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**TREE IMPROVEMENT —
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**AMÉLIORATION DES
ARBRES UN
EFFORT COOPÉRATIF**



CANADIAN
TREE IMPROVEMENT
ASSOCIATION

ASSOCIATION
CANADIENNE
POUR L'AMÉLIORATION
DES ARBRES

PROCEEDINGS
TWENTY-FIRST MEETING
PART 2

TRURO, NOVA SCOTIA
AUGUST 17 - 21, 1987

COMPTES RENDUES
VINGT-ET-UNIÈME CONFÉRENCE
2^e PARTIE

TRURO, NOUVELLE-ÉCOSSE
DU 17 AU 21 AOÛT 1987

EDITORS/RÉDACTEURS
E.K. MORGENSTERN
T.J.B. BOYLE

PROCEEDINGS
OF THE
TWENTY-FIRST MEETING
OF THE
**CANADIAN TREE IMPROVEMENT
ASSOCIATION**

PART 2:
SYMPOSIUM ON
TREE IMPROVEMENT-PROGRESSING TOGETHER

HELD IN
TRURO, N.S.
AUGUST 17-21, 1987

EDITORS:
E.K. MORGENSTERN & T.J.B. BOYLE

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Distributed to Association members and to others on request to the Editor,
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COMPTES RENDUS
DE LA
VINGT ET UNIÈME CONFÉRENCE
DE
**L'ASSOCIATION CANADIENNE POUR
L'AMÉLIORATION DES ARBRES**

PARTIE 2
COLLOQUE SUR
**AMÉLIORATION DES ARBRES - UN EFFORT
COOPÉRATIF**

TENUE À
TRURO (N.-É.)
DU 17 AU 21 AOÛT 1987

RÉDACTEURS:
E.K. MORGENSTERN & T.J.B. BOYLE

**Partie 1. Procès-verbaux et rapports des
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**PROCEEDINGS OF THE TWENTY-FIRST MEETING OF
THE CANADIAN TREE IMPROVEMENT ASSOCIATION**

With the compliments of the Association

Enquiries may be addressed to the authors or to Mr. J.F. Coles, Executive Secretary, C.T.I.A./A.C.A.A., c/o Ontario Tree Improvement Council, Johnston Hall, University of Guelph, Guelph, Ont. N1G 2W1.

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The Twenty-second Meeting of the Association will be held in Edmonton, Alberta, August 14-18, 1989. Speakers will be invited to address the topic of "Test results and their application in tree improvement". Canadian and foreign visitors are welcome. Further information will be distributed in the winter 1988 to all members and to others on request. Enquiries concerning the 22st Meeting should be addressed to: J.I. Klein, Canadian Forestry Service, Northern Forestry Centre, 5320-122 Street, Edmonton, AB T6H 3S5.

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La vingt-deuxième conférence de l'association aura lieu à Edmonton, en Alberta, du 14 au 18 août 1989. Des orateurs seront invités à adresser le sujet de << Résultats d'expériences et les applications à l'amélioration des arbres >>. Les intéressés au Canada et à l'étranger sont les bienvenus. Des renseignements supplémentaires seront distribués au cours de l'hiver de 1988 à tous les membres et à tous ceux qui en feront la demande. Si vous avez des questions à poser concernant la 22^e conférence, veuillez les adresser au: Canadian Forestry Service, Northern Forestry Centre, 5320-122 St., Edmonton, AB T6H 3S5.

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I would also like to extend thanks to the members of the '86-87 executive who made my term as Chairman a rewarding, yet painless experience: Kris Morgenstern (symposium), Howard Frame (local arrangements), Jim Coles (Executive Secretary), Kit Yeatman (Treasurer) and Tim Boyle (Editor). Special recognition must also be given to the other members of the Planning Committee for the 21st meeting whose extraordinary efforts made for an extraordinary meeting: Ted Bulley, Penny Chapman, Don Fowler and Brian White. Thanks are also due to Bill Selkirk and Barb Ballantyne of Petawawa National Forestry Institute for the completion of the Proceedings and for organizing and editing the French abstracts, respectively.

T.J. Mullin, Chairman

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CONFÉRENCIERS INVITÉS

INVITED PAPERS

THE STATUS OF PROVENANCE RESEARCH IN CANADA

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ABSTRACT

Between 1940 and 1992, approximately 900 ha of provenance experiments will have been established in the 10 provinces of Canada, involving 27 coniferous and 12 deciduous species. British Columbia leads in provenance test area established (301 ha), followed by Ontario (215 ha) and Quebec (209 ha). Establishment peaked in the 1970-1974 period and is now declining. Maintenance and measurement of some older key experiments will continue. Of particular interest is the trend in survival which may lead to further modification of seed and breeding zones. Much useful information has become available for the major species but for species of minor importance experimental results are scarce. Some of these species are now becoming more important in reforestation than they have been in the past.

It is concluded that provenance research is making important contributions to silviculture and tree improvement as well as to our understanding of the basic biology of the species.

RÉSUMÉ

De 1940 à 1992, environ 900 ha servant à des tests de provenance auront été établis dans les 10 provinces du Canada; ces expériences auront porté sur 27 essences de conifères et 12 de feuillus. La Colombie-Britannique arrive en tête dans ce domaine avec 301 ha et devance l'Ontario (215 ha) et le Québec (209 ha). Le nombre d'hectares établis a atteint un sommet dans la période 1970-1974 et il est maintenant en voie de déclin. Le maintien et la mesure de certaines anciennes expériences fondamentales se poursuivