large that the disease can grow dramatically without leaving the initially infected tree (Heybroek, 1982); hence, mixing resistant and susceptible trees may not be of benefit and, as mentioned above, actually may result in greater infection of the resistant trees.

Contrary to popular belief, mixing varieties may not be helpful, especially if too much of the stand is affected for the remaining trees to compensate with added growth. The forest manager must know the attributes of the specific disease of interest prior to determining the deployment strategy. Blind mixing of varieties will lead to useful reduction in disease impact only in rare cases (Heybroek, 1982). Mosaics of single variety stands seem to represent a good compromise of issues.

Economic Considerations: As forest geneticists, forest managers, and mill managers begin to discuss their common interest in the forest stands, and resultant raw material, grown from genetically improved varieties, several economic leverage points become apparent, many of which are related to varietal deployment (Nance, 1986; Talbert, 1993). These issues center around the value of (1) increased uniformity of the raw material for processing, (2) increased uniformity of the regeneration and forest management processes and the resultant increased survival of trees and enhanced stand growth and yield, and (3) the capability to produce targeted products from the forest.

Increased uniformity of raw material allows savings in harvesting and processing as well as production of a higher value product. Deployment of mosaics of single variety stands or mixtures of very similar varieties will lead to uniformity of raw material. Aracruz Florestal S.A. has already achieved substantial gains in fiber processing efficiencies due to their development, deployment and use of superior *Eucalyptus* spp. clones (Zobel et al., 1983; Demuner et al., 1991).

Maintaining separate varieties through the cone and seed processing, seedling or rooted cutting production, and stand management processes leads to substantial improvements at each step (Duzan and Williams, 1988). The various managers at each stage can learn the peculiarities of each variety and then manage the process accordingly. The end result should be overall cost savings and increased yield of the forest stand.

Purposeful deployment of improved varieties provides the opportunity for the forest manager and mill manager to produce a known quality of raw material at the best location. For example, an organization could choose to utilize their most superior varieties on the highest site quality land nearest an appropriate mill (Talbert, 1993). This deployment strategy should result in the highest financial return on their investment. Another strategy might entail the mixing of two varieties in a stand. One variety may be noted for its production of high quality sawlogs while the other is noted for its fiber quality for pulp and paper production as well as being able to efficiently use its available growing space (crop ideotype; Cannell, 1978). The second variety would be systematically removed during thinnings to leave the first variety for the final harvest. These options represent only a few of those possible once forest geneticists, forest managers, and mill managers begin working together to fully utilize the available improved varieties. Detailed information on each variety is crucial to enable these types of strategies.

EXPERIMENTAL DESIGNS TO INVESTIGATE THE RELATIONSHIP BETWEEN DEPLOYMENT, GENETIC DIVERSITY, AND STABILITY OF GROWTH AND YIELD

Few studies are in place which will provide information to guide forest geneticists and forest managers in development of efficient deployment strategies. Since the studies are long-term by nature, it is imperative that they be established soon, so that the information will be available as soon as possible. Two experimental designs will be discussed as well as some suggestions on how to utilize the information in growth and yield modeling.

Replacement Series with Competition Diallel Design

In the mid-1980's, Warren Nance (USDA Forest Service, Southern Forest Experiment Station, Gulfport, Mississippi, USA) spearheaded an effort to install several studies, using different species, with a more-or-less common experimental design. This design was a replacement series with two-variety pairs determined through the use of a competition diallel (Hill and Shimamoto, 1973; Hill, 1974). Separate multi-location studies were installed with slash pine, loblolly pine, and eastern cottonwood. The design for the eastern cottonwood study is given by Knowe et al. (in review) and Foster et al. (in review) and partially in Table 1 of this paper. The other element that was not previously mentioned is that the studies all contain a treatment which has row plots of all varieties in a single plot and another treatment which consists of multi-tree, non-contiguous plots of all varieties in a single plot. Some of the studies also have an unselected checklot as a treatment, and some have half of the blocks per location at one spacing and the other half at another spacing. The data coming from the studies will be used in a variety of analyses, both genetics and growth and yield oriented, which will be developed by somewhat different teams of scientists.

Replacement Series with Crossing Diallel and Competition Diallel

The studies described above have used either open-pollinated families or clones as the varieties. While the genetic analyses will be useful, there are several genetic parameters and their interactions with competition that can only be obtained by using full-sib families as the varieties. I designed the following study as one possible way to explore, in detail, the intergenotypic interactions as influenced by fully pedigreed individuals. The study has not been implemented yet, but I would encourage others to consider using it.

Design: Eight parent trees will be crossed using a partial diallel mating plan (Table 3) in order to create the eight full-sib families needed to install the mixing study. Each full-sib family will be coded (Table 4) to facilitate the development of the diallel competition design for use in field test establishment.

Table 3. Diallel mating design used to guide controlled-crossing.

Female parent	Male parent						
	2	3	4	5	6	7	8
1	X						X
2		X					
3			X				
4				X			
5					X		
6						X	
7							X

Table 4. Coding of the full-sib families for use in the competition diallel (See Table 5).

Full-sib family	code	Full-sib family	code
1 x 2	1	5 x 6	5
2 x 3	2	6 x 7	6
3 x 4	3	7 x 8	7
4 x 5	4	1 x 8	8

The diallel competition design will be used to determine which families to combine in the binary mixtures. The design is similar to 2 four-parent half diallels in a disconnected design. The diagonal elements represent monoculture plots (e.g., pure family) (coded as "a" in Table 5), and the binary mixtures provide two distinctive competitive environments in a genetic sense: (1) binary mixtures of two full-sib families which share one common parent (i.e., the two families are half-sibs) (coded as "b" in Table 5) and (2) binary mixtures of two full-sib families which share no common parentage (i.e., unrelated) (coded as "c" in Table 5).

Table 5. Diallel competition design used to guide treatment formation in the field study.

	Family							
Family	1	2	3	4	5	6	7	8
1	a	b[x]	c	c				
2		a	b	c				
3			a	b				
4				a				
5					a	b	c	c
6						a	b	c
7							a	b
8								a

[x] Each of the plot types b and c will also be represented in two treatments: 75% family T:25% family R and 25% family T:75% family R.

In addition to the monoculture plots and binary mixture plots, there will be two other types of mixture plots. The first will involve mixing equal numbers of seedlings of each of the eight full-sib families at random in a single plot, which is a multiple-tree non-contiguous mixture (termed a "blend" for the purposes of this study plan). The other plot type is a row plot of each of the eight full-sib families within a single plot (termed a "row mix" for this study plan).

Eight full-sib families will be tested with the following plots:

8	monoculture plots
24	binary mixtures (12 mixtures x 2 ratios)
2	blends (1 blend plot in each of 2 sets)
2	row mix (1 row plot in each of 2 sets)
36	test plots per replication

Due to the large number of treatments, the plots will be divided in half to form two sets of treatments. Each set will contain one of the four-family half diallels. Hence a set will contain 18 plots. For statistical accuracy each set/rep will contain one blend plot and one row mix plot. Two test plantations will be established, each with four replications.

Genetic Background of Varieties in Competition Studies

The potential value and inferences from the proposed studies hinges on the pedigrees and genetic constitution of the varieties included in them. The varieties could have the same or differing levels of heterozygosity or inbreeding level or could constitute different effective population sizes. The coancestry among the varieties could vary. The goal is to develop hypotheses about the relationships among genetic diversity, deployment, and stability of growth and yield.

Important Functions Within Growth and Yield Modeling

There are at least five major functions involved in growth and yield modeling which have the potential of being affected by genetic variation. Researchers should investigate each of these in their experiments. These functions include: height over age (site index) function (Buford and Burkhart, 1987; Knowe and Foster, 1989), diameter distribution function (Knowe et al., in review), survival function, stem taper function (Schmidtling and Clark 1989), and yield-density (-3/2 power law) function (Nance et al., 1987; Schmidtling, 1988; Buford, 1989). Studies which investigate intergenotypic competition are quite large, so few varieties can be tested in each study. This strongly limits the number of varieties that can be tested, hence, the necessity for building reliable models which can be used to simulate test results of previously untested varieties.

CONCLUSIONS

With improved varieties available, forest managers can now design their forests both at the stand and at the landscape level. The relationship between genetic diversity and forest health is a major issue with scientists, managers, and the general public. Fortunately, the level of genetic diversity in new forest stands can be manipulated to minimize risk of stand failure and also adverse public opinion. Unfortunately, most of the information needed to guide this deployment of forest tree varieties is lacking, hence, experiments need to be conducted to assess the basic relationships between intergenotypic competition, genetic diversity, and stability of stand growth and yield. Since the needed studies are large and long term by nature, they must be established now to yield needed information as soon as possible.

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EVALUATING THE ECONOMICS OF ALTERNATIVE BREEDING AND DEPLOYMENT STRATEGIES FOR NORTHEASTERN CONIFERS

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ABSTRACT

Conventional seed orchard programs are compared with non-conventional improvement strategies that incorporate cloning. A financial analysis is described that extends standard discounting techniques to include allowable-cut effects and changes in stumpage value resulting from genetic improvement. The effects of these factors are illustrated by a series of graphs showing the present value of expected gains, compared with the extra cost of nursery stock resulting from seedling- and clone-based programs.

INTRODUCTION

Several conifer species are used for reforestation throughout the Maritimes where forest nurseries ship about 70-80 million trees annually (Lanteigne et al., 1992a, 1992b). Over the past 15 years, cooperative tree improvement programs in this region have concentrated their efforts on producing genetically improved material for all important reforestation species. To date, breeding work and deployment of improved stock has relied totally on planting materials grown from seed. However, refinement of rooted-cutting procedures and recent advances in micropropagation through cell culture have stimulated considerable interest in the potential of clonal propagation to enhance the returns from breeding and reforestation programs. The question that now faces breeders and reforestation planners alike is: "Does clonal propagation offer potential gains and management benefits which might justify the higher cost anticipated for planting stock production?". This paper reports the results of an economic analysis conducted as part of a larger study, initiated under the Canada/New Brunswick COOPERATION Agreement for Forestry Development, to investigate factors that should be considered in the evaluation of clonal propagation as a breeding tool and deployment method for reforestation (Mullin 1992).

OVERVIEW OF TREE BREEDING AND REFORESTATION OPTIONS

Conventional approaches to tree improvement

Conventional breeding and reforestation programs are designed to ship planting stock grown from seed. Superior phenotypes are selected to form a breeding population which is managed to produce genetic improvement through recurrent selection. Parents in the breeding population are inter-mated and their progeny established in test plantations. The plantations provide information to assess the genetic value of each parent and the best progeny are selected to form the next generation of the breeding population. In each generation, the best parents are established in wind-pollinated seed orchards and the seed produced is used for production of operational planting stock.

In the "ideal" seed orchard, no contamination will occur from pollen sources outside the orchard, all of the orchard parents will produce equal numbers of gametes, will mate randomly with all other parents, and will refrain from self fertilization. Of course, all orchards will fall short of this ideal, and in some cases, orchards will only produce a fraction of the genetic improvement possible from the selected orchard parents. Although careful location and management can mitigate contamination and non-panmictic mating in wind-pollinated seed orchards (Di-Giovanni and Kevan 1991), the only way to completely eliminate the potential negative effects is to fully control the source of pollen. This is commonly done when performing controlled pollination for the purposes of breeding, but is more difficult to accomplish on a large scale for operational seed production.

Non-conventional approaches using clones

The term *clonal forestry* is often applied rather loosely to refer to any use of cloning technologies in breeding and reforestation operations. There are, however, three distinct ways in which clones may be utilized:

1. *clonal replication* of genotypes in test plantations may be used to enhance testing efficiency;
2. *vegetative multiplication* of planting stock may be used to amplify the number of plantable trees from a limited quantity of seed; and finally,
3. *clonal selection* may be combined with vegetative multiplication to deploy operational planting stock originating from tested individual clones or clonal mixtures.

In this paper, the term *clonal deployment* refers to any use of clones in breeding or reforestation, whereas *clonal forestry* is used in the narrower sense where selection at the clonal level is carried out. It is important to realize that while clonal forestry is often promoted as a means to maximize genetic gain, it is not itself a breeding method (Burdon 1989). Continued genetic gain over successive generations still requires the inter-mating of parents in a base population, followed by recurrent selection to produce progressively higher numbers of alleles with favourable additive effects.

Although clonal selection may result in gain from the entire range of additive and nonadditive effects expressed in a population of genotypes, vegetative multiplication can only increase the extent to which gains from polycross or full-sib family selection are realized. If sufficient numbers of seed from selected families are available to supply the entire reforestation requirement, vegetative multiplication will not contribute to additional genetic gain. However, even in cases where sufficient numbers of seeds can be produced, differences in performance between vegetative propagules and seedlings may offer other opportunities for yield or quality improvement and reductions in silvicultural costs (Arnold 1990; Arnold and Gleed 1985; Gemmel et al., 1991; Klomp 1988).

Clones may also be used to replicate test material in the field and thus enhance the effectiveness of ranking based on clonal means, rather than individual values (Libby 1964; Shaw and Hood 1985). When family relationships exist among the clones in the test, it may also be possible to partition nonadditive sources of genetic variance which would otherwise be confounded with the error variance (Mullin and Park 1992). Clonal replication may be advantageous, even if operational planting stock is to be grown from seed. In this case, it is not necessary for the growth of the test propagules to resemble that of seedlings, provided that rankings between clones and seedlings are highly correlated.

OVERVIEW OF STRATEGY DECISION FACTORS

There are many benefits one might hope to achieve using clones beyond what is usually described as increased genetic gain; these have been discussed in detail by others, e.g., Carson (1986), Libby (1990), and Libby and Rauter (1984). Although the emphasis in the present study is on the economic viability of alternative methods using clones, economic returns and cost-benefit ratios are by no means the only strategic decision factors. As stated by Burdon (1989), improvement strategies which utilize clones must also satisfy criteria of technical feasibility and biological safety.

Technical feasibility

Obviously, any consideration of opportunities for integration of cloning into breeding and reforestation strategies is largely academic unless clonal propagation is technically possible and readily accomplished in an operational setting. To date, the only cloning system which has been used on an operational scale for northeastern species is based on the rooting of cuttings from very young seedlings, producing *stecklings*. Maturation of donor plants has long been recognized as a major hindrance to vegetative propagation (Bonga 1987; Greenwood 1987). As donor plants age, cuttings become more difficult to root and often display poor form and a decline in growth rate. However, cuttings from very young donor plants of spruce and larch species are relatively easy to root and, for the most part, appear indistinguishable from seedlings once planted in the field. Other species such as jack pine have proven to be much more difficult and attempts to produce steckling stock have not had much success. Large quantities (i.e., 1 million or more trees per year) of vigorous steckling stock have been produced vegetatively from young donor plants of spruce species in operational settings. Although technically feasible, the labour-intensive nature of striking and setting operations creates logistical problems when annual production levels rise to more than a few million, requiring a high quality nursery labour pool, careful production scheduling, and improvements in mechanized handling. New technology for vegetative propagation could have a profound impact on the feasibility of producing large quantities of clonal planting stock. Tissue culture methods for micropropagation of forest trees have attracted much attention (Thorpe et al., 1991). Most recently, the production of plantlets by means of asexual embryogenesis has renewed interest in tissue culture propagation, particularly in spruce (Adams et al., 1993).

The integration of cloning into breeding and reforestation programs poses other logistical problems. The pursuit of additional gain through clonal selection will require additional testing of individual clones to capture nonadditive genetic variance. Such gains represent a genetic "dead end" which cannot be carried forward to the next generation, and effort expended in their pursuit must come at the expense of efforts to advance additive gain through recurrent selection. A trade-off must be recognized between clonal testing and advancement of the breeding population (Burdon 1986, 1989).

Additional logistical problems are introduced when operational deployment of clones is required to achieve a given level of diversity or a particular spatial arrangement of clones. There may be a requirement to deploy clones in particular mixtures or in mosaics of individual clonal blocks. Maintaining the identity of many clones in the nursery and producing particular clonal mixtures will pose a challenge to nursery managers and will certainly add to the cost of production.

Biological safety

For many people, the phrase "clonal forestry" conjures up disturbing images of widespread plantations composed of a single, super-yielding clone. No one would argue that such a scenario would be anything short of foolish. All of our important reforestation species are out-breeders and have evolved to maintain genetically diverse populations in nature. Genetic diversity in a breeding population will minimize the risk that adverse sampling during sexual recombination will render progeny susceptible to a particular pest or environmental stress factor. Diversity is also known to inhibit the spread of a pathogen through a population, even if some genotypes are highly susceptible, but interspersed among other resistant genotypes (Burdon 1989; Carson and Carson 1991). Nevertheless, there remains a great temptation to select only the very best genetic material for operational deployment; and this is true regardless of the deployment method used.

If deployment of only one genotype is hazardous, how do we know when a planting mixture has a sufficient level of diversity to be regarded as "safe"? The question is legitimate but difficult to answer since diversity, particularly at the allelic level, is difficult to observe directly. The problem is not unique to clonal mixtures; seed-orchard populations must also represent an adequate level of genetic diversity to ensure that seedling progeny will not be at risk. Several authors have considered the problem of ensuring adequate diversity while maximizing genetic gain from mixtures (e.g. Hühn 1986; Huehn 1987; Kleinschmit 1979; Libby 1982; Lindgren et al., 1989; Roberds et al., 1990). The number of clones required for planting mixtures has even been subject to legislation in Sweden and Germany (Muhs 1988).

Benefits and costs

Once the prerequisites of technical feasibility and biological safety are satisfied, the decision to use clones comes down to a weighing of expected advantages and costs. The objectives of forest management will dictate how the assessment is performed and how the results are compared with other silvicultural options. Economic comparisons of benefits and costs often concentrate on financial aspects, and commonly employ investment analysis techniques such as present value (PV) analysis and internal rate of return. These analyses have the appeal of quantifiable comparisons and the appearance of precision. However, investment analysis techniques typically employ data such as discount rates and future stumpage values which are characterized by a high degree of uncertainty. Moreover, they focus the attention of the decision maker to returns at the stand level, rather than the impact of management to output from the forest as a *whole* (Reed and Baskerville 1991). Nevertheless, PV analysis is well accepted as a means to evaluate individual components of the silvicultural investment portfolio.

Most economic analyses have been cautiously optimistic about the financial viability of clonal forestry options, assuming that significant genetic improvement will be achieved (Hasnain et al., 1986; Smith 1986; Timmis 1985; Wilhelmsson 1991). Invariably, economic assessments of particular situations conclude that the viability of clonal strategies is highly sensitive to the cost of propagation (Gill 1983; Lundkvist and Gullberg 1981; McKenney et al., 1988; Smith 1991; Wilhelmsson 1991). Unfortunately, many of these studies used simple discounting and valued the extra volume increment at regular stumpage rates; an approach which fails to recognize the overall effect on harvest flows, cost reductions resulting from closer wood supply, and gains from quality and value enhancement (Reed 1989).

EVALUATING COSTS OF ALTERNATIVE STRATEGIES

This study considered four areas where alternative seedling and clonal strategies might differ in cost: *(i)* breeding and testing; *(ii)* production of improved genetic material; *(iii)* nursery propagation of planting stock; and *(iv)* plantation establishment and management. Cost estimates for each of these components are discussed and form the basic input to the financial analysis.

Breeding and testing

The pursuit of genetic gain through recurrent selection in advanced generations is common to all breeding strategies. This will normally involve polycross testing of selected parents to verify genetic quality, followed by controlled crossing among selected parents for establishment of selection plantations. The method of deployment of planting stock has no bearing on the cost of these operations.

If clonal selection is to be used to pursue additional nonadditive gains within a breeding cycle, an additional screening operation is required. Capturing these additional effects contributes nothing to the advancement of gain in the next cycle, and this leads to problems when integrating clonal forestry with management of breeding populations (Burdon 1986, 1989, 1991). It is clear that screening of clonal material will either raise the total cost of testing, or will come at the expense of efficient advancement of the breeding population. Since resources available to breeding operations will normally be constrained, one would expect that clonal testing will likely compete for the same resources used for advancing the breeding population. Obviously, the additional returns from clonal selection must not only compensate for higher costs of deployment, but must also compensate for lost efficiency in testing for advancement of the breeding population.

When spread over the operational production of planting stock, the contribution of breeding to the cost of reforestation is generally considered to be small. In this study, breeding activities were assumed to be relatively intensive and contribute $5 per thousand to the cost of planting stock.

Production of improved genetic material

The most significant differences in costs among the strategies considered in this study are related to management of the production population and nursery propagation of the planting stock. While production costs for conventional orchard production are available from several sources, costs for alternative control-pollinated orchards and clonal strategies are more difficult to estimate due to lack of experience and the early stage of development of some of the technologies. In the following sections, the current status of cost estimates is reviewed and, where possible, related to the cost of conventional seed-orchard and nursery operations.

Seed orchards - conventional

Costs of production in wind-pollinated seed orchards can, of course, vary widely depending on species and orchard productivity. Considered separately from the costs of breeding and seed collection, seed-orchard management entails a wide range of operational costs, including land acquisition, site preparation, irrigation, fencing, propagation and establishment of orchard stock, protection, soil amendments, and crop monitoring. While these costs are spread over the average production of the orchard throughout its lifespan, the contribution of these costs to plantation establishment is also strongly influenced by the efficiency of seed usage by the production nursery (South 1987).

Calculation methods used in the literature vary, but estimates of the contribution of seed-orchard management to planting stock production costs are in the neighbourhood of $8-15 per thousand (Arnold 1990; McKenney et al., 1988; Talbert et al., 1985). Allowing for a conservative evaluation of additional costs which might experienced in northeastern orchards, a working cost estimate in the range of $10-20 might be appropriate. The calculations in this report assumed that seed-orchard costs contribute $15 per thousand to the cost of planting stock.

Seed orchards - alternative designs

In recent years, several modifications to the classical Syrach Larsen orchard have been proposed and prototypes established. All are characterized by an attempt to achieve higher seed yields with more favourable pollination conditions. The first proposal by Sweet and Krugman (Sweet and Krugman 1977) described separate male and female orchards with controlled pollination. The concept of "meadow" orchards, with trees established at ever-increasing densities, was further developed in New Zealand where the first commercial-scale installation was established in 1987 (Arnold 1990). Most recently, the New Zealanders have developed aqueous pollination systems which achieve parental control without the use of isolation bags (Sweet et al., 1990).

Given the changing state of control-pollinated orchard technology, cost comparisons quickly become outdated and must be viewed with caution. In New Zealand, control-pollinated seed was commercially available in 1989 for about 10x the cost of conventional wind-pollinated orchard seed. Costs are expected to drop substantially, with controlled-pollinated seed from older-style orchards and "meadow" orchards stabilizing at about 5.2x and 3.6x the cost of conventional orchard seed, respectively. When controlled pollination is carried out without isolation, the price is expected to drop to about double the cost of conventional orchard seed (Arnold 1990).

Proponents of indoor, containerized seed orchards feel that similar costs can be achieved by producing seed on intensively managed ramets growing under controlled conditions, and that production levels can be increased rapidly to meet the entire reforestation requirement at reasonable cost (Ross et al., 1986). Certainly, a controlled environment has a clear advantage when it comes to using treatments such as heat, moisture stress, and photoperiod control as a means of stimulating seed production, and would probably enhance the efficiency of gibberellin treatments and aqueous pollen application.

One way or another, it appears that improved orchard technology will make it possible to produce seed of known parentage, for about double the cost of managing conventional wind-pollinated orchards. The main areas of uncertainty appear to be the scaling-up problems and the time to reach full production.

Management of clonal donor material

The strategies addressed in this study which rely on clonal deployment of planting stock, do not require the management of a large seed-orchard production population. Even if seeds from controlled crosses are used as the starting material, multiplication rates of 50-100x can be achieved by the most crude cloning system, requiring a very small initial amount of seed material. There is however, a requirement for continuing management of clonal donor material. In the case of rooted cuttings, this would be a stool bed where hedged plants are managed as a source of cuttings. For micropropagation and embryogenic systems, tissue must be maintained under suitable culture conditions, or placed in cryo-storage.

Many authors consider these costs to be insignificant, or include them as part of the cost of producing clonal planting stock. McKenney et al., (1988) recognized that repeat crossing and maintenance of donor material could be relatively expensive for a small program; up to $10 per thousand plantable trees. However, for planting programs larger than 500 ha per year, these costs should remain constant at about $5 per thousand.

Nursery propagation of planting stock

Most of the planting stock produced in eastern Canada is grown as containerized seedlings; very little is produced as bareroot stock, due to higher production and planting costs. Procedures vary among nurseries and species, but most container-grown trees are started from seed in heated greenhouses and spend at least the first portion of their nursery life in a controlled environment. Accounting procedures vary among nurseries, particularly in terms of how capital costs are assigned to stock output, but general production costs are reported in the range of $135 to $170 per thousand, with $150 as a median figure.

If production costs for seedling stock are poorly documented, then costs for clonally produced planting stock are almost nonexistent and tremendously varied, as illustrated in Table 1. Relatively few estimates are based on actual operational production experience. Black spruce steckling programs in Ontario and Quebec have indicated that their clonal stock produced from juvenile rooted cuttings costs in the range of 1.8 to 4.5x the cost of normal seedling stock. However, both of these programs are limited to 1 or 2 million stecklings per year (Rogers 1990; P. Miville, pers. comm.) and are using expensive facilities which presumably have a higher capacity.

There is a trend towards lower costs associated with programs which have gained practical experience and produce larger quantities of steckling stock. In Nova Scotia, juvenile cuttings were used in the mid-1980s to amplify limited quantities of black spruce seed for planting on the highlands of Cape Breton. Annual production of black spruce stecklings reached 2.3 million at an estimated cost of 1.5 to 3x the cost of container seedlings with costs continuing to decrease as the program was phased out (B. White and K. Thomas, pers. comm.). In Sweden, Hilleshög has considerable experience in the production of steckling stock for Norway spruce (*Picea abies* [L.] Karst.) on a commercial scale. In the mid-1980s, Hilleshög reported that steckling costs were about double those of comparable seedling stock (Bentzer 1986). Since then, annual production has increased to about 3 million and prices for stecklings now run 60 to 70% higher than seedling stock (B. Bentzer, pers. comm.). Other nurseries in Germany and Ontario with considerable experience in steckling production have even reported cost estimates for rooted cuttings as low as 20% higher than regular seedlings (Kleinschmit and Schmidt 1977; McKenney et al., 1988).

Production systems are constantly changing and operational programs are actively investigating ways to reduce costs. On the other hand, steckling stock is highly labour intensive and production in the tens of millions could very well introduce new problems as programs attempt to scale up. Nevertheless, it seems that an operational program of sufficient size with experienced management could probably achieve production costs for stecklings which are 1.5x the cost of container-grown seedlings, or roughly $225 per thousand.

Costs of production for micropropagation and embryogenic systems are even less certain. Proponents of these emerging technologies predict great reductions in cost which will make these techniques increasingly attractive, but there are no hard data to support this speculation. Estimates in the literature for micropropagation costs run the range from double to 10x the cost of seedling stock (Table 1). Despite these uncertain costs, some forest companies have made a significant commitment to micropropagation on an operational scale; e.g., Tasman Forestry Ltd. in New Zealand opened a tissue culture laboratory in 1988 to produce 2.5 million plantlets per year (Gleed 1991).

Table 1. Comparison of vegetative propagation costs.

Species	Location	Cost per 1 000 plants		Source
		Stecklings	Micropropagation	
Populus tremuloides	USA	US$320-390	US$780-880	Hall et al., 1990
Eucalyptus spp.	France		3 000F (2x cost of seedlings)	Franclet and Boulay 1983
	USA		US$140	Mascarenhas et al., 1988
Pinus radiata	New Zealand	NZ$223 (5.6x price of seedlings)		Arnold and Gleed 1985
			7 to 10x cost of seedlings	Smith 1986
		NZ$350-450 (2.7-3.5x price of seedlings)	NZ$640-800 (4.9-6.2x price of seedlings)	Smith 1991 (1989 prices)
			NZ$320-712	Aitken-Christie and Davies 1991
		NZ$87 (1.7x cost of seedlings)	NZ$450	Menzies 1985
Picea abies	Russia	25% less than bareroot transplants		Rutkovskii and Kharina 1987
	Germany	1.2 to 1.5x cost of transplants		Kleinschmit and Schmidt 1977
	Sweden	2x cost of seedlings		Bentzer 1986
		1.6 to 1.7x cost of seedlings		B. Bentzer, pers. comm. 1991
Picea sitchensis	U.K.	UK£13-50 (1.8 to 3.6x cost of seedlings)		Gill 1983
Various conifers	U.K.	UK£57-120	UK£80-300	Dixon 1987
Juniperus spp.	USA	US$260		Badenhop 1984
		US$131		Bluhm and Burt 1983
Picea mariana	Ontario	$225-325 (1.3-1.9x cost of seedlings)		McKenney et al., 1988
		$725 (4.5x cost of seedlings)		P. Nitschke, pers. comm. 1993 (private-grower contract)
	Quebec	$550 (1.8x cost of seedlings)		P. Miville, pers. comm. 1993
	Nova Scotia	1.5 to 3x cost of seedlings		B. White, pers. comm. 1989

Cost estimates for embryogenic systems in conifers are virtually nonexistent, as protocol development for somatic embryogenesis is still at an early stage. Certainly, mechanization is seen as having major potential to reduce the high cost of labour currently associated with tissue culture and embryogenic techniques (Aitken-Christie 1991; Aitken-Christie and Davies 1991; Harrell and Simonton 1986). Liquid culture systems hold the greatest promise for automated handling of somatic embryos (Levin et al., 1988), although our important conifers have not responded well to culture in liquid media (J. Bonga, pers. comm.).

It is early yet to speculate on the real potential for cost reductions resulting from tissue and embryogenic culture systems. The progress made in the past few years with respect to protocol development has been nothing short of phenomenal, and there seems little doubt that, even if never used for the production of operational planting stock, these techniques will prove invaluable for the long-term maintenance of clones and insertion of new genes, and may assist in the achievement of real rejuvenation of mature material. If used for operational deployment of clonal stock, these new systems will compete on a cost basis with rooted cuttings, and must yet demonstrate favourable, true-to-type performance in the field.

Plantation establishment and management

One might ignore the possibility of cost differences between seedling and clones after the planting stock have left the nursery. To do so would require the assumption that management requirements for clonal plantations will be similar to those established with seedlings; an assumption which has yet to be well tested in our important species. However, at the very least, we might expect that faster growth rates will lead to differences in management costs during the life of the plantation, perhaps reducing the need for competition control and pest protection.

Experience with radiata pine in New Zealand has indicated that other differences may also have an important effect on the cost of plantation management (Arnold and Gleed 1985). It has been determined in this case that initial planting density may be reduced from 1200-1500 stems per ha, to 750, since the cuttings do not require early competition to achieve acceptable form, and this reduces the loss of trees during precommercial thinning operations. Pruning costs were also reduced. Thus, even including the cost of steckling stock, the overall cost of management was reduced and a case could be made supporting clonal deployment, without *any* expectation of genetic improvement for growth rate.

There are no data available to indicate what differences, if any, may exist in the costs of plantation management between clones and seedlings of northeastern conifers. Although the safest assumption at this point would probably be that clonal plantations will be equally expensive to manage, there remains the possibility that cost reductions will become apparent once sufficient experience has been gained with clonal stock in the field.

Net cost differences between seedling and clonal strategies

In the financial analysis carried out in this study, the emphasis was on evaluating the PV of benefits, primarily higher per-hectare stand value. Since many of the strategy costs contribute directly to higher cost of planting stock, the benefits are also expressed per 1000 planted trees. For purposes of comparing alternative reforestation strategies, only the *differences* in benefits and cost are really important. This simplifies the task somewhat and focuses the decision on identifying a justifiable threshold price for improvement of nursery stock.

In a management environment where costs are changing continually, the decision maker must constantly re-evaluate the impact of cost on forest management investments. Point estimates of cost components of nursery stock production, used for illustration in this report, are summarized in Table 2. In this summary, the difference between the cost of improved seedlings resulting from a conventional wind-pollinated orchard strategy and unimproved stock is estimated to be $20 per thousand, whereas clonal stock produced from control-pollinated seed will cost $85 more per thousand than unimproved stock. The decision maker will compare these extra costs against the PV of benefits expected to accrue from the use of improved stock.

Table 2. Point estimates of cost components ($ per thousand) for production of unimproved and improved seedlings, and improved clonal planting stock.

	Stock type		
Cost component	Unimproved seedling stock	Improved seedling stock	Improved clonal stock
Breeding population	-	$5	$5
Production population			
Seed orchard operations	-	15	-
Donor material management	-	-	5
Nursery propagation			
Seedlings	$150	150	-
Stecklings	-	-	225
	==========	==========	==========
Total cost per '000	$150	$170	$235
Cost differences	<---- $20 ---->		<---- $65 ---->
	<---------- $85 ---------->		

OTHER ECONOMIC FACTORS

Although genetic gain and differential costs are important, financial comparisons of alternative improvement strategies requires consideration of several other market and non-market factors, including: *(i)* rate of discount; *(ii)* scheduling of harvest and regeneration activities; *(iii)* stumpage value; *(iv)* expectation of yield from plantations; and *(v)* the size of the planting program (Thomson 1989).

Discount rate

When financial analyses are used in decision making, no factor is more controversial than the choice of discount rate to apply to future costs and benefits. The whole concept that the future ability of the forest to provide benefits is somehow *less* valuable than present consumption is abhorrent to many and has been attacked by reputable forest management experts, e.g. Baskerville (1991). It could even be argued that discounting of forest management benefits is inconsistent with the philosophy of sustainable development, and therefore inappropriate for modern decision making in forest management, particularly for publicly owned forests. Nevertheless, analytical practices used for the bulk of decision making which drives our economy accept the principle of discounting.

Many economists have discussed the selection of an appropriate rate of discount for analysis of forestry investments. Most agree that a "real" rate of interest should be used, i.e., without inflation. The real discount rate will be affected by many market factors and the social objectives which influence management activity in the forest (see Fraser 1985; Harou 1983). A low "social" rate of discount may be applicable to silvicultural investments, especially when they occur on publicly owned land (Harou 1985). Very low rates may not be acceptable in the real world of corporate business, while high rates will be a strong disincentive against any investment which occurs over a time period as long as a forest rotation. Rates in the range of 3 to 5% have been suggested (see Ondro and Constantino 1990, for a discussion of Canadian and US rates.)

The effect of stumpage appreciation can be included by adjusting the real interest rate. This effective discount rate does not equal the real interest rate minus the rate of stumpage appreciation; rather, the correct adjustment, as given by Binkley (1980), is

$$i' = \frac{(1+i)}{(1+h)} - 1$$

where i' is the discount rate adjusted for stumpage appreciation; i is the real rate of interest; and h is the stumpage appreciation rate.

A study by Deloitte & Touche Management Consultants used a real interest rate of 4% to discount economic benefits of budworm control in eastern Canada (Deloitte & Touche 1991). They argued that timber values may appreciate at a higher rate than other goods and services, resulting in an estimated 1% appreciation in stumpage value. In an economic analysis of a tree improvement program for western larch (*Larix occidentalis* Nutt.), Fins et al., (1984) used real discount rates of 4 and 6%, and assumed that stumpage rates would appreciate at the rate of 2%. Although the choice of discount rate is most appropriately made by the decision maker, calculations presented in this paper used real interest rates of 4 and 6%, with and without a 1 or 2% appreciation in stumpage.

Scheduling of forest harvest and regeneration

Tree improvement activities are generally designed to meet the requirements of a given regeneration program. However, the dimensions of a regeneration program can be affected by both market and nonmarket factors (Thomson 1989). Changes in management objectives, market conditions and constraints to forest management will affect the timing of harvest and, thus, the timing of regeneration. Changes in the reforestation program may also come about as a result of losses to fire, disease or insects.

The conventional wind-pollinated orchard is designed from the outset to meet a given seed demand. Its value would be maximized if reforestation requirements were to coincide with the development of orchard productivity. More likely is that orchard output at any point in time will be somewhat below or exceed the actual requirements for stock production, and this will decrease the PV of the investment in orchard establishment. Compared to the conventional seed orchard, clonal production populations, and even other alternative orchard systems, are somewhat more flexible and can more readily accommodate changes in regeneration requirements.

Harvest scheduling also has an impact on the ability to realize early benefits from an improvement program. Planting improved material will result in an increase in the overall productivity of the forest estate, which, in turn, will raise the allowable cut, the so-called Allowable-Cut Effect (ACE). Provided there is sufficient standing timber to provide an even flow of material throughout the life of the plantation, the ACE will result in an increased allowable harvest equivalent to the increase in growth rate resulting from the investment. The ability of the forest to provide the required harvest flow throughout the investment period has a major impact on the ACE (Binkley 1980, 1984; Schweitzer et al., 1972). Unfavourable age-class distributions are common throughout the northeast, and these will have an impact on the ability of the forest to maintain the even flow of increased harvest volumes which would be necessary to realize the maximum benefit from the ACE. The PV of any stand establishment treatment, such as tree improvement, will be greatly affected by harvest scheduling and silvicultural investments in *existing* forest stands which will permit the ACE to be realized.

Stumpage value

It is obvious that the future value of stumpage will have a major effect on the benefits realized from investment in tree improvement. Market forces could cause stumpage to appreciate at a faster or slower rate than other goods and services. More surprising is the difficulty that the decision maker will experience when attempting to make a true determination on current stumpage. The value of stumpage to a woodlot owner selling to a harvesting contractor may be much lower than that determined by a government land-owner who places considerable value on the impact that forest production has on employment and other economic activity. A higher "shadow" price for stumpage may be appropriate when the investor values the investment as being socially attractive (Harou 1984).

Deloitte & Touche (1991) used a residual timber value approach to determine the current value of stumpage on the Restigouche-Tobique Crown license in New Brunswick. Starting with marketing board mill-delivered prices for pulpwood and sawlogs, deductions were made for harvesting, transportation, additional costs of management on Crown land, and an allowance for profit and risk. The residual value of the average pulpwood-sawlog mix was determined to be $\$15.52/m^3$. Although considerably higher than stumpage fees paid to the Crown or to private forest owners, this stumpage value is thought to better reflect the *true* value of the material. Even so, stumpage value of wood from genetically improved stands may actually be higher yet, due to value gains from factors such as increased piece size, lower harvesting costs, higher lumber recovery rate, shorter average hauling distance, release of forest land for other purposes, and reduction of risk (Reed and Baskerville 1991). The analyses presented in this paper used a stumpage value estimate of $\$15.50/m^3$, and provided for increases in value resulting from genetic improvement.

Expectation of yield from plantations

Gains from tree improvement, or other plantation management treatments, must be related to the yield from unimproved plantations. Unfortunately, information about site quality of future planting sites and their expected yields is very poor, although research is under way to rectify this problem. Returns from tree improvement will be maximized if selected stock is established only on the best planting sites. The challenge is to describe growth and yield from the mean site quality expected to receive improved stock. In this study, reference is made to growth and yield estimates from New Brunswick, courtesy of the NB Department of Natural Resources and Energy (NBDNRE).

In an earlier study of black spruce tree improvement in New Brunswick, Cornelius and Morgenstern (1986) considered an "intermediate" site which they described as reaching peak mean annual increment (MAI) at 46 years, with 296 m^3/ha and a mean height of 18.1 m. The New Brunswick plantation growth and yield model has been revised considerably since the Cornelius-Morgenstern study was published (V. Zelazny, pers. comm.), and now describes an "average" spruce site (SI_{50} = 15.2 m) as achieving maximum MAI at 60 years with a merchantable volume of 276 m^3/ha and mean height of 17.5 m. Even an "above average" site (SI_{50} = 17.2 m) does not achieve peak MAI until 51 years, with a merchantable volume of 278 m^3/ha and height of 17.4 m.

The current New Brunswick model was used in this study, with the assumption that improved stock will be deployed at a density of 2,500 trees per hectare on "above average" sites (SI_{50} = 17.2 m). Several approaches can be used to determine "optimal" rotation ages (see Newman 1988), but in the present study, only two rotation ages are considered: *(i)* minimum operable age, considered by the NBDNRE to be reached when average tree size exceeds 0.12 m^3; and *(ii)* the age when MAI is maximized. Wood-supply pressures may require that fibre from plantations enter the harvest stream as soon as the stands are operable. According to the NBDNRE model, minimum operable age on "above average" sites is 40 years, yielding a merchantable harvest volume of 211 m^3/ha and MAI of 5.28 m^3/ha. If wood-supply planning will permit, production will be maximized if harvest is delayed until MAI reaches its peak of 5.45 m^3/ha at 51 years, when merchantable volume will be 278 m^3/ha.

Size of the planting program

Gains from improvement efforts do not accrue until improved trees are planted. Obviously, a large reduction in the size of the planting program would have a negative impact on the returns from investment in any tree improvement strategy. This factor is not considered in the financial analyses reported in this study; comparisons are made among strategies which fully utilize the material available from the production population. However, it should be recognized that strategies which require a large capital investment in seed-orchard development or construction of expensive breeding-hall facilities will be particularly vulnerable to changes in planting requirements. Changes in output can be achieved more efficiently when cloning is used to amplify control-pollinated seed or deploy tested clones. Although this flexibility may be a major advantage of clonal strategies, the requirement, and therefore the value, of flexibility may be difficult to assess.

CALCULATING THE PRESENT VALUE OF IMPROVED PLANTING STOCK

The financial analysis procedure reported here extends standard present value techniques to incorporate ACE and changes in stumpage value resulting from genetic improvement. The calculations were performed within a spreadsheet which reports the PV of improved seedlings growing on a specific site, for a given present stumpage value, with and without ACE, over a range of effective discount rates incorporating the effects of stumpage appreciation with the real rate of interest.

Regardless of the vegetative propagation method, stecklings and emblings will likely remain more expensive than planting stock propagated from seed. Assuming that the productivity and quality of this stock is higher, managers should be willing to pay a premium price for such stock. Calculations were made to determine the maximum justifiable increase in the cost of improved planting stock, depending on the expected gain in value at time of harvest. The gain in value is considered to have two components: *(i)* gain from extra volume increment, and *(ii)* gain in stumpage value due to genetic improvement, as discussed earlier.

The PV of these gains may be calculated as the sum of the two components, using standard discount techniques:

$$V_0 = \left[\frac{n \cdot I \cdot G_I \cdot S}{(1+i')^n}\right] + \left[\frac{n \cdot I \cdot (1+G_I) \cdot G_S \cdot S}{(1+i')^n}\right]$$

where:

n	is the rotation age;
I	is the mean annual increment at age n;
G_I	is the gain in volume increment, as a proportion;
G_S	is the gain in stumpage value of the stand (as a proportion);
S	is the stumpage value; and
i'	is the effective discount rate.

In this equation, the first term describes the PV of gains in volume production, while the second term accounts for the increase in stumpage value due to improvement. The assumption is made that no benefit will accrue from the investment until the stand is harvested at year *n*. However, as discussed earlier, the increase in plantation yield may have an impact on the allowable cut for the entire forest, throughout the rotation of planted trees. Provided that an even flow of increased harvest material can be maintained throughout the rotation, this ACE will accrue as an annuity. While the ACE will have an impact on the volume component of the PV equation, the stumpage value component remains unchanged:

$$V_0 = \left[I \cdot G_I \cdot S \frac{(1+i')^n - 1}{i'(1+i')^n} \right] + \left[\frac{n \cdot I \cdot (1+G_I) \cdot G_S \cdot S}{(1+i')^n} \right]$$

where the terms are as defined previously.

Results of present value analyses

These equations were used to explore the effect of various factors on the PV of planted stands under various management and economic scenarios. Figure 1 shows the outcome of PV analysis for unimproved plantations on "above-average" spruce sites. The calculations were made, both with and without ACE at the minimum age of operability, 40 years, and at age 51 when MAI reaches its peak. The value of the ACE is immediately apparent, as is the dramatic impact of effective interest rate. The total ACE realized by plantations will be somewhat less if untreated sites achieve merchantable, albeit lower, increment via natural regeneration. Without an ACE, the value is generally higher if the plantation is harvested at the minimum operable age. However, when the ACE is considered, PV continues to increase for harvest ages beyond that of peak MAI. The figure also relates the cost of unimproved seedling stock ($150 per thousand) to the PV of the plantation, and demonstrates that, without the ACE, the value of the plantation is not sufficient to cover even the cost of planting stock, let alone other costs of establishment and management, at interest rates higher than 5%. Obviously, PV analysis without ACE does not give strong support for investment in plantation forestry under this scenario. The fact that planting programs exist at today's moderately high level is an indication that decision makers value impact of silvicultural investment on harvest flows from the whole forest, and/or accept a low social discount rate and high shadow price for stumpage. These same values should also be applied to investments which increase the return from plantations.

In Figures 2 and 3, the PV of volume gains from different levels of genetic improvement are shown at harvest age 40 and 51, respectively. The figures demonstrate that the assumption of rotation age does not have a critical effect on the economic viability of investment in genetic improvement. This observation supports a similar finding by McKenney et al., (1988) who determined that optimal rotation age is *not* significantly affected by tree improvement. The analysis also confirms that returns from wind-pollinated seed orchards can easily justify the extra cost to produce improved stock from wind-pollinated seed orchards. Even without the ACE, the conventional seed-orchard program is justified at low levels of genetic gain; 5% gain when an effective interest rate of 3% is used, and about 10% gain when effective interest rate is 5%. If the ACE is considered, then conventional orchards are viable at conservatively high interest rates, even if genetic gain is less than 5%. To be profitable, clonal deployment of improved stock requires much higher levels of genetic gain to be achieved, particularly when the ACE is not available. However, if harvest levels can be increased during the rotation, clonal deployment is viable with genetic gains of less than 10% when the effective real interest rate is less than 3.5%, and at 15% genetic gain when the interest rate is 6%. Figure 3 also illustrates the sensitivity of the net PV of clonal deployment to the level of genetic gain achieved and the actual cost of propagation. If clonal stock production costs are 50% higher than estimated in this study, genetic gain must be 7 to 8 percentage points higher to achieve the same cost-benefit ratio, even if an ACE can be realized.

One might hope to maximize returns from genetic improvement by concentrating the investment on the best sites. Figure 4 illustrates the range of returns that would be expected on spruce sites ranging from "average" to "best" quality. Upgrading the planting site by one class, equivalent to a change in SI_{50} of 2 metres, would increase the PV of the plantation by roughly $35 per hectare, or $14 per thousand planted trees. This will not only justify a higher cost for nursery propagation, but will also produce additional fibre which may be badly needed to fill a hole in the wood supply.

In addition to the extra volume that genetic improvement would hope to achieve, several factors could also contribute to a higher stumpage value for wood from improved plantations. The concentration of fibre closer to the mill, lower harvesting costs and increased piece size will certainly add to value of harvested material. Depending on the mill process and market, value may also increase due to improvement of fibre quality. Figure 5 illustrates the effect of varying degrees of quality improvement on the PV of investment which results in a 20% gain in volume. In this example, a 10% gain in stumpage value is approximately equal to a 1% reduction in the effective rate. If the effective interest rate were considered to be 4%, this same gain in stumpage value would increase the value of planting stock by $42 or $28 per thousand, with and without the ACE, respectively.

In Figure 6, a sample comparison is given for the PV of gains from wind-pollinated orchards, control-pollinated orchards and clonal forestry. If these gain assumptions are considered reasonable, the analysis demonstrates that all of the strategies are economically viable if an ACE is available. Even when no ACE is available, the clonal forestry option is viable at effective rates of real interest below 3.8%. If we assume that, compared to wind-pollinated orchards, control-pollinated orchard seed will increase the cost of improved stock by $15 per thousand, this option is viable without an ACE at interest rates below about 4.75%. The impact of the extra cost of producing clonal planting stock is readily apparent in this figure. Although the 50% premium used here to estimate the cost of clonal stock is probably reasonable for moderate quantities of steckling material, it may not be possible to produce sufficient quantities to supply the majority of reforestation stock as clones, unless automated micropropagation techniques are employed, for which the cost premium is much less certain. If clonal propagation costs are double those used in this analysis, viability of clonal forestry would likely require an ACE, and assurance that gains can actually be achieved would become even more critical.

Although useful for comparing approaches for enhancing plantation performance, PV analysis, by itself, will not identify the optimal place of genetic improvement in a strategic silvicultural plan that seeks to achieve a stated objective for the sustainable flow of benefits from the whole forest. In developing a strategic silviculture plan, one would first identify lowest-cost, short-term treatments that have a high probability of contributing to the overall harvest requirement, while satisfying constraints imposed by non-fibre objectives. If the wood supply objective has not been met once all available areas have been identified for treatment, additional higher-cost, longer-term and higher-risk treatments must be added to the silviculture strategy until the objective is satisfied. The cost of individual treatments added to the mix may be very high, but what is really important is the overall cost of the silvicultural strategy designed to meet the objectives of the whole forest. This is the *real* cost of doing business in forestry. Once the optimal mix of treatments has been identified, additional investment opportunities can then be assessed in terms of their potential to provide attractive returns and opportunity for additional economic activity. Although qualitative statements can be made regarding benefits such as risk reduction, release of forest land for other uses, and maintenance of biodiversity, quantitative techniques which bring these factors into decision making are not well developed.

The financial analysis described here goes beyond the standard discounted cost-benefit techniques commonly applied at the stand level and incorporates the impact of increased fibre value and the ACE derived from genetic improvement. This modification may be quite appropriate for some landowners, but those with large capital investment in processing facilities and managers of publicly owned forests must also consider the maintenance of harvest flows to sustain economic activity. For these decision makers, a strategic planning approach is most appropriate; one that seeks to minimize the cost of achieving a sustainable harvest objective, rather than simply analysing investment opportunities. Strategic planning must analyze the distribution of inventory and treatment opportunities, to determine the least-cost mix of silviculture expenditures to satisfy objectives for the sustained flow of benefits from the whole forest, while remaining faithful to other constraints imposed by social values. This kind of planning will help identify where high-cost, long-term treatments such as clonal forestry are warranted to deal with shortfalls in harvest objectives which cannot be overcome by low-cost treatments alone.

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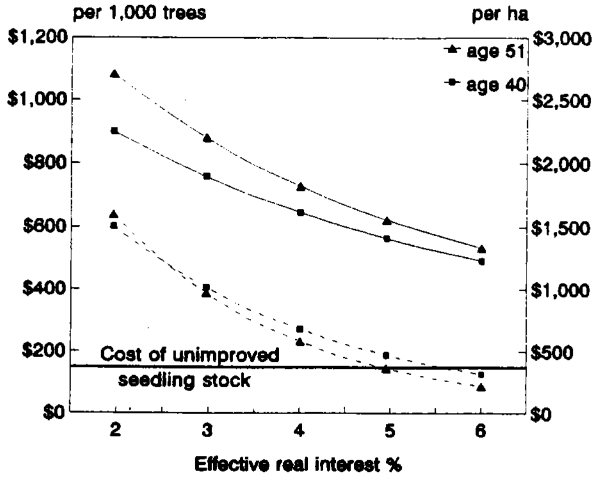

Figure 1. Present value of a spruce plantation established with unimproved seedlings on an "above-average" site, harvested at age 40 or age 51, relative to the cost of planting stock production. Calculations assumed stumpage at \$15.50 per m^3. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

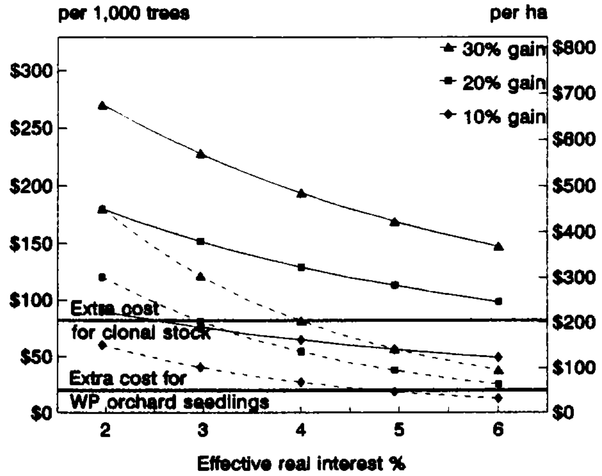

Figure 2. Present value of volume gains on an "above-average" spruce site, harvested at age 40 (minmum operable age), relative to the extra cost of producing improved seedling and clonal planting stock. Calculations assumed stumpage at \$15.50 per m^3, with no increase in value due to genetic improvement. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

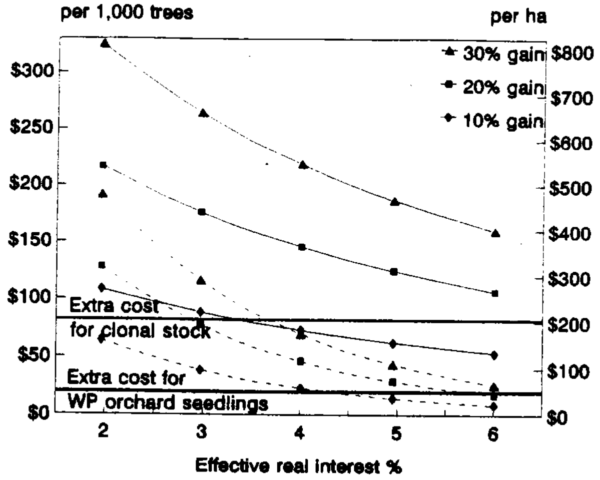

Figure 3. Present value of volume gains on an "above-average" spruce site, harvested at age 51 (age of peak MAI), relative to the extra cost of producing improved seedling and clonal planting stock. Calculations assumed stumpage at $15.50 per m^3, with no increase in value due to genetic improvement. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

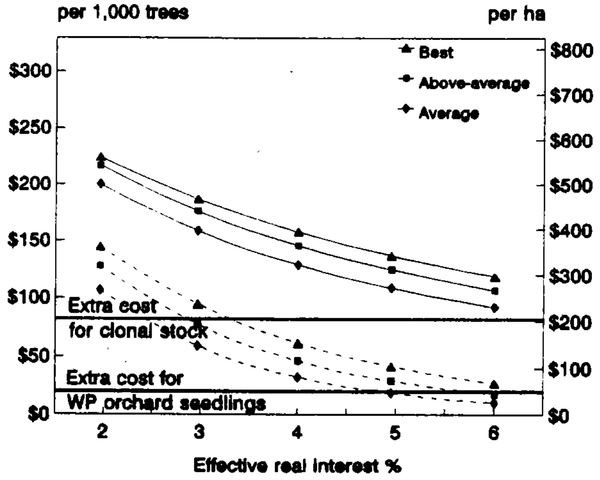

Figure 4. Present value of a 20% genetic gain in volume on "average", "above-average", and "best" spruce sites, when harvested at peak MAI. Calculations assumed stumpage at $15.50 per m^3, with no increase in value due to genetic improvement. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

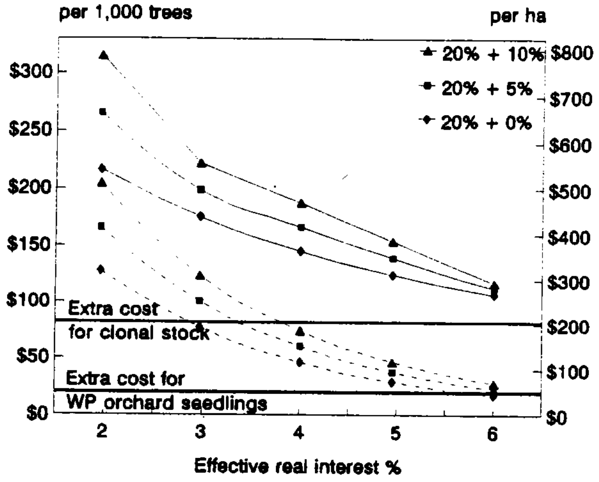

Figure 5. Present value of gains on an "above-average" spruce site at age 51, when a 20% gain in volume is combined with 0%, 5% and 10% increases in fibre value due to genetic improvement. Calculations assumed stumpage of unimproved fibre at \$15.50 per m^3. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

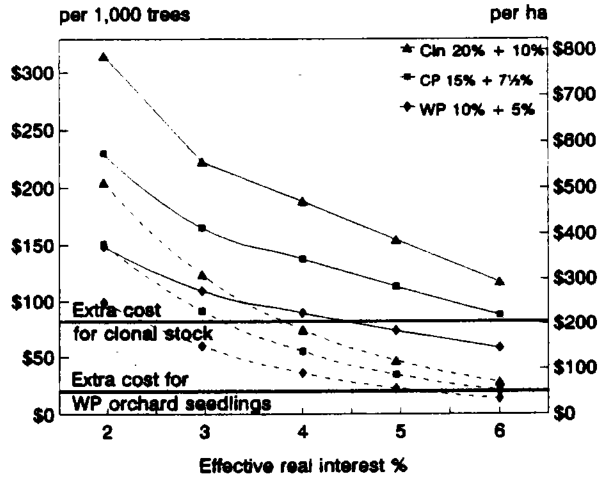

Figure 6. Present value of gains on an "above-average" spruce site at age 51, for different breeding strategies where wind-pollinated orchards are assumed to produce a 10% gain in volume, combined with a 5% gain in stumpage value; control-pollinated orchards and clonal forestry are assumed to produce gains equivalent to 1.5 and 2 times those achieved by wind-pollinated orchards. Calculations assumed stumpage of unimproved fibre at \$15.50 per m^3. Solid and broken lines refer to present values calculated with and without allowable-cut effects, respectively.

CONTRIBUTED PAPERS

GENETICS OF SEED AND SEEDLING TRAITS IN YELLOW-CEDAR (*CHAMAECYPARIS NOOTKATENSIS* D.DON SPACH.)

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Introduction

Conifer species in the Pacific Northwest region of North America have shown to follow alternative strategies in adapting to heterogeneous environments (Rehfeldt 1984). Coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), Sitka spruce (*Picea sitchensis* (Bong.) Carr, and western hemlock (*Tsuga heterophylla* (Raf.) Sarg) are relatively more specialized, that is, population gene frequencies have changed in response to environmental differences, as compared to white pine (*Pinus monticola* Dougl.) and western redcedar (*Thuja plicata* Donn), which tend to be more generalized, with little genetic differentiation among populations (e.g. Kuser and Ching 1980, Rehfeldt 1984, Lines 1987, Campbell 1986, Ying 1990).

Genetic variation among populations for specialists tend to be adaptive (i.e. correlate with environmental site descriptors), such that, trees from seed collected in relatively mild and /or wet climates grow faster, grow later into the season, and are more cold susceptible than populations from colder and/or drier climates. Generalists, on the other hand, display more phenotypic plasticity (i.e. the degree to which phenotypic expression varies under different environmental conditions), and more genetic variability within populations than among (Rehfeldt 1984, Sultan 1987).

Yellow-cedar has a similar north-south distribution on the west-coast as redcedar, western hemlock, and Sitka spruce, occurring over 20 latitude from northern California to southeast Alaska. However, within this latitudinal range, yellow-cedar occupies a unique geographic distribution. It occurs strictly at high elevations (over 1200 m) in both the Siskiyou Mountains in northern California and southwest Oregon, and the west side of the Cascade Mountains in Washington and Oregon. It occurs at high elevations in both the Olympic Mountains in Washington and in the Coastal Mountains in southern British Columbia. From approximately 51° N. latitude and northward, yellow-cedar occurs from sea level to timberline, however, it is restricted to a narrow longitudinal band along the coast. Two isolated stands occur over 200 km inland from the most easterly coastal populations, in central Oregon and southern British Columbia.

Yellow-cedar has a wide ecological amplitude throughout its distribution. In the southern part of its range (south of Mt. Ranier), yellow-cedar is found on wet to dry sites, and occurs in generally open-habitats from bogs to rocky ridges (Antos and Zobel 1986). In British Columbia, yellow-cedar occurs on moderately dry to wet soils, and on nutrient very-poor soils to very-rich soils (Klinka 1991). The most productive sites in British Columbia occur on very- moist and nitrogen very-rich soils in montane, very wet maritime climate where it competes with amabilis fir (*Abies amabilis* (Dougl.) Forbes) (Krajina 1969).

Although yellow-cedar has a wide ecological amplitude, it does not occur on many sites that it seems capable of occupying. On disturbed sites, amabalis fir comes in quicker and is more shade tolerant, thus replacing yellow-cedar in a closed canopy forest (Antos and Zobel 1986). On deep, well drained soils, other species such as redcedar, western hemlock, and Sitka spruce outgrow yellow-cedar. The main limiting factor to the distribution of yellow-cedar seems to be its inability to compete because of slow initial growth and not because of limited ecological amplitude.

Given its wide ecological amplitude, especially if considered in the absence of competition, and its indeterminate growth habit, yellow-cedar should exhibit substantial phenotypic plasticity in fitness traits, typical of a generalist. At the same time, given its wide latitudinal distribution and the occurrence of disjunct, isolated populations (i.e. decreased gene flow), the likelihood of the evolution of a specialist mode, represented by the differential changes in gene frequencies among populations in response to environmental selection pressures seems plausible.

Study Objectives

A study was undertaken to determine the amount of genetic variation present in seed, and seedling morphological and physiological traits of yellow-cedar that sampled the developmental sequence of events that influence the adaptation of a population to its environment. In particular, how much of the genetic variability is attributed to differences among populations as opposed to families within populations? Is any of the genetic variability associated with populations adaptive? Finally, how does this compare to other Pacific Northwest conifers commonly associate with or similar distributions as, yellow-cedar?

Study Design

Seed was collected from, and kept separate by, three to 10 individual trees from each of 33 rangewide populations. Seedlings from a total of 171 open-pollinated families from the above populations were grown in a common garden trial at Cowichan Lake Research Station on Vancouver Island, British Columbia (48 49 N. Latitude, 124 09 W. Longitude, 175 m elevation) for three years, and measured during this time for shoot growth, growth rhythms, and cold-hardiness.

A subset of 18 populations, nine of which were represented by 27 open-pollinated families, were grown under two photoperiods and two moisture regimes in a greenhouse during the second growing season. The photoperiods, which approximated the southern and northern extremes of yellow-cedar's range, started at the beginning of the growing season and continued until the fall equinox. The moisture treatments included regular watering, and a drydown which extended over a 6-week period in mid-summer. The seedlings were measured for periodic and total shoot growth, biomass accumulation, growth rhythms, cold-hardiness, gas exchange, and water relations.

Summary

From this study, the following generalities for morphological and physiological seedling traits that measured the range of the annual developmental sequence of yellow-cedar, can be stated:

1. significant population and family within population genetic variation existed for many of the traits measured;

2. genetic variation among families, for most traits, was 2 to 16 times greater than population variability;

3. seedling traits were under moderate to strong additive genetic control;

4. genetic variation at the population level was moderately correlated with seed origin for most traits;

5. genotype by environment interaction at the population and family (population) level was not significant for all traits;

6. drought resistant ecotypes were evident with seedlings from xeric habitats, and;

7. seedlings exhibited phenotypic plasticity in response to environmental changes.

The above generalities on population effects and associations with geography were more relevant when all populations were considered. If southern populations were removed, (i.e. Oregon populations), then correlations of traits with seed origin were, for the most part, nonsignificant. As well, morphological and physiological responses to drought were only apparent among populations from areas that greatly differed in moisture availability (i.e. coastal, windward versus coast:interior transition).

Although yellow-cedar appears to occupy a unique and limited niche within the Pacific Northwest, its range is extensive latitudinally and it exhibits a wide ecological amplitude in response to soil moisture and nutrient availability. In the absence of competition from other associated conifers, the range of yellow-cedar would most likely be (or has been in the past) more extensive. This view is supported by past climatic and geological events (Critchfield 1984) and the existence of isolated, inland populations in central Oregon and southern British Columbia. The combination of an extensive latitudinal range and geographic isolation, coupled with a wide ecological amplitude and indeterminate growth habit, would seem to suggest aspects of both a specialist and generalist adaptive mode.

Thus, it seems possible that yellow-cedar populations in the extreme environmental ranges of the species, (i.e. southern and continental populations), have responded to environmental selection pressures, most likely aided by reduced gene flow due to spatial isolation and poor sexual reproduction, by changes in gene frequency. At the same time, however, the species has maintained a substantial amount of both genetic variation and phenotypic plasticity within populations. Yellow-cedar seems to have evolved an intermediate adaptation mode with less genetic differentiation associated with geography than coastal Douglas-fir, Sitka spruce, and western hemlock, and more geographic differentiation than western white pine and western redcedar.

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EFFECTS OF HORMONAL AND PHYSIOLOGICAL STRESSES ON ALLOZYME VARIANT SEGREGATION IN *PICEA GLAUCA*.

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ABSTRACT

Hormone treatment alone or in conjunction with physiological stresses have been used to induce flower production in white spruce. Such treatments may change the genetic make-up of the offspring produced from the induced trees. Biochemical analyses were performed on seed tissue from both induced and control trees. Allozyme variants from thirteen loci, resolved on cellulose acetate gels, were analysed to test this hypothesis. The total numbers of detected deviations from the expected Mendelian segregation ratios were low in both cases and did not differed significantly. Therefore, no evidence of a stress-dependent selection in favor of a particular allele at any locus has been found in any of the treatments applied. These results support the belief that flower induction has no direct or residual effects on gene inheritance in white spruce so that the quality of seeds produced on induced trees is similar to the quality of seeds produced under natural conditions.

INTRODUCTION

The reproductive cycle of white spruce, an indirect-flowering plant (Grainger, 1938), is extended over two years. During the first spring of this cycle, undetermined and vegetative apices pass through a plastic phase during which environmental and endogenous factors interact in controlling differentiation (Owens and Blake, 1985). It is at this stage that flower induction treatments are used to concentrate the plant's energy towards reproduction.

The newly differentiated flower buds become dormant and remain as such over the winter period. In the next spring, meiosis takes place followed by pollination and fertilization of the ovules. Cones mature over the summer and shed their seeds early in fall.

Deviation from Mendelian segregation were reported in an annual species following physiological stresses. Controlled pollination among stressed radish plants showed over three times as much deviants as the unstressed controls (Ellstrand and Delvin, 1989). In conifers, flower induction treatments often cause visible damage to the tree such as needle yellowing and shedding. Even if induction treatments are not applied at the time meiosis occurs, we believe that their residual effects could affect the trees in some other ways.

The objective of this study is to verify whether flower induction treatments applied a year before has some residual effects on meiosis causing an haploid phase selection in favor of one allele, therefore affecting the maternal contribution to the offspring generation. Allozyme variants in megagametophyte tissue from heterozygote trees were used.

MATERIALS AND METHODS

Genotypes and Treatments

Genotypes were chosen in the spring of 1992 based on their high level of heterozygosity and their occurrence in the two study locations in the vicinity of Quebec City.

In the first location, that is Cap Tourmente National Wildlife Area, grafted trees, in five tree row plots, were grown in the field. One tree per plot was induced to flowering. The treatment consisted in injecting in the trunk 100 mg of gibberellic acid 4/7 and 10 mg of naphthalene acetic acid. The smaller trees received a half dose. The treatment was applied in June when shoots had, on average, completed 68% of their elongation.

In the second location, that is Valcartier Forest Experiment Station, potted grafts were treated under a controlled environment (greenhouse). The treatment consisted in root pruning the trees in the spring, hormone injection in the trunk (50 mg of gibberellic acid 4/7) in June when average shoot elongation was 82% and heat stress applied in July (day/night temperatures of 30-35°C/20°C, 16-hour daylength).

The female strobili were pollinated in May with a polymix. Whenever seed supply was sufficient, 50 megagametophytes from seeds of induced trees and controls were analysed for each site.

Electrophoresis

The megagametophytes were ground in 3 *ul* of NADP (2 mg/ml) and 10 µl of Ferret buffer (Ferret, 1971) containing sodium metabisulfite (3,3 g/l). The samples were centrifuged at 10 000 rpm for 5 minutes. Electrophoresis was performed on a cellulose acetate gel in a cool chamber (4°C) and run at 200 V for 30 min for all systems except for the leucine amino peptidase (LAP) which was run at 300 V for 20 min. The isozymes assayed, their acronyms, number of loci scored and buffer systems used are listed in Table 1. Stains were prepared following recipes giving by Hebert and Beaton (1989) for all systems except for the glutamate deshydrogenase for which an adaptation of Cheliak and Pitel's recipe was used (Cheliak and Pitel, 1984). For systems controlled by more than one locus, the loci were numbered from anode to cathode. The banding patterns for the PGI, PGM, GDH , IDH and 6PG corresponded to the patterns observed by Cheliak *et al.* (1984).

Table 1. Isozymes, acronyms, E.C. number, number of loci scored, and electrophoretic buffer used.

Isozyme	Acronym	E.C. No.	Nb. of loci	Buffer system*
ISOMERASES				
Phosphoglucose isomerase	PGI	5.3.1.9	2	B
Mannose phosphate isomerase	MPI	5.3.1.8	1	B
LYASES				
Fumarase	FUM	4.2.1.2.	1	B
Aconitase	ACO	4.2.1.3	1	B
OXYDOREDUCTASES				
Isocitrate deshydrogenase	IDH	1.1.1.42	1	A
6-Phosphogluconate deshydrogenase	6PG	1.1.1.44	2	A
Glutamate deshydrogenase	GDH	1.4.1.3.	1	B
PEPTIDASES				
Leucine amino-peptidase	LAP		2	B
TRANSFERASES				
Phosphoglucomutase	PGM	2.7.5.1.	2	B

*A. Solution from Cheliak and Pitel(1984) B. Solution from Hebert and Beaton (1986)

Plates and chambers: Tris 0,125M
pH7 with citric acid 1,0M

Data Analyses

Data analysis for each genotype and treatment was performed on an individual tree basis. A maximum likelihood test ratio, G test (Sokal and Rolf, 1969) was used to test for 1:1 segregation of alleles in the megagametophytes from trees identified as heterozygous at a particular locus. The number of trees that showed Mendelian segregation and the number of those showing distortion were determined. The percentages of both classes were compared using a Z-statistics (Pfaffenberger and Patterson, 1981).

RESULTS AND DISCUSSION

In Cap Tourmente, 29 genotypes were analysed and Mendelian segregation was confirmed in 133 of the 137 ratios (97%) examined for the injected trees and in 128 of the 135 ratios (95%) for the controls (Table 2).

In Valcartier, seed production was low and only 19 out of the potential 33 genotypes could be used for the analysis. Seeds were produced mostly on the treated trees (14 out of the 19). For the induced trees, 96% (70/73) of the ratio examined behaved as simple codominant markers and for the controls, 92% (12/13) fitted the expected Mendelian segregation ratios.

There were no significant differences between ratios for both treatments and locations. For all the loci analysed, the genetic contribution of the female parent was not influenced by the induction treatments.

Table 2. Frequency of deviant segregation ratios for treated and controls trees at two study locations.

Isozyme locus	Cap Tourmente				Valcartier			
	Induced		Controls		Induced		Controls	
	Normal	Deviant	Normal	Deviant	Normal	Deviant	Normal	Deviant
ACO	15	0	15	0	6	0	1	0
FUM	8	0	7	0	6	1	1	0
GDH	12	0	11	0	6	0	1	0
IDH	8	1	9	0	7	0	1	0
LAP-1	9	2	9	2	5	0	4	0
LAP-2	17	0	16	1	9	0	2	0
MPI	7	0	7	0	3	0	0	0
6PG-1	3	0	3	0	1	0	0	0
6PG-2	10	0	10	0	4	1	1	0
PGI-1	2	0	2	0	1	0	0	0
PGI-2	19	0	19	0	12	0	1	1
PGM-1	11	1	10	2	3	1	0	0
PGM-2	12	0	10	2	7	0	0	0
Totals	133	4	128	7	70	3	12	1

$P<0,05$

Our results on white spruce differed from those obtained by Ellstrand and Delvin (1989) on radish. They observed an increased level of deviation in physiologically stressed plants (18% versus 6% for controls). We found less than 8% distortion in segregation in white spruce and it was not associated with a particular locus, genotype and treatment. No significant differences could be found between the treated and the control trees.

The difference in response between the two species could be explained by the fact that the physiological stresses were applied during meiosis and seed development in the experiment on the radish. In white spruce, it did not seem that residual effects of the induction treatments done during the first year of the reproductive cycle played a role in the second year when meiosis, pollination and seed set occurred. These residual effects although causing needle yellowing and shedding during the seed production year on some genotypes, may have been dissipated before meiosis began. It is also possible that abortion hides real effects of these treatments on meiosis. Comparison of percentages of empty seeds obtained from induced and control trees would allow us to verify this hypothesis.

In conclusion, based on our results, there is no reason to believe that the genetic constitution of the offspring produced on induced trees may be different from those obtained under natural conditions. It is of prime importance to certify the quality and integrity of this material as these techniques are currently used in Quebec by the provincial government for mass seed production.

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GENETIC-INDUCED VARIATION OF CONTAINER NURSERY-CULTURED INTERIOR SPRUCE IN BRITISH COLUMBIA

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ABSTRACT

Foresters in British Columbia have expressed concern about the morphology, operational logistics and physiology of interior spruce seedlings derived from seed orchard seed collections when compared to those of stock obtained from natural stand (wild) seed collections. Most of the perceived problems can be related to nursery culture. It was difficult to assess the extent of the spruce, seed orchard issue because of the paucity of information on expected variation (morphological and physiological) of wild seedlot collections grown in container nurseries. Therefore a pilot project was established at Red Rock Research Station in February 1993 to assess genetic-induced variation of 50 interior spruce seedlots from different sources; registered natural stand collections, registered seed orchard collections and full sib collections from the research program. Variation was and will be assessed for the: i) germination period (nursery and laboratory); ii) nursery growth and bud development stages (under 'control' and 'blackout' conditions for selected seedlots); iii) frost hardiness and dormancy induction phases (a wide range of seedlots grown under different photoperiod treatments); and iv) post planting phenology and growth stages of the nursery-cultured stock (in a common garden at Red Rock). Variation observed during germination and early nursery growth was considerable. However, seed orchard seedlot variation was within the range observed for natural stand collections albeit at the upper end. Nursery culture, short day treatment in this case, can overcome the logistical and morphological concerns expressed by field foresters. Field implications of cultural modifications have yet to be ascertained but to date look promising.

INTRODUCTION

Seedlings derived from seed orchard seed have a controversial past in British Columbia (BC). The controversy arises not because of documented short comings but because of perceptions based on anecdotal observations. Coastal, Douglas-fir seed orchard seed destined for bareroot culture has been through this cycle (Mueller et al. 1981). Now, it appears to be the turn of seed orchard products of interior spruce (Hawkins 1993a).

Foresters in the interior of BC recently began expressing a reluctance to take seedlings originating from seed orchard or A class spruce seed. They were and are concerned about the stock's morphology, operational logistics and physiology. The stock was tall, spindly with poor diameter and root system development; the height variability was thought to be greater for these seedlots than for those from natural stand collections. Logistically the stock was not being delivered on time for a high elevation summer plant program. This program has a very narrow planting window and late delivery upsets the entire high and low elevation summer planting program. Physiologically, it was thought that stock grown for spring planting, from a single seedlot, was placed into freezer storage with different levels of physiological quality, dormancy and stress resistance. The physiological variability could result in seedling damage and mortality on the planting site. Whether correct or not, these concerns are not surprising because uniform planting stock is desirable, for many reasons, both in the nursery and at the planting site (Lang 1989). Many of the above concerns can be addressed through modifications to existing nursery culture.

Short day, photoperiod manipulation or blackout treatment applied during nursery culture is an effective means of height control in forest seedlings (Arnott and Mitchell 1981, D'Aoust and Cameron 1981, Hawkins and Draper 1991, Bigras and D'Aoust 1993). The purpose of this treatment is to expose seedlings to a day length shorter than the critical day length, thus simulating a later time in the growing season (Hawkins and Draper 1988). These treatments not only regulate crop height but increase uniformity and promote frost resistance and dormancy induction (Silim et al. 1989, Bigras and D'Aoust 1993). A caveat however needs to be added: extremes of this treatment can have negative effects on phenology and frost resistance in the field (Hawkins and Hooge 1988, Odlum and Colombo 1988, Bigras and D'Aoust 1993).

Due to the paucity of information available on nursery growth norms for natural stand collection (wild) seedlots, a ready reply was not available for field foresters. Therefore a joint Research Branch -Silviculture Branch - Forest Region, research trial was established in February 1993 at Red Rock Research Station, near Prince George. The objectives were to investigate nursery and field variation within and among various interior spruce seed sources. The sources were registered natural stand and operational seed orchard seedlots and full sib families from the research program.

This paper reports on nursery results from sowing through to mid season and summer planting in July 1993, and it provides preliminary conclusions based on these data.

MATERIALS AND METHODS

Fifty spruce (*Picea glauca* (Moench) Voss, *P. engelmannii* Parry and their naturally occurring hybrids) seedlots (ranging in origin from the U.S. to the Yukon border, from the Alberta border to the western boundary of the Central Plateau and from 450 to 1800 m in elevation) were sown on 17 February 1993 at Red Rock Research Station (53°45' N and 122°41' W) into 415B (112, 105 mL cavities per container) styroblocks (Beaver Plastics, Edmonton, AB) and germinated and grown using a modification of the regimes described by Draper and Hawkins (1989) and Hawkins and Draper (1991). Germination results were obtained for all 50 seedlots (Hawkins 1993b). However due to logistical constraints, only 11 seedlots (Table 1) representing the three seed sources were non destructively sampled at 7 to 14 day intervals from the end of the germination. Both germination and nursery height growth phases are reported for these seedlots.

Because this project was also a pilot trail for operational implementation, a secondary objective was to determine a practical and functional series of measurements for the nursery phase. Parameters monitored or to be monitored over the course of the experiment include; i) germination for each seedlot and treatment, ii) morphology for each seedlot and treatment (destructive sampling), iii) detailed height growth for 11 seedlots (serial, non destructive sampling), iv) bud development, v) acquisition of frost resistance, vi) dormancy induction, vii) root growth phenology in the nursery and after summer planting, viii) post planting field phenology and ix) field growth over several seasons. This large body of data will be used to establish a cost effective series of assessments for future operational nursery studies and to examine the effects of treatment on physiological response.

Short day treatment was applied to 28 of the 50 seedlots. This treatment was initiated when mean seedlot height was ≈ 10 cm. The earliest treatment was applied on 27 May and the latest application commenced on 10 June 1993. All treatments were for 17 days. Seedlots whose latitude of origin was N of 53°N were given a 14 h day and those whose origins were S of 53°N were given an 11 h day. These day lengths and durations were based on earlier work at Red Rock (Hawkins and Draper 1991). Height control was affected in additional styroblocks of all 50 seedlots, at the same time, via water and nutrient regulation. These seedlings are referred to as controls. Blackout treated seedlots and their untreated control counterparts were planted into a common garden at Red Rock in six seedling rows, 30 cm spacing within and between rows, 17 days after the seedlot was removed from short day treatment. The remainder of the crop was held for fall lift, freeze storage and spring planting (Hawkins 1993b); this will be reported later.

RESULTS AND DISCUSSION

Germination was variable among full sib seedlots but even the poorest was considerably better than the overall worst germinator from a natural stand collection. Germination of seed orchard seedlots were also variable but they were at the upper end of the overall response spectrum (Figure 1). Natural stand collection seedlots had germination expressions ranging between the overall poorest to best germinating seedlots (Figure 2). Regardless of seed source, greenhouse germination capacity (percent of seedlot germinating) was a few percent lower than laboratory tests would predict. Only two seedlots had a germination fall down that would be considered operationally significant, 15 to 20 percent (eg. seedlot 8791, Figures 1 & 2). The rate of germination (steepness of curve) was also highly variable. This is not surprising as variability of germination has been reported for seed source (Allen 1961) and family (El-Kassaby et al. 1992). Overall the most rapid and complete germination was observed in a seed orchard seedlot. Roche (1969) reported that spruce populations from higher latitudes exhibited earlier and more rapid germination than did low latitude ones. This does not appear to be the case for these seedlots.

The germination responses displayed by the seed orchard seedlots were not outside the range observed for seedlots from natural stands. They were at the upper end of the germination response spectrum. They were not unusual or more variable when compared to wild seedlots. In fact from a nursery perspective, they were 'good' germinators.

The rate of seedlot germination had little if any affect on early seedling height growth for all three seed sources. The seed orchard seedlots had quite different germination rates, yet there was no difference in early height growth (Figure 3, from ≈ days 30 to 90). Natural stand seedlots also demonstrated little relationship between germination rate and early height growth (Figure 4). Generally if any differences were observed, the more rapid germinators tended to have slower early height growth. For example, seedlot 30664 had an average rate of germination (Figure 2) yet it was the most vigorous of all seedlots (Figures 4 & 5). Current nursery dogma in BC suggests that rapid germination rates and high germination capacity correlate to rapid initial height growth. The data in this experiment tend not to support this view.

Short day treatment application started between 99 and 113 days after sowing and was determined when mean seedlot height was ≈ 10cm (Figures 3 & 4). Seed orchard seedlots responded well to short day treatment and height was effectively controlled. Somewhat surprisingly, the more vigorous (faster height growth) of the seed orchard seedlots responded more to blackout (stopped growing sooner) than did the less vigorous (slower height growth) seedlot (Figure 3). This supports the observation of Silim et al. (1989) that the response to short days is more than a reflection of the vigor of seedlot height growth. If the response were vigor related, fast growing seedlots would be more difficult to stop than slower growing seedlots.

Natural stand seedlots also responded well to short day treatment (Figure 4). In some instances, height growth of the control seedlots was little more than that of the treated ones. This indicates bud initiation in response to the local Red Rock photoperiod at about the same time as short day treatments were applied. The small height differences arose under quite different photoperiods, ≈19 h (Red Rock) and 14 h (treatment). This response appears to be characterized in local (≈ 54 °N) and high latitude seedlots. The phenomenon is better observed when the height growth of the control treatments for the 11 seedlots are displayed (Figure 5). The range in height growth for the 11 seedlots was about 11 cm between the shortest and tallest. The most (30664) and least (4073) vigorous seedlots were both from natural stand collections. Short day treatment controlled height and reduced the range between seedlot extremes to about 5 cm (Figure 6). This too demonstrates the effectiveness of the treatment and its promotion of uniformity (c.f. Silim et al. 1989). The 'dip' in height growth following short day treatment reflects detection of a bud. Prior to that time, the location of the shoot (bud) tip was 'estimated' by feel. The dip is characteristic of serial height assessments in spruce.

The coefficient of variation (CV) for mean seedlot height was reduced by short day treatment for all seedlots from about 24.5 to 19.7 percent (not presented). During the season the CV decreased as the seedlings proceeded towards maturity. This has been observed previously for white spruce (Silim et al. 1989) and black spruce (Stoehr and Farmer 1989). The CV for the control seed orchard seedlots was greater than the grand mean but short day treatment reduced the CV an amount similar to that observed for full sib and wild seedlots. This indicates the utility of the treatment for inducing crop uniformity (c.f. Silim et al. 1989); even in more variable and vigorous seed sources.

Overall variation in nursery height growth was greater in seed orchard seedlots. However the absolute height of these seedlots was within the range observed for natural stand collections treated in a similar manner. The perceptions of field foresters and nursery workers that seed orchard seedlots were overly vigorous when compared to wild seedlots is not correct. Seed orchard seedlots perform similar to the better growing wild seedlots. There does not appear to be any poor nursery performing seed orchard lots as there is with collections from natural stands. This fact results in the incorrect observation about the vigor of the former.

Shortly after the end of blackout treatment, July 5 to 15 (between 138 and 148 days since sowing), all 28 short day treated seedlots had detectable buds. At this time, these treated seedlots and their untreated controls were planted into a common garden at Red Rock. Major concerns of summer planting are the stock is too succulent and will succumb to the rigors of planting or the bud is not adequately developed (set) and the seedling will flush shortly after planting. The flush could then be lost to summer drought or early frost events. To date (7 September 93), mortality is 0.06 percent and no seedling has reflushed. This indicates the early success of the treatment. However, it should be noted that this stock was not subjected to the usual transportation rigors of summer planting. Transport distances in excess of 500 kilometers are common for this program.

Also, short day treatment can have post planting affects on bud phenology and frost resistance (Hawkins and Hooge 1988, Odlum and Colombo 1988, Bigras and D'Aoust 1993). It appears the more severe the short day treatment, the greater the potential perturbation in the field (Hawkins and Hooge 1988, Odlum and Colombo 1988, Bigras and D'Aoust 1993). Day lengths used in this study are thought to be moderate rather than severe treatments. Therefore little nursery treatment 'carry over' is expected. The answer to these questions will not be available until at least the end of next growing season.

PRELIMINARY CONCLUSIONS

Seed orchard seedlot nursery variation for germination and height growth is at the upper end of the range observed for natural stand seedlots. However, these are not unusual seedlots. There are no poor nursery performers as can be found with the wild seedlots. Nursery culture, short day treatment specifically, can overcome the morphological variability concerns that have been expressed about seed orchard seedlots by foresters. The chosen nursery treatment also allows logistical requirements for a summer plant program to be met. The physiological concerns were not dealt with in this paper. The field implications of these cultural modifications have yet to ascertained. However to date, it looks promising; no reflush and mortality of much less than one percent.

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Table 1. Description (elevation (Elev) m; latitude (Lat) and longitude (Long) degrees and minutes) of the eleven seedlots reported in detail for this report and their germination as predicted by laboratory testing.

Seedlot	Source	Origin	Elev	Lat N	Long W	Germ %
1	full sib	PG 21 X PG 87	-	≈54	≈124	96
5	full sib	PG 145 X PG 28	-	≈54	≈124	100
6863	orchard	Central Plateau Low	740	54.57	124.54	93
6913	orchard	Shuswap Adams Low	749	51.08	119.15	93
6914	orchard	Shuswap Adams Mid	1270	51.03	119.18	93
4073	wild	Hungary Creek	1190	53.46	121.30	69
4140	wild	Elizabeth Creek	900	56.01	122.19	71
8779	wild	Alvin Creek	1067	55.44	122.02	82
30664	wild	Birch Island	480	51.36	119.57	94
31460	wild	Mt. Robson	1500	53.02	119.19	89
35075	wild	Prophet River	450	58.25	122.55	96

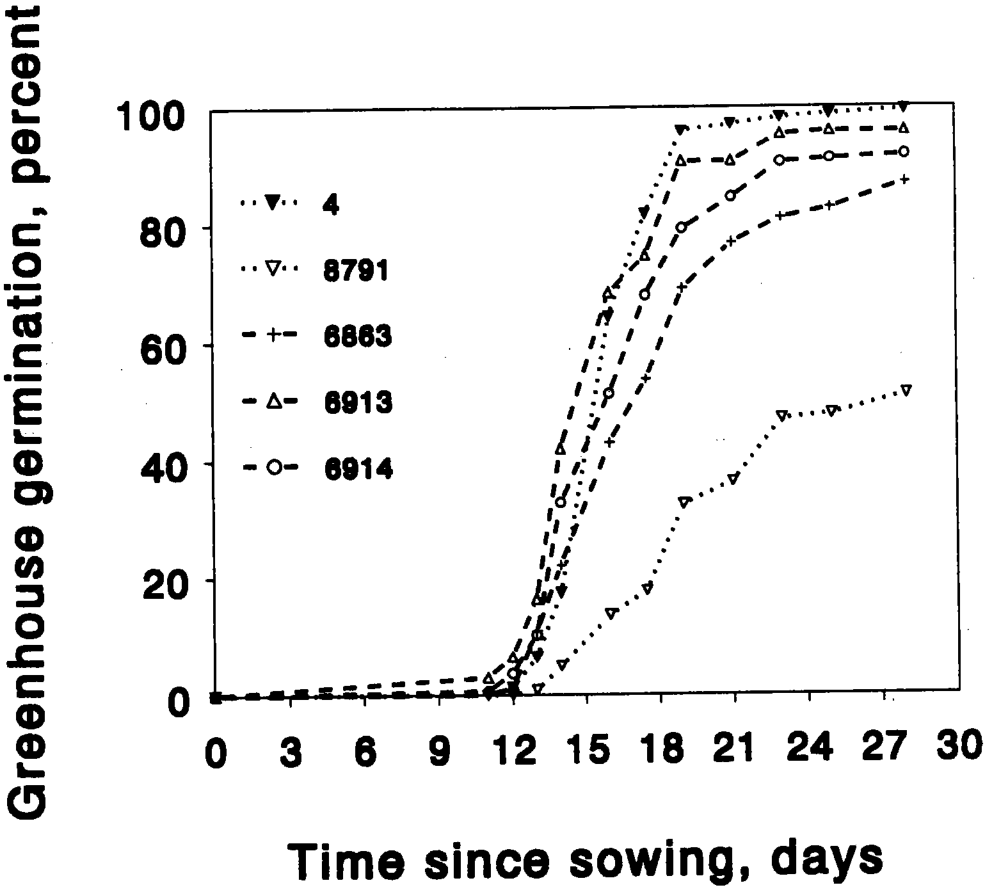

Figure 1. Seed orchard seedlot (dashed lines) germination compared to the best (seedlot 4 a full sib) and poorest (seedlot 8791 a natural stand seedlot) germinators (dotted lines). n = 12, 14 cavity rows per seedlot.

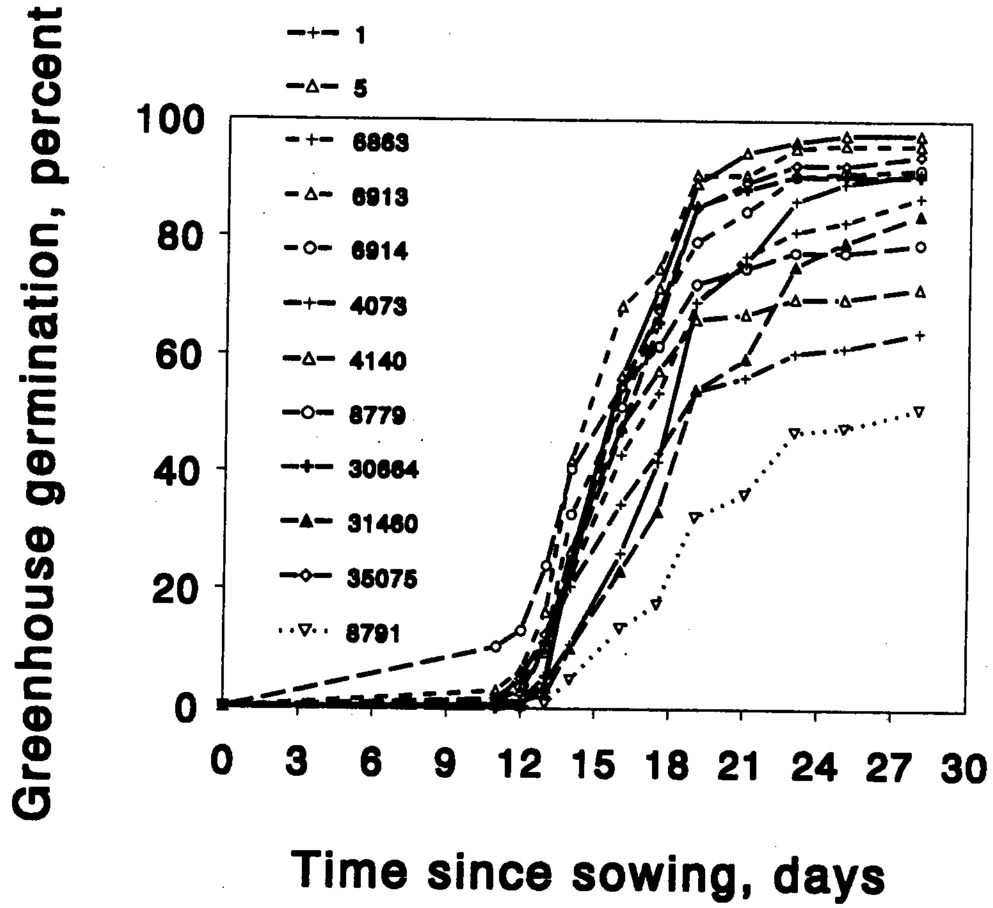

Figure 2. Full sib (solid lines), seed orchard (short dashed lines) and natural stand (long dashed line) germination in comparison to the poorest germinating seedlot (dotted line). n = 12, 14 cavity rows per seedlot.

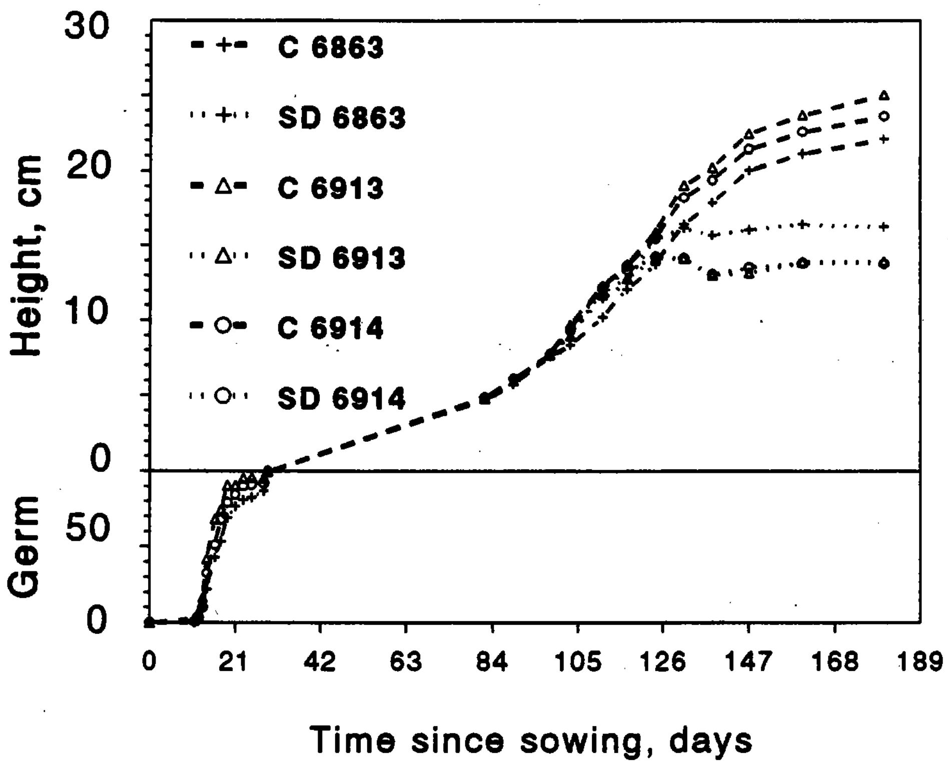

Figure 3. Germination for the control (short dashed line) seed orchard seedlots followed by serial nursery height growth for control and short day (dotted line) nursery treatments for the orchard seedlots. Short day treatment (SDT) was applied between days 99 and 113. n = 168 seedlings per seedlot treatment.

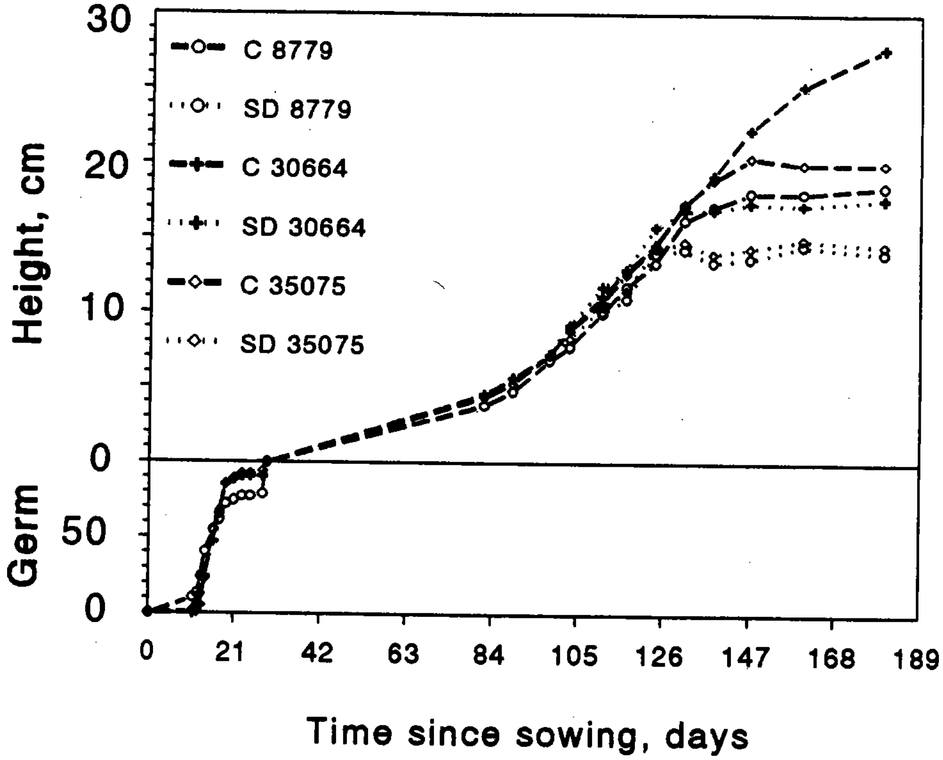

Figure 4. Germination for a sub set of the control (long dashed line) natural stand seedlots followed by serial nursery height growth for control and short day (dotted line) nursery treatments for the wild seedlots. SDT applied between days 99 and 113. n = 168 seedlings per seedlot treatment.

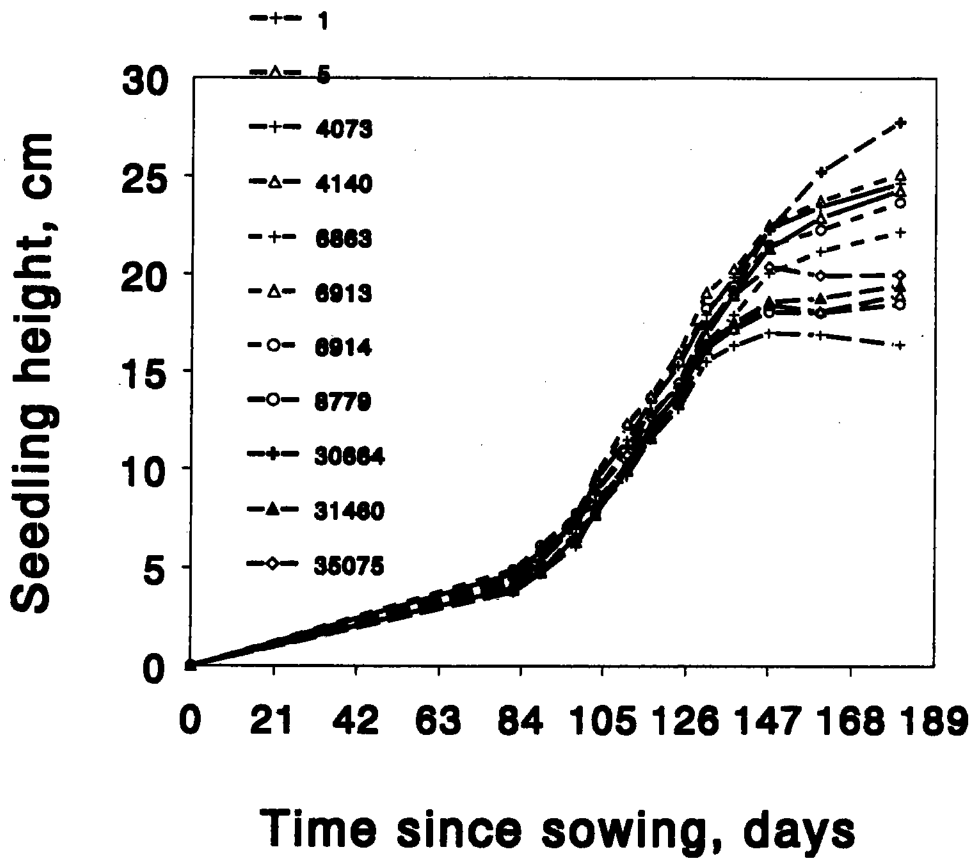

Figure 5. Serial nursery height growth for control nursery treatment of full sib (solid line), seed orchard (short dashed line) and natural stand (long dashed line) collection seedlots. Stock was summer planted between days 138 and 148. n = 168 seedlings per seedlot treatment.

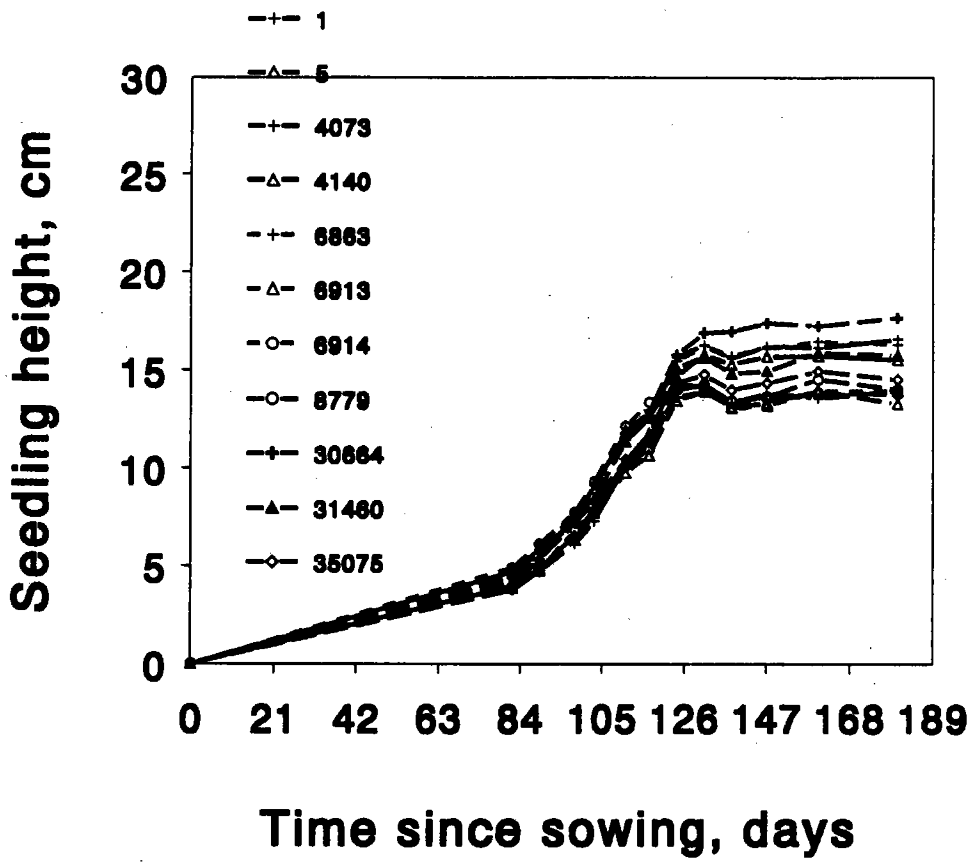

Figure 6. Serial nursery height growth for short day nursery treatment of full sib (solid line), seed orchard (short dashed line) and natural stand (long dashed line) collection seedlots. SDT applied between days 99 and 113 and stock summer planted between days 138 and 148. n = 168 seedlings per seedlot treatment.

OPERATIONAL CONE INDUCTION IN BLACK SPRUCE SEEDLING SEED ORCHARDS: WHAT DO WE KNOW AFTER FIVE YEARS?

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INTRODUCTION

In the Maritimes, seed production in first-generation black spruce seedling seed orchards began in the mid-1980s. Although over 120 ha of orchard had been established, seed demands exceeded production and methods to increase seed production were needed. Effective methods for injecting gibberellin (GA) into tree stems had yet to be developed thus cone induction with GA necessitated 4 - 8 weekly foliar sprays. Until such time as refinements to GA methodologies made that tool more cost-effective and operationally practicable, fertilization was deemed as having the greatest potential.

In 1987, a series of cooperative fertilizer trials in black spruce seedling seed orchards was established. These trials involved seven plantings in five orchards. Initial results were reported (Smith and Phillips 1989; Smith et al. 1989; Wang and Smith 1989) and final papers are in preparation. The focus of this paper will be to summarize the results from these trials and to:

I. Discuss cone production responses to fertilizer applications;
II. Discuss some preliminary work on studying the effects of fertilizer treatments on orchard tree health and corresponding effects on cone and seed yields; and
III. Recommend a generalized fertilizer schedule to increase cone and seed production in black spruce.

I. Effects of Applying Ammonium Nitrate on Cone and Seed Production

Four main benefits from fertilizing with ammonium nitrate were demonstrated:

a. increased numbers of cones;
b. earlier seed production;
c. increased seed yields; and
d. improved seed quality.

a) ***Increased numbers of cones***: Increased cone production in response to fertilizer applications has been well described in the literature (see Owens and Blake 1985). However, results from these trials are often inconsistent and, except for some work in the southern pines (Schmidtling 1983) and Douglas-fir (Ebell 1972), of only 1-2 years duration. While cone induction in response to fertilizers has been observed, the long-term effects of repeated fertilizer applications on tree health and, therefore the indirect benefits (i.e., increased numbers of potential flowering sites and the possible improved ability of the orchard trees to support the cone crops) has not been adequately studied.

Ammonium nitrate applications significantly increased black spruce cone production. The magnitude of the response varied with site, tree age/size, year, rate, and timing. With these potential confounding factors, it is not surprising that results from trials spanning only 1 or 2 years on single sites, are often inconsistent and consequently do not accurately assess the potential utility of fertilizing for cone induction as a management tool.

b) ***Earlier cone production:*** Time of first cone production in black spruce is strongly correlated with tree size (age) (Caron and Powell 1989; Simpson and Powell 1981). In order to investigate whether or not the time to first significant cone production could be shortened, trees of three different ages growing on a uniform orchard site were fertilized.

Cone production on the youngest fertilized trees equalled that of control trees on the same site that were 2 years older, as well as that of control trees on a second site that were 3 years older. Although comparing sites is somewhat more tenuous, the data demonstrate that annual fertilizer applications caused trees to produce significant quantities of cones 2-3 years earlier than unfertilized controls.

c) ***Increased seed yields:*** Intuitively, one would think that a healthier, more vigorous tree would be able to produce bigger cones with more seed. Single fertilizer applications generally had little effect on seed quality. Extraction and testing of approximately 200 additional seedlots are not yet completed, but initial results indicate a positive effect of fertilizer on seed yields. Cones from fertilized trees were larger, with increased seed yields due both to greater numbers of cone scales (greater seed potential), and to larger cone scales. The latter observation could translate into a greater operational extraction efficiency. These early results need to be studied further.

d) ***Improved seed quality:*** It is generally assumed that seed resulting from induced cones is comparable in quality to cones from non-induced trees (Owens and Blake 1985). Given that cone induction treatments such as GA applications are of relatively short duration and occur, in the case of black spruce, the year before maturation, this is probably a reasonable assumption. However, benefits of fertilization may not be restricted to the year of application. Early results indicated a positive effect of fertilizer on seed vigor as measured by accelerated ageing stress tests (Wang and Smith 1989). An additional 100 seedlots are being tested which should provide sufficient additional data for a better evaluation of effects on seed vigor.

II. Correlating Cone Production with Tree Nutrition

a) ***Site quality effects on orchard productivity:*** Seed orchards are often located on sandy or droughty sites deemed conducive to flowering. These types of sites often have a very poor nutrient base resulting in slow tree growth. This delays the time at which trees begin flowering, and often results in fewer numbers of cones being produced. Under these circumstances, applying ammonium nitrate may increase cone production by removing nitrogen (N) as a limiting factor to tree growth, which indirectly benefits flowering.

b) ***Changes in nutrient availability over time:*** Few studies have tried to evaluate how the ability of a site to provide nutrients changes during the development of an orchard. Nutrient dynamics and their effects on tree growth and development, and orchard productivity have been largely ignored, or missed in the research reported to date. These fertilizer trials have been able to provide some insight into this area.

The theory that crowns of orchard trees should not overlap (avoid reducing cone production due to shading), combined with a reasonable family test measurement schedule, has contributed to determining the age for first roguing at age 10 and an initial planting spacing of 2 m X 1 m for black spruce seedling seed orchards in the Maritimes. Even though upper-crown branches, the seed cone-bearing zone, do not overlap by age 10, root competition and the ability of a site to provide nutrients, e.g., site carrying capacity, appear to limit seed production prior to roguing. Furthermore, under the aforementioned roguing schedule, considerable overlapping of lower branches within rows does occur which, it can be assumed, has a negative impact on pollen production.

Although comparing cone production between years introduces potential errors in estimating response to roguing and fertilizer, some interesting trends still warrant discussion. Approximately 55 - 60% of the trees were removed in the first roguing. Cone production in the fertilized rows had recovered to pre-roguing levels by the second year. However, when comparing controls with fertilized blocks, they produced less than half the number of cones. This is based on operational treatments, pooling all cones from all fertilized blocks. Fertilized trees in the 'best' rows, produced four to five times more cones than unfertilized trees. This approaches the magnitude of response obtained from applying GA.

When an orchard is rogued, the site is opened up which should result in a general warming of the soil, thereby changing the nutrient dynamics of the site. An attempt was made to indirectly assess the net effects on the trees remaining following roguing by evaluating cone production. For control trees, cone production decreased for 1 or 2 years prior to roguing. Applying ammonium nitrate reduced this trend, presumably by reducing the degree to which nitrogen had become limiting. This is being substantiated by foliar analyses.

c) ***Setting target nutritional levels:*** Cone initiation and seed maturation involve a series of developmental stages spanning 2 years in black spruce. There is an obvious problem in trying to select a single point measure of tree nutrition that correlates well with cone production. While increases in foliar N following fertilization have been reported, this does little to indicate whether the addition of fertilizer induced an increase in cone production directly, or if the increase was due to alleviation of a nutrient deficiency, or both. Despite these inherent problems, attempts have been made to use foliar N as an indicator since additions of N have most frequently increased cone production when compared to additions of other nutrients.

As part of the fertilizer trials, annual foliar samples were collected, and analyses of macronutrient levels are now being completed. The following discussion is based on preliminary results.

Developing cones act as strong nutrient sinks preferentially diverting nutrients away from developing foliage (Dickman and Kozlowski 1969). Similarly, growth is often reduced in heavy cone production years. This study indicated that within a given year, there was a positive correlation between foliar N and cone production. Yields (volumes of cones) appear more closely correlated with N in the year of maturation than in the year of initiation. Nutrient levels during the year of initiation were not significantly correlated with cone production the following year. Ingestad ratios were only slightly more positively correlated with cone production than nutrient levels.

Additional analyses underway include trying to refine Diagnosis and Recommendation Integrated System (DRIS) indices for black spruce cone production. Although the database is somewhat limited at present, as additional data are collected, a better evaluation of the potential utility of this approach should be possible.

III. Implementing a Routine Fertilizer Schedule for Increased Cone Production

The benefits of routinely fertilizing orchard trees to optimize their health during the establishment phase is generally accepted (Simpson and Smith 1988). The rationale is that, other factors being equal, larger trees will produce cones earlier and in greater numbers than smaller trees. In practice, however, once the trees are well established, they are often not fertilized as often as perhaps they should be. Assessing the desirability and/or feasibility of implementing a routine fertilizer program involves answering several questions;

a) *What are the seed demands?* Seed of the 'best' genetic quality will always be in demand. Therefore, the first step is to set quantity and quality objectives and, once this is done, the practicality and efficiency of the different methods to obtain that seed can be evaluated.

b) *What effects will treatments have on the genetic quality of the seed?* Two common problems that detract from panmixis in conventional open-pollinated orchards are pollen contamination and lack of balance in flowering between genotypes (e.g., 80:20 rule). Operational fertilizer treatments may be used to reduce the effects of pollen contamination in black spruce orchards. Although only seed cone production was assessed in these fertilizer trials, earlier work showed that ammonium nitrate applications increased cones of both sexes, although the optimum rate differed for the two (Smith 1985). The greatest impact of fertilization on pollen production would be expected to result from the increased vigor of lower branches, the pollen producing part of the crown. Effects of induction treatments on the relative proportions of seed and pollen cones between genotypes or their gametic contributions in the resulting seed have not been adequately studied. Both GA and fertilizer treatments generally increase the number of seed cones produced, but do not induce recalcitrant genotypes to flower.

c) *Gibberellin or fertilizer - which is the better tool?* There is little doubt that, timed correctly, GA induces more seed cones per tree than fertilizer. However, gibberellins do nothing to improve tree vigor and in fact may have a negative effect, especially if phytotoxicity occurs. It has yet to be demonstrated that trees treated regularly with GA will produce more cones than fertilized trees over the longer term. Increases in seed cone production following GA application often occur at the expense of pollen production, which could have a negative impact on genetic quality of orchard seed.

Combining GA and fertilizer should also be considered as a possible management option. Cooperative studies in clonal orchards of black, Norway, red, and white spruce as well as one black spruce seedling orchard all indicated moderate to large increases in pollen production on trees receiving both treatments over those receiving GA alone and the controls. There was no doubt that GA was the more consistent and effective treatment in stimulating seed cone production.

d) *What are the costs?* Seedling production costs are usually expressed as dollars per 1000 seedlings. The additional orchard management costs of fertilizing for cone induction can be expressed similarly (Figure 1). Assuming seedlings cost $150.00/1000, fertilization costs represent only 1-3% of this figure even with modest increases in cone production. Expressing the 'extra' management cost this way should facilitate justifying the expenditure, especially when seed supplies are limited.

In seedling seed orchards, with 5000 trees per ha before roguing, manpower quickly becomes limiting to operational cone induction using GA. Conversely, when applying fertilizer, most costs are associated with materials, while labor costs change little with the amount of fertilizer used. For orchards in which fertilizer application can be mechanized e.g., by using a tractor with a side-banding kit, labor costs become insignificant.

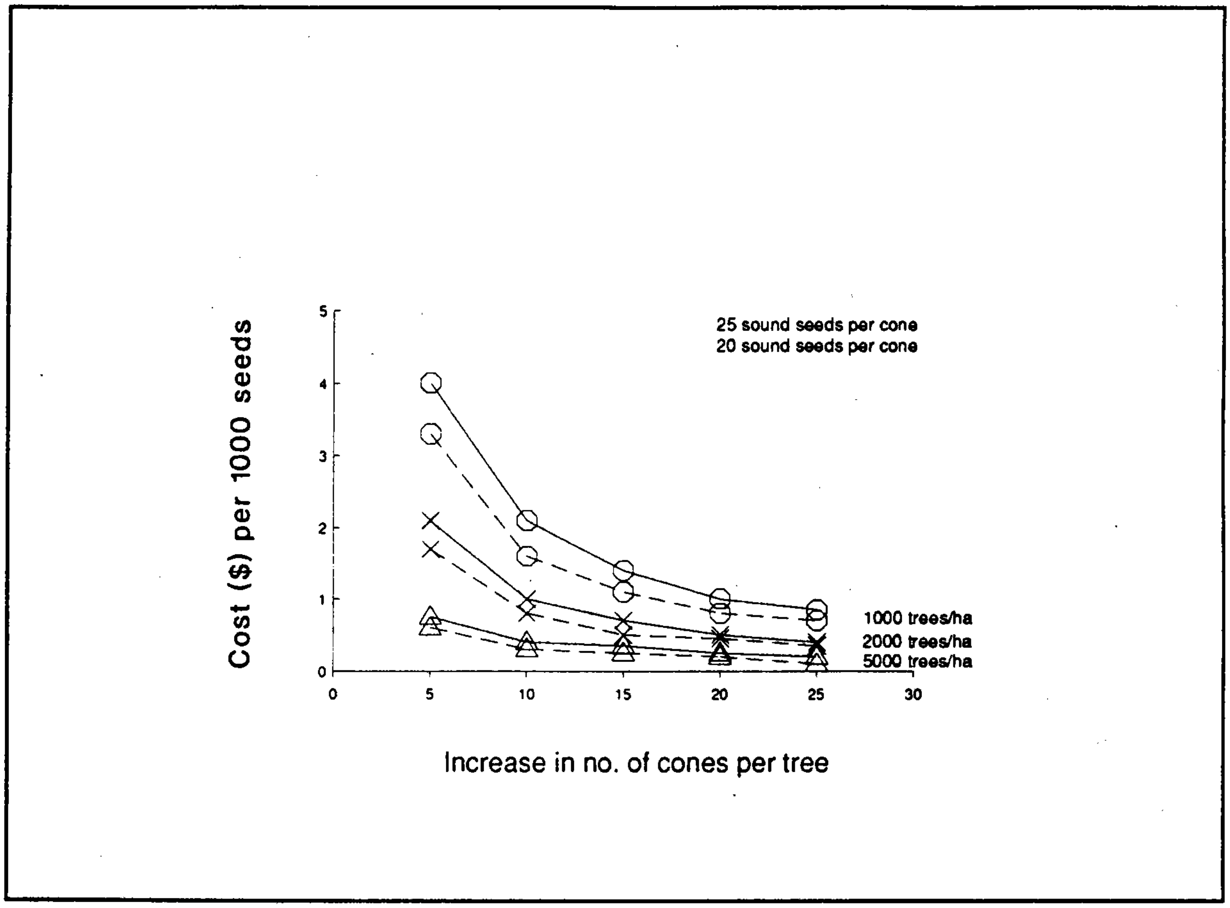

Figure 1. Costs per 1000 seed of fertilizing black spruce seedling seed orchards. Assumptions: a) fertilizer applied at 1000 kg/ha (5.0 kg/50 on two sides of rows, b) cost of 34-0-0 = $300.00/tonne c) labor = $50/personday d) two-person crew can fertilize 1.0 ha per day. Increases in the number of cones per tree represent conservative values obtained in the fertilizer trials.

RECOMMENDATIONS

How much: The optimum rate of fertilizer increased with increasing tree size (Table 1). Similarly, the amount of fertilizer required to elicit a response increased with the amount of ground cover, e.g., the optimum rate for one orchard located on a cutover with a non-continuous ground cover (ferns and miscellaneous species) was lower than for an orchard planted on a field containing thick sod cover. Trees fertilized each year did not significantly outproduce those receiving fertilizer over the first 2-3 year period. In subsequent years, trees receiving annual applications began to outproduce controls and those receiving biennial applications.

Table 1. Recommended schedule for fertilizing black spruce seedling seed orchards for cone production. Fertilizer to be applied in bands along the edge of crown dripline.

Average tree height (m)[1]	**Rate (kg 34-0-0) per 50 m tree row**	**Band(s)**	**Total fertilizer per ha (kg)**
1.5 - 2.0	5.0	1 side	500
2.0 - 3.0	5.0	2 sides	1000
3.0 - 4.0	7.5	2 sides	1500

[1]. Trials commenced when the oldest trees averaged less than 4.0m in height. At the end of the 5-year period, the oldest trees were 5-7m tall.

When: Fertilize in mid-May to early June. This can be timed to coincide approximately with the end of the female receptive period.

SUMMARY AND CONCLUSIONS

Seed requirements for black spruce are currently being met by first-generation seed orchards in New Brunswick and Prince Edward Island, while in Nova Scotia, demands should be met in the near future. These first-generation orchards will continue to supply the majority of seed for reforestation programs in the region for the next 5-10 years when second generation orchards come into production. Therefore, their continued productivity is critical to realizing the benefits from the region's tree improvement programs.

Regular applications of ammonium nitrate have been demonstrated to reliably and significantly increase cone and seed production in black spruce. The per seed costs are very low even when increases in yields per tree are very modest. Fertilizer, while not as reliable as gibberellins in cone induction per se, has an additional positive effect on tree health which increases over time. The compounding effect of annual or biennial fertilizer applications results in increasing yields over time in addition to those associated with increasing tree size. Regular fertilizer applications also reduce some of the site-imposed limitations to cone production, and as well, induce trees to produce larger quantities of cones earlier.

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Doug Matthews, New Brunswick International Paper, and
Tom Matheson, Scott Worldwide.

Information from gibberellin and fertilizer trials in black, red, white, and Norway spruce has also been used. Participants in these studies include:

Stan Kempton, Bowater Mersey;
Greg Adams, J.D. Irving, Ltd.; and
Peter Nitschke[1] and Al Smith, Nova Scotia Dept. Natural Resources.

Last but not least, sincere thanks go to Laurie Yeates for technical assistance and valuable contributions during all phases of this work.

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[1] Now with Ontario Ministry of Natural Resources

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ABSTRACTS OF WORKSHOP CONTRIBUTIONS

Tree Seed Working Group

Tree Seed Working Group

Front Row: Stéphan Mercier, Peter DeGroot, Guy Caron

Back Row: Frank Schnekenburger, Dave Kolotelo

SEED TESTING FREQUENCY

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The current schedule of testing all 4500 seedlots stored at the Tree Seed Centre (TSC) every two years was reevaluated to determine the extent of species differences in storability. In order to formulate a new schedule a simple linear regression (SLR) approach was used to evaluate the storability of coniferous seeds using the available repeated measures of germination over time. The objective of this analysis was to i) determine species differences in deterioration, ii) formulate a revised retest schedule on a species basis taking into consideration the deterioration rate and the total number of tests which can reasonably be performed by staff in one year.

The variables available were the original germination capacity (GC), the GC at each subsequent retest and the elapsed storage time for each of these retests. Using storage time as a variable to predict germination on a species basis [GERM = a + b * STORAGE TIME] provides an estimate of deterioration based on the slope (b) of the equation ($\Delta GC/\Delta time$). This simple method provides a general estimate of a species deterioration rate and can be expressed in realistic terms as the average decrease in germination per year. For species with a history of more than one test type the test type deterioration rates weighted by sample size was used as the species average and only regressions significant at $\alpha = 0.05$ were considered reasonable species estimates. The species deterioration rates of major species ranged from 0.4%/year (interior Douglas-fir) to 3.7%/yr (western hemlock).

The deterioration rates are useful as a general reference, but how can they be used to aid in formulating a retest schedule. If our objective was test seedlots at intervals to define a 5% change in germination then our frequency for western hemlock would be to test every 1.4 years and for interior Douglas fir every 14 years. This last figure is unreasonable and retesting frequencies should have practical bounds - the TSC has decided that retesting frequencies should be between 1 and 4 years. Other agencies may have different ranges based on the number of seedlots to be tested, species and storage conditions. A curve with the formula [retest frequency = $1 + 3 * (1.4/(e^{\Delta GC/yr}))$] is being used as a guide for determining retesting frequencies, although practical considerations concerning the total number of tests or ongoing research may modify these retest frequencies. This curve was drawn to provide good separation among species as most fall below a 1% change in germination per year.

For species with non-significant regressions the retest frequency was set at 1.5 years to obtain more data on species not following this relationship and to ensure accurate GC results. For species with a variety of tests a reduction in the number of tests performed aids in reducing the number of tests per year. With the proposed reduction of the number of tests and increased efficiency by testing species relative to their deterioration the workload has the potential of decreasing from an estimated 5168 tests/year to 2759. Future work will focus on within species variability at the seedlot level. For those wishing further information a handout was prepared for the CTIA meeting and is available on request.

DORMANCY IN TREE SEED: A PHYSIOLOGICAL APPROACH AND SOME SOLUTIONS

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We can define dormancy as "the state of a healthy and mature seed which does not germinate even in optimal environmental conditions." In fact, the germination mechanisms as well as the type and depth of dormancy of seed lots vary among species, collection sites and years of harvest. Of course, there is no miracle solution which works for every seed species; it is more a case of looking at seed lot by seed lot. Generally, two types of dormancy are described: embryo dormancy and coat-imposed dormancy. Seed may experience one or the other (or both at the same time). Currently, only these two types of dormancy have been described, but it also likely that more exist.

First of all, we must consider that the depth of dormancy may depend on harvesting date, handling conditions as well as the conservation of the seeds. In some cases harvesting seeds at the right time allows seed lots which have reached maturity but not yet deep into dormancy to be obtained. However, there is not sufficient information available about the influence of the harvest date on seed dormancy. Another possible approach involves eliminating abscisic acid (ABA) by producing a constant ventilation during seed conservation. Abscisic acid is derived from mevalonic acid which can be converted either into gibberellic acid or ABA. Gibberellic acid is an important hormone which stimulates the germination process in seed, among others. Germination is always inhibited by the presence of ABA. In other words you can add as much gibberellic acid as you want but germination will not begin until almost all the ABA has been eliminated. In order to bring the seed out of dormancy, it is not adequate to increase the amount of gibberellic acid; the amount of ABA in the seed must also be reduced. This ventilation was made possible by a chimney system or polyethylene 4-mil perforated bags.

Dormancy is still a poorly understood phenomenon. Breaking dormancy in deciduous trees is particularly difficult; in some cases it becomes a limiting factor in the production of these plants. New methods for breaking dormancy are being consequently discovered. All these techniques, while successful on an experimental level, become less effective when performed at an operational level. More research is still needed to develop better methods for breaking dormancy, and to improve the synchronization of germination after dormancy is broken.

ABSTRACTS OF
WORKSHOP CONTRIBUTIONS

Forest Genetics in Light of Climate Change

Chaired by K. Johnsen, Natural Resources Canada

THE ROLE OF PROVENANCE RESEARCH

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Implications of the predicted doubling of CO_2 and rise in mean annual temperature of 2.8 to 4.2°C during the next 60 years are discussed. Western grasslands may extend almost to the Arctic Ocean and eastern cool temperate forests to James Bay, leaving only a narrow strip of boreal forest south of the Arctic Ocean. If such temperatures are reached as rapidly as predicted, there will be insufficient time for ecosystems to migrate as happened after glaciation. If the changes are not as fast, escape into higher elevations in the south and migration north may be possible. Ex-situ gene conservation will be the only option under extreme conditions. One of the expected effects is natural selection by temperature in tree populations under conditions of stable photoperiods, since these will not change. Provenance experiments will play an important role in the monitoring and adjustment to these conditions.

GENECOLOGY AND CLIMATE CHANGE

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Within each species, each natural population of trees has an adaptive profile. That is, a genetic constitution that conveys adaptation to a limited range of environments. Stock transfer beyond these environmental limits results in increased levels of plant stress and damage because the inherent growth rhythm of the stock is not synchronized with the host environment. Over the last 15 years, short-term testing techniques have been developed that assay how closely populations are adapted to local environmental conditions. When these techniques are applied to a sampling of a large number of populations representing the ecological amplitude in a geographic region, models describing the pattern of adaptive variation can also be developed. These models can be used to build biologically sound seed zones and to identify the most appropriate seed sources for reforesting specific locations. When this understanding of adaptive variation is combined with high quality climatic change information, the potential for maladaptation in natural stands can be evaluated. Should natural populations become grossly maladapted due to the rate of climate change, these models can also provide the base for guiding human intervention.

PHYSIOLOGICAL GENETICS RESEARCH ON TREE RESPONSES TO CHANGING ENVIRONMENTS

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Atmospheric CO_2 concentrations are increasing and there is a very high probability that they will double during the next century. Increased CO_2 can influence many aspects of tree performance including growth, dry matter partitioning, water use efficiency and phenology. Increased CO_2 may also result in climate changes such as increased temperatures and drought frequency. Although there is some information on forest tree genetic variation in responses to varying temperatures and drought, there is virtually no information on genetic variation in CO_2 responses.

Decades of provenance testing have clearly shown that forest tree species exhibit substantial gross adaptation to "current" conditions. In some cases, notably shown by the work of Gerry Rehfeldt and Tom Ledig, trees display quite "fine-tuned" physiological and phenological adaptation to the environment. If rapid climate change occurs, there is a potential for increased maladaptation of trees in both natural and plantation forests.

Physiological genetics research can provide information to aid in the prediction of climate change effects on forests and on establishing genetic management options for mitigating climate change effects. But, to have faith in any predictions, we need some more success stories on relating process component traits to presently observed G and G x E. To accomplish this, it is imperative that reliable sources of G and G x E be utilized for study (real genetic differences!). Also, we need to stop searching for the "Holy Grail" of traits which universally explains differences in growth. The importance of any one trait or traits in contributing to differences in productivity and/or survival is most likely population and/or environment specific. In addition, due to compounding, large differences in productivity can accrue due to small differences in instantaneous physiological rates. Situations where, for instance, differences in photosynthetic rates account for genetic differences in productivity will likely occur only when other aspects of tree form and function are similar and where carbon gain limits productivity. The detection of photosynthetic differences of the magnitude expected requires high sampling intensity, quality measurements, and the use of new technologies such as stable isotopes.

Although not new news to forest geneticists, tree physiologists need to be persuaded to use the proper genetic material for study. Experiments can be conducted using "pedigreed" material (both old and newly established) to partition and quantify among and within stand variation in responses to "future environments". Genetic correlations and their structure should be studied (regional, provenance and within-stand levels) in responses to both current field conditions (such as drought) and simulated future conditions (such as elevated CO_2). It is important that we also search for any "hidden" ramifications of current breeding efforts. This includes studying the correlations between fast growth and stress responses of "winners" and "losers" to predicted climate change conditions.

RESPONSE OF FOREST TREES TO CLIMATE CHANGE: DO WE KNOW ANYTHING?

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Predictions of global warming over the next 100 years leave considerable room for doubt. However, there is little doubt that climate has, and will continue to change. In this presentation, I examine the ability of forests to tolerate climate change of any kind, in general. Two kinds of forests are considered: (1) natural forests and (2) plantations of improved trees.

Historical records show that tree species migrate in response to changing climate. But, concerns have been expressed that species migration may be too slow to keep pace with the rapid climate change predicted under most warming scenarios. The expected result is widespread forest death. This argument assumes that species, or populations, cannot exist outside their current environments. However, records of species distributions in the past and man's planting efforts in the present indicate that species *can* inhabit climates outside their current native ones. The tolerances of species to climate conditions are often underestimated by assuming range limits are controlled solely by climatic factors. In many cases, species ranges are determined, instead, by competition with other species. If the physiological tolerances of extant populations are not exceeded, then we might expect gradual invasion of more competitive species without abnormal forest mortality. But, what are the physiological tolerances of a population? The answer to this question is largely unknown. However, it is clear that tree populations harbor generous amounts of within stand genetic variation, presumably providing substantial buffering against changing conditions. Furthermore, provenance tests suggest that trees may be moved to much warmer climates (5°C or more) with little threat of mortality (although colder and droughtier environments present a greater challenge). To more accurately predict the effects of postulated climate change on vegetation, it will be necessary to incorporate both genetic buffering and physiological homeostasis into current vegetation models. Knowledge of host-pest interactions will also be required. The network of provenance tests established by forest geneticists provides a rich source of materials from which much of the required information may be obtained.

Trees that are actively undergoing domestication differ from their progenitor populations due to genetic drift and the application of artificial selection pressures. As in other domesticated organisms, we should expect, over time, (1) reduced variation and (2) departures from optimal fitness under natural conditions in improved populations. Evidence of both may be found in current programs. For example, rare alleles appear to be absent from loblolly pine seed orchards, and several studies have shown a negative correlation between growth rate and stress resistance. In the short-term, however, these changes should induce little increase in vulnerability. The changes themselves are small (given the large base populations and conservative selection strategies employed), and the intensive management that attends improved plantations should reduce stress and increase tree health. In the long-term, however, it is likely that domesticated populations will show greater vulnerability to climate change than natural populations. To assess risk, it would be wise to monitor changes in traits of obvious adaptive significance during the improvement process. Risk may then be reduced by moving improved plantations to suitable sites and/or altering selection criteria. It is also wise to breed, or at least collect, populations of trees having alternative sets of characteristics. Such practices are already being implemented by most aggressive tree improvement programs.

ABSTRACTS OF
WORKSHOP CONTRIBUTIONS

Breeding Strategies of Important
Tree Species in Canada

Chaired by Y.S. Park, Natural Resources Canada, Maritimes Region

Breeding Strategies Workshop

Front Row:	Kris Morgenstern, Yill Sung Park, Greg Adams
Second Row:	Gilles Vallée, Ante Stipanicic, Dale Simpson
Third Row:	Dennis Joyce, Jack Woods, Tim Mullin
Back Row:	Peter Nitschke

BREEDING PROGRAMS AND STRATEGIES FOR DOUGLAS-FIR IN NORTH AMERICA

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Douglas-fir breeding programs in western North America are discussed and compared. The existing programs have followed one of two basic strategies in the first generation; diallels or open-pollinated testing. Large differences in breeding zone size exist between programs. Advanced-generation strategies have been developed and are in progress for some programs. Where these strategies are fully designed, a complementary mating design is being implemented to estimate second-generation parental breeding values separate from material for forward selection. The integration of the breeding programs with operational planting is an important factor in breeding strategy, particularly with regard to subline structure.

ADVANCED GENERATION BREEDING STRATEGY FOR JACK PINE IN ONTARIO

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The foundation of the proposed advanced-generation breeding strategy is based on: the conservation of adaptive gene complexes by ensuring that breeding populations and zones are biologically sound, the maintenance of a minimum effective population size of 100 for managing inbreeding, and the inclusion of marginal gains analyses in determining the optimum selection age. A nucleus breeding system together with a simple mating design will be used to promote continued rapid genetic gains while minimizing the human and financial resource required. Farm-field testing procedures will be used for assessing genetic potential and small-scale field performance tests used for estimating realized gain. A pilot breeding population will be used to work out the details for implementation.

A REVIEW OF BREEDING PROGRAMS AND STRATEGIES FOR LARCH SPECIES

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An outline of the present and future status of larches in Canadian reforestation programs and the lumber industry. The most important pest problems are enumerated, and breeding programs and strategies are described by province. Breeding programs described deal with western larch (*Larix occidentalis* Nutt.) in British Columbia and Alberta; tamarack (*L. laricina* (Du Roi) K. Koch) in Alberta, Ontario, Quebec, New Brunswick, Prince Edward Island, and Newfoundland; Siberian larch (*L. sibirica* Ledeb.) in Alberta, Saskatchewan, and Manitoba; and European larch (*L. decidua* Mill.), Japanese larch (*L. kaempferi* (Lamb.) Carr) and their hybrids in Ontario, Quebec and the Maritimes. For western larch, tamarack and Siberian larch the breeding strategy is based on clonal or seedling seed orchards combined with half-sib progeny tests to evaluate general combining ability. For European larch, Japanese larch, and their hybrids, the breeding strategy is based on intra- and inter-specific mating designs between selected trees to make intraspecific improvement and to evaluate specific combining ability for the production of interspecific hybrids.

AN UPDATED BREEDING STRATEGY FOR BLACK SPRUCE (*PICEA MARIANA* (MILL.) B.S.P.) IN NEW BRUNSWICK

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As an alternative to the current conventional seed orchard breeding, strategies using controlled breeding followed by vegetative multiplication procedures are proposed for implementing the first set of second-generation black spruce selections. The 80 parents are divided into four 20-tree sublines, one of which is an elite subline consisting of the best 20 parents. One purpose for using the elite subline is to use the best parents intensively in the production population. A two-tiered breeding and testing scheme is followed, involving the elite subline and the regular sublines. Within the elite subline, a disconnected diallel mating is used to produce progeny for genetic testing, including clonally replicated, accelerated tests in greenhouse. For the regular Conventional progeny tests, including the families for the elite subline, will be carried out and, subsequently, converted to selection plantations for the third-generation selection.

Papers presented at the workshop on Breeding Strategies of Important Tree Species in Canada

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ABSTRACTS OF POSTER EXHIBITS

C.T.I.A.-TREE SEED WORKING GROUP IS TEN YEARS OLD

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The Canadian Tree Improvement Association's Tree Seed Working Group is celebrating its tenth anniversary this year. Since the group was formed at the 1983 CTIA meeting its membership has increased from 93 to 250. Between 1984 and 1992 the proportion of members working in research and pest control has increased while the proportion working in the seed industry and in tree improvement has decreased. Currently 21% are involved in tree improvement, 18% in seed orchard management, 17% in seed research, 12% in using seed, and 11% are involved in pest control. The balance, in proportionally smaller numbers, work in the seed industry, seed testing or certification, or in seed procurement/processing. Most members are also interested in a variety of seed biology and technology subject areas that are outside the sphere of their employment.

The Group has arranged for tree seed-related subjects to be the topic of workshops or sessions at each CTIA meeting, has twice yearly issued a Newsbulletin, and has, as a Group and as individuals, continually voiced support for tree seed research and technology activities.

CONIFERYL ALCOHOL OXIDASE - A KEY ENZYME OF LIGNIFICATION IN CONIFERS

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The appearance of Coniferyl Alcohol Oxidase (CAO) firmly bound to walls of lignifying tracheids, has been reported to bear a perfect correlation with the occurrence of lignification during wood formation in *Pinus strobus, Abies balsamea, Larix laricina, Picea rubens* and *Pinus banksiana* (Savidge & Randeniya, 1992). As confirmed by combined gas chromatography - mass spectrometry analysis, this enzyme catalyses the oxidation of coniferyl alcohol to dehydrodiconiferyl alcohol and pinoresinol. Under aerobic *in vitro* conditions, CAO converts coniferyl alcohol into hydrophobic globules that are identical in emission color to guaiacyl lignin under UV microscopy (Savidge & Udagama-Randeniya, 1992).

Extraction of a semi-purified solubilized oxidizing factor ("SL PP SOF") containing CAO activity by enzymic digestion of lignifying tissue of *Pinus strobus* was previously reported (Savidge & Udagama-Randeniya, 1992). Isolation and biochemical characterization of CAO, a glycoprotein, is reported here. CAO was purified to homogeneity by buffer precipitation of "SL PP SOF" followed by gel filtration. The purified native enzyme had an approximate molecular weight of 105,000 ± 3,000 Daltons, and deglycosylated CAO was 67,000 ± 1,000 as estimated by SDS-PAGE, hence the enzyme contains 36% carbohydrate. The pH optimum of the enzyme was 6.3 while the isoelectric point defined by isoelectric focussing was 7.45.

Purified CAO possesses a characteristic brown tinge as opposed to the classical blue color of laccases. Attempts at confirming the presence of copper in the protein have not been successful so far. However, extensive substrate specificity studies suggest CAO could be a laccase. The UV/Vis profile of CAO showed absorption maxima at 210 and 258 nm and appeared to be identical to that of *Pyricularea oryzae* laccase (Sigma Chemical Co.) and an in-house purified laccase from *Rhus typhina* latex. The *Rhus typhina* laccase is identical to CAO in both its molecular weight and isoelectric point.

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EFFECTS OF EXOGENOUS INDOLE-3-ACETIC ACID AND GIBBERELLIC ACID ON *IN VITRO* WOOD FORMATION IN "CHIPS" FROM WHITE ASH STEM

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This research attempted to simulate *in vivo* wood formation. Twenty-year old "chips" containing cambia sandwiched between mature phloem and xylem were cut from large-diameter (7 cm) stem regions of a healthy white ash tree shortly before bud-break, and cultured on modified Wolter and Skoog (1966) media. Exogenous IAA and GA_3 (10 mg/L each, both filter-sterilized) were applied individually or in combination to the media. Transverse and radial sections from central parts of chips were stained by the Maule reaction and the Wiesner reaction.

The medium without growth hormones supported cambial cell-division activity, but not differentiation of xylem cell types or lignification. Exogenous IAA and GA_3 individually or in combination promoted cambial cell division, differentiation and lignification of derivatives. IAA typically stimulated vessel differentiation and lignification. GA_3 typically promoted formation of sclerenchyma. IAA and GA_3 in combination promoted differentiation and lignification of vessel elements and also formation of sclerenchyma. With few exceptions, IAA and GA_3 individually stimulated abnormal cambial growth, while together they promoted relatively normal cambial growth. The Maule reaction indicated that exogenous IAA and GA_3 individually or in combination promoted the production of syringyl lignin among both vessel elements and sclerenchyma in newly formed xylem (Srivastava 1966; Sarkanan and Ludwig 1971), and the amount was about the same between these two types of cells. The amount of syringyl lignin appeared to be lower than that of previous-year latewood fibres, but higher than that of previous-year latewood vessel elements.

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ANTI-IAA MONOCLONAL ANTIBODY FOR SCREENING AND EARLY SELECTION

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Monoclonal antibody (McAb, IgG_2) raised against indol-3-ylacetic acid (IAA)-linked through to bovine serum albumin had a high specificity for both acetic acid and imine moities on IAA, and cross reacted with neither the C1 (carboxyl) nor the C3 (indole nucleus) position of related indole derivatives. Enzyme-linked immunosorbent assay based competitive inhibition of anti-IAA McAb combined specificity and sensitivity to a high degree, providing the potential for analysing impure biological samples, such as the vascular cambium. The anti-IAA McAb thoroughly inhibited *in vitro* aerobic oxidation of IAA by both horseradish peroxidase and crude peroxidase preparations from the cambium of jack pine (*Pinus banksiana*). Binding between paratope and IAA epitope inhibited the transition of peroxidase and related IAA* radical formation. No IAA-degradation products could be identified by GC-MS or spectrophotometry when IAA was incubated with peroxidase + anti-IAA McAb. These results indicate that the anti-IAA McAb will be a useful probe in understanding the effects of biosynthesis and catabolism of IAA in tree growth and development, and in employing ELISA for screening and early selection in tree improvement programmes.

THE USEFULNESS OF ANTI-IAA MONOCLONAL ANTIBODIES FOR TREE IMPROVEMENT STUDIES

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Phytohormones are thought to act as agents of integration in growth and morphogenesis. Research over many decades has characterized the effects of exogenous auxin but the involvement of endogenous auxin in these processes has been less than adequately ascertained. Evidence shows that indole-3-acetic acid (IAA) is involved primarily in radial enlargement of cambial derivatives but also affects cellular division and secondary cell wall production to varying degrees while its polar transport is necessary for maintenance of the orientation of the cambial fusiform cells (Little and Savidge, 1987. Plant Growth Regul. 6: 137-169). Antibodies raised to specifically recognize IAA could permit experimentation to focus on the distribution and function of endogenous IAA through immuno-cytochemical techniques such as immunoaffinity purification, immunoassays and immuno-localization. As preliminary steps to such work, anti-IAA monoclonal antibodies were tested for affinity to endogenous IAA as well as for cross-reactivity with other substances native to the cambium of *Pinus strobus*. Through GS-MS analysis of the endogenous substances isolated by immuno-affinity purification, most anti-IAA monoclonal antibodies were found to have significant cross-reactivity but one demonstrated significant binding to only IAA. This antibody is being further characterized and tested for immuno-localization.

REGULATION OF BORDERED PIT DEVELOPMENT IN *LARIX LARICINA* (DU ROI) K. KOCH

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Keywords: Bordered-pit, auxin, cambial development, phytohormones, xylogenesis.

The presence of bordered-pits on the cell walls of vascular land plants has been dated back nearly four hundred million years to the late Silurian period. Bordered-pits can be found in varying degrees throughout the plant kingdom. Variability existing both within and between species exhibits the adaptability of species to better survive and compete in their environment.

As the cambium develops through the growth season, bordered-pits develop to eventually mature and serve as a pathway for water movement up the stem.

Stem "chips" containing dormant cambium sandwiched between mature xylem and phloem from merchantable stem regions of *Larix laricina* (Du Roi) K. Koch were grown as *in vitro* cultures on the surface of an agar medium that has been shown to support normal cambial cell division and xylogenesis. Using 1-naphthalene acetic acid (NAA), an auxin, fusiform cambial cells underwent periclinal divisions to produce enlarged, bordered-pitted, secondary walled, lignified and autolysed earlywood tracheids. The presence of bordered-pits in both tangential (uncommon in earlywood) and radial (normal in earlywood) cell walls suggests a missing factor that controls orientation and localization of the bordered-pit.

Using light and electron microscopy to aid in data collections, different phytohormones alone and in combination and also variations in the medium components will be tested to see how this affects bordered-pit regulation and development.

MULTIPLE-TRAIT COMBINED SELECTION OF JACK PINE FAMILY TEST USING BEST LINEAR PREDICTION

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Best linear prediction or BLP, is a method of estimating breeding values that makes efficient use of unbalanced data. The procedure has some similarities with selection index but differs in providing more precise breeding value estimates to entries with more data.

BLP was used to estimate breeding values for 5 traits, of jack pine trees growing in family test plantations at three locations. Height and diameter of each tree were adjusted for microsite effects by subtracting average within-family deviation of neighboring trees. Variation among families was partitioned into portions due to source areas, stands in source areas, and families in stands. The analysis of variance used locations as the blocking variable, ignoring replications, since the neighbor adjustment effectively reduced within-family variation. Variance components were estimated using the restricted maximum likelihood method, and method of moments height x diameter covariance components were estimated using type III sums of cross products. A file was created with a record for each tree, and variables for tree identity, tree and multi-level family effects for both traits, and family structure values (trees/family-in-location, family, stand, and area, families/stand and area, stands/area) for the tree.

Values were calculated for each element of the matrices required by BLP for each tree, using the tree's family structure values along with the test's variance and covariance components. The matrix algebra expression characteristic of BLP then generated the height and diameter breeding value estimates for each tree.

Observations or scores on stem quality, western gall rust incidence, and wood density were taken on trees chosen subjectively for average or better height and height growth. A procedure similar to that for height and diameter was followed to estimate breeding values of these traits, and of height and diameter in the shorter list of trees to reflect inter-trait relationships. The analysis was simplified by ignoring stands. The two sets of trait breeding values were merged for the short list of trees, then ported to a spreadsheet for more convenient weighting of trait values to calculate total score, followed by sorting and ranking to complete selection.

DENSITÉ DU BOIS ET PRODUCTION VOLUMIQUE DE 22 PROVENANCES D'ÉPINETTE DE NORVÈGE

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L'étude des caractéristiques de l'épinette de Norvège (*Picea abies* [L] Karst) a été faite sur 22 provenances d'Europe Centrale établit en 1969 à Valcartier, Québec. Le diamètre à hauteur de poitrine et la hauteur totale ont été mesurés pour estimer le volume moyen par arbre. Des carottes de sondage de 12 mm de diamètre, prélevées à hauteur de poitrine, sur 20 arbres par provenance, ont servi à évaluer la densité du bois et la largeur des cernes annuels. Les résultats ont démontré la présence de liens négatifs modérés entre le taux de croissance radiale et la densité du bois. Aucune différence significative entre les valeurs moyennes de densité du bois juvénile n'a été détectée entre les provenances. Dû à la présence d'une interaction provenance-bloc significative, il n'est pas possible de conclure sur les différences entre les provenances au niveau de la croissance en hauteur et du patron de variation radiale de la densité. Ces deux caractères sont fortement corrélés avec le lieu d'origine des provenances. Les provenances du Nord-Est ont généralement une plus forte croissance en hauteur et un bois plus homogène que les provenances du Sud-Ouest. Il reste tout de même une forte variabilité individuelle chez ces caractéristiques, 60 à 88 % de la variation totale étant expliquée par les différences entre les arbres d'une même parcelle. Ainsi, la sélection des meilleures provenances suivie d'une sélection massale en deux étapes permettrait de maintenir la densité moyenne du bois, d'améliorer l'homogénéité de la densité du bois juvénile et de doubler la production en volume. Ces résultats confirment le potentiel pour la sélection de provenances d'épinette de Norvège à croissance rapide avec un bois d'une excellente qualité et une bonne adaptation aux conditions pédoclimatiques du Québec.

RELATIVE DENSITY AND VOLUME YIELD OF 22 NORWAY SPRUCE PROVENANCES

A study of Norway spruce (*Picea abies* [L] Karst) characteristics was done on 22 Central European provenances planted in 1969 at Valcartier, Quebec. Diameter at breast height and total height were measured to estimate average volume yield per tree. Increment cores of 12 mm diameter, taken at breast height, on 20 trees per provenance, were sampled to estimate relative density and radial growth. Results indicate a moderate negative correlation between radial growth and relative density. There was no significant difference of average values of juvenile wood relative density between provenances. Due to a significant provenance-bloc interaction, we can not conclude on the differences between provenances for the radial variation of relative wood density and height growth. These two characteristics were strongly correlated with the geographic coordinates of the origin of the provenances. Even though significant differences between provenances exist, 60 to 88% of the total variation is explained by the variation between trees in the same plot. Thus, selection of the best provenances followed by a two-step massal selection should make it possible to maintain the average relative density, to decrease the radial variation of relative density and to double the volume yield. These results

confirm the potential for selecting fast growth Norway spruce provenances with excellent wood quality and good adaptation to Quebec's soil and climatic conditions.

MODIFIED AUGMENTED DESIGN ADAPTED TO NORWAY SPRUCE PROGENY TEST

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Within the Norway spruce genetic improvement program, progeny tests have been planned for each breeding zone. A randomized complete block design adapted from the modified augmented design was developed with respect to our constraints: more than 250 progenies to test, block of a maximum size of 0.2 ha and reasonable establishment and follow-up costs. This type of design uses whole plots comprising control plots and subplots to investigate the general pattern of soil variation. It will make it possible, through ANOVA, to answer such important questions as: (i) is soil variation in the field homogeneous? (ii) is soil variation in one or two directions? (iii) if it is two-directional, are these effects additive? If soil variation is shown to be heterogeneous, we could use three different methods to adjust the data. Three tests were established in 1992 in the Appalachian breeding zone and the tests for the Laurentian breeding zone are presently in the nurseries. We feel that this type of design will be more accurate for analysing the data as we control soil variation.

LE DISPOSITIF ENRICHI MODIFIÉ ADAPTÉ POUR LES TESTS DE DESCENDANCES D'ÉPINETTE DE NORVÈGE

Dans le cadre du programme de génétique et d'amélioration de l'épinette de Norvège, nous avons planifié des tests de descendances pour chacune des zones d'amélioration. Un dispositif de type blocs complets aléatoires a été adapté du dispositif enrichi modifié afin de respecter nos exigences: plus de 250 descendances doivent être testées, le bloc doit avoir une superficie maximale de 0,2 ha et les coûts pour l'établissement et le suivi du dispositif doivent être raisonnables. Ce type de dispositif utilise le principe des grandes parcelles qui comprennent des parcelles témoins principales et secondaires pour vérifier l'homogénéité du sol à l'intérieur des blocs. Il permettra par l'ANOVA de répondre à des questions importantes: (i) est-ce que les variations du sol sont homogènes dans le bloc? (ii) est-ce que les variations du sol sont dans une ou deux directions? et (iii) si elles se produisent dans deux directions, est-ce que leurs effets s'additionnent? Si les variations du sol sont hétérogènes, nous pourrons utiliser trois méthodes différentes pour ajuster les données. Trois tests ont été établis en 1992 dans la zone d'amélioration des Appalaches et les tests pour la zone des Laurentides sont actuellement en cours en pépinières. Comme ce dispositif tient compte des variations du sol, nous croyons qu'il permettra d'améliorer la précision des analyses.

UTILISATION DU CHARANÇON DU PIN BLANC DANS LE PROCESSUS DE SÉLECTION DE L'ÉPINETTE DE NORVÈGE

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Le charançon du pin blanc (*Pissodes strobi* Peck) peut s'attaquer à la plupart de nos épinettes et pins. Essence à haut potentiel ligneux, l'épinette de Norvège (*Picea abies* L. Karst) est une des espèces de prédilection du charançon. Les dommages observés en plantation sont, à ce jour, si important, que ce problème remet en question l'utilisation de l'espèce dans le programme de reboisement au Québec. Toutefois, on observe des variations de susceptibilité au sein des provenances. Il apparait donc possible d'améliorer cette espèce afin d'augmenter sa résistance à l'insecte; l'amélioration génétique pourrait ainsi offrir une solution viable à long terme. Il a été vérifié que l'insecte peut démontrer des préférences alimentaires lors de tests de phagostimulation. L'utilisation de l'insecte comme "bio-indicateur" de la résistance serait un outil précieux de sélection. Afin de vérifier si l'insecte était en mesure de faire une discrimination, sur la base de ses préférences alimentaires, nous avons effectué des tests de phagostimulation au choix et forcés. Le matériel végétal utilisé au cours de ces tests provenait de 45 paires d'arbres jugés résistants et susceptibles à l'intérieur de 23 plantations. Par la suite, des tests de ponte ont été réalisés afin de vérifier si la résistance observée en milieu naturel s'explique par une réduction de la ponte. Lors des tests de phagostimulation, le charançon n'a démontré aucune préférence alimentaire. De plus, à l'analyse des résultats des tests de ponte, nous avons observé la même tendance à savoir que le niveau de ponte est équivalent pour les deux classes d'arbres. Les tests effectués jusqu'à maintenant ne laissent ressortir aucune tendance significative d'une *résistance passive* de la part des arbres sélectionnés comme étant résistants. L'absence de différences significatives dans les tests de laboratoire suggèrent que 1) ces tests ne reproduisent pas fidèlement les conditions naturelles permettant aux insectes de faire leur choix ou que 2) la résistance observée devrait davantage être considérée comme de la tolérance puisqu'il y a développement de tiges de qualité malgré les attaques de l'insecte.

GENETIC-INDUCED VARIATION OF CONTAINER NURSERY-CULTURED INTERIOR SPRUCE IN BRITISH COLUMBIA

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Field foresters in British Columbia have expressed concern about the morphology, physiology and logistics of seedlings derived from seed orchard seed collections compared to that of stock obtained from wild seed collections for interior spruce. Most of the perceived problems can be related to nursery culture. A decade ago, similar concerns were voiced about Douglas-fir stock derived from the seed orchard program, albeit, the stock was mainly bareroot (Mueller et al. 1981). Hawkins (1993) described greater nursery morphological and physiological variation for seed orchard seedlots than that observed for a limited number of wild collection seedlots. Overall, it is difficult to assess the extent of the spruce, seed orchard issue because of the paucity of information on expected variation (morphological and physiological) of wild seedlot ('natural') collections grown in container nurseries.

A pilot project was established at Red Rock Research Station in February 1993 to assess genetic-induced variation of 50 interior spruce seedlots from different sources (a, wild collections ranging from the U.S. to the Yukon borders and from the Alberta border to the western boundary of the Central Plateau; b, seed orchard collections from the Prince George and Shuswap Adams zones; and c, full-sib collections from the Prince George breeding zone (grown in pure and mixed-sib blocks)). Variation will be assessed for the: i) germination period (nursery and laboratory); ii) nursery growth and bud development stages (under 'control' and 'blackout' conditions for selected seedlots); iii) frost hardiness and dormancy induction phases (a wide range of seedlots grown under different photoperiod treatments); and iv) field phenology and growth stages of the nursery-cultured stock (in a common garden at Red Rock). Variation observed during germination and early nursery growth was considerable. It was least in the full-sib collections and greater but of similar extent for the wild and seed orchard seed collections. At this very preliminary stage, the data suggests that seed orchard seedlot nursery variation is not outside the range observed for wild seedlot collections.

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DEVELOPING PROVISIONAL SEED TRANSFER GUIDELINES FOR WHITE SPRUCE IN QUÉBEC

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Ecological regions are currently used to guide seed transfer in Québec because no information is available on patterns of genetic variation and the degree of their relationships with the patterns of environmental variation. Our objectives in this study are: 1) to determine the patterns of genetic variation among seed sources and their relationships with environmental variation for white spruce in Québec and Ontario, and 2) to develop provisional seed zones to guide seed transfer for white spruce in Québec. Seventy-nine seed sources of white spruce were sampled in Québec and Ontario, with each source containing one to five separate open-pollinated families (a total of 342 families). Data were collected on total seedling heights (1-, 2- and 3-year) and 3-year budburst and budset in the greenhouse and nursery as well as 8- and 13-year heights in three field test sites. All traits differed significantly among seed sources and families within seed sources, and they were intercorrelated at both seed source and family levels. Principal component analysis was used to summarize genetic variation in all traits among seed sources into a few principal components. Two principal components accounted for about 87% of the total seed-source variation in all traits, with the first component having high loadings from field traits and the second from seedling traits. Factor scores derived from the two principal components were related to geographical variables: latitude, longitude, and elevation. The regression models accounted for about 50% of the seed-source variation for the two principal components. We will also test the validity of the models by examining the significance of lack of fit to the models by using families as repeats as well as by using different seed sources in other field tests. Provisional seed zones will be developed from the models describing the patterns of genetic variation among seed sources.

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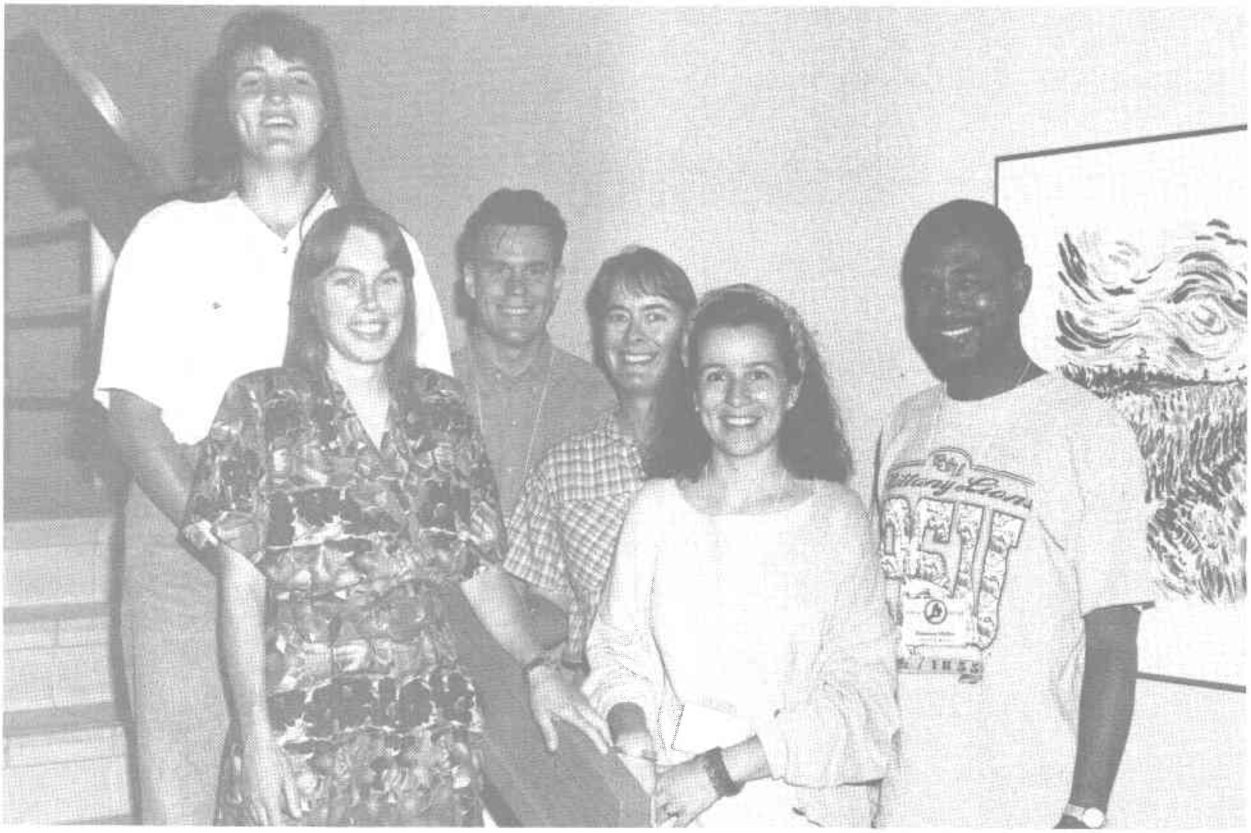
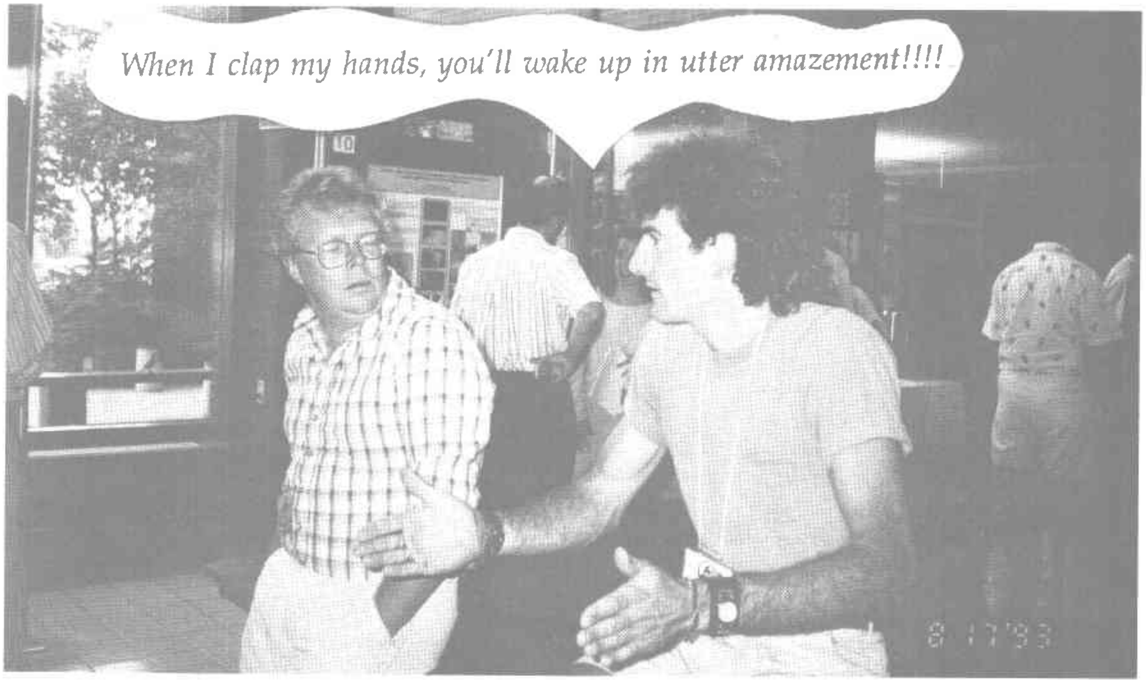
PHOTO-OPS

CTIA/ACAA Sponsored Students



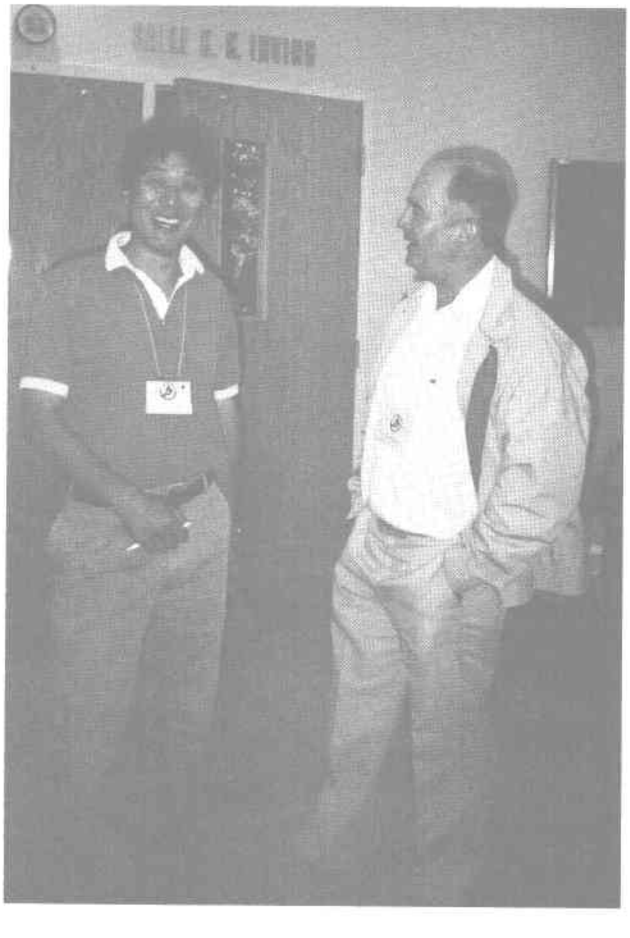
Marilyn Cherry (UBC)
Nathalie Isabel (Laval)
Christine Hansen (U of A)
Kristjen Cameron Vitols (U of T)
Stephanie Nicholas (UNB)
Emanuel Sildor (U de M)

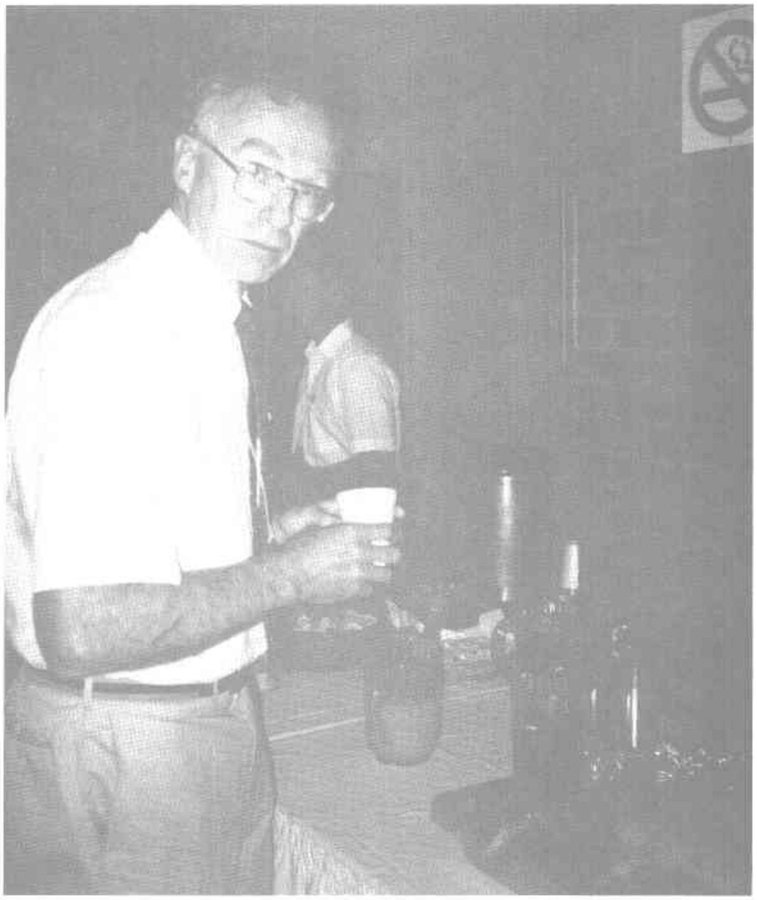


Dave Steeves and Matt Leitch

Gordon, Look at the
camera and smile
they're taking our picture.

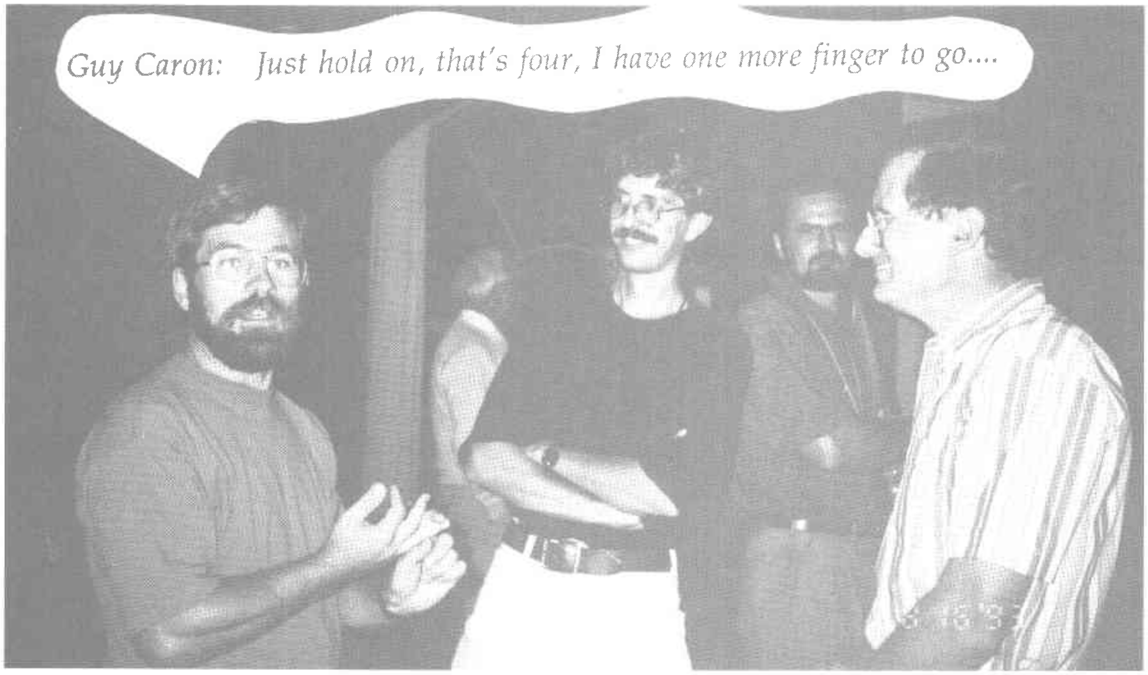
Yill Sung Park and Gordon Murray





Mike Greenwood

Caught going for seconds.



Onlookers: Stephan Mercier, Jean Beaulieu and Ante Stipanicic (anxiously waiting)

I told you to meet me under the apple tree!!

Kathy Tosh and Tony Elders

Greg Adams advising controlled pollination techniques:

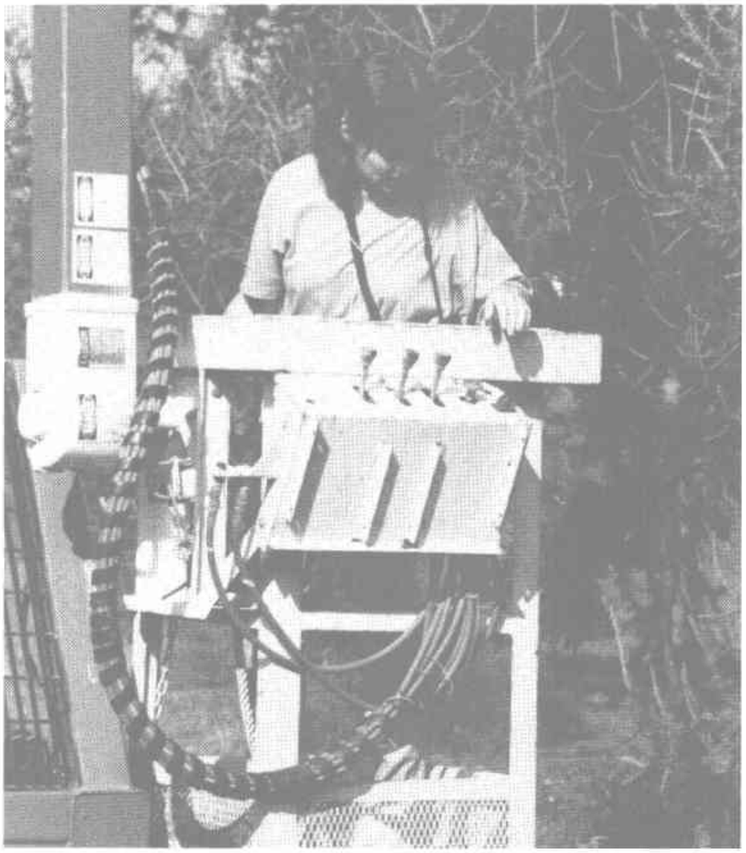
We squeeze them till their buds pop!

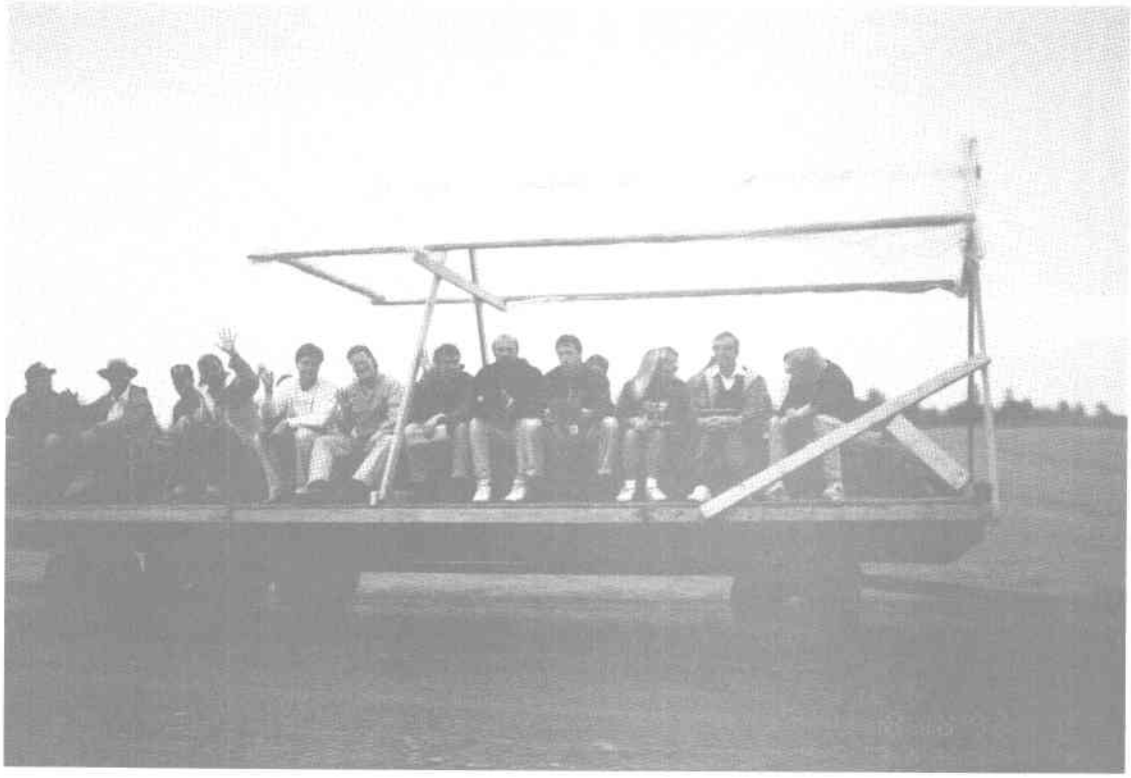
We don't mean to preach, but
if you will turn to spruce:6 and recite...

Diane Roddy...

wondering ...

what will happen when I
push this button?





Which mode of transportation do you prefer?????

Kingsclear Banquet

3rd degree Complex situation's need food for thought...

Howard and Nancy Frame "Lobster Attack"   Score: Lobster = 3
Howard = 1

A HISTORIC DATE

These members attended the
Fourteenth meeting held in Fredericton, N.B. August 28-30, 1973

Thanks, for the history...
of Meetings long ago and friendships that have grown.

Don Fowler, Ante Stipanicic, Bruce Dancik, Jim Coles, Al Gordon,
Kris Morgenstern, Gilles Vallée, Jerry Klein, Graham Powell

The Band

The Jig

The Happy Observer's

Jon Sweeney
Dale Simpson
Yill Sung Park
Ben Sutton
John Russell

And last, but certainly not least,
Sharon Moreau
of the Petawawa National Forestry Institute

Without Sharons excellent computer manipulation skills,
the proceedings of the 24th meeting could never have been published.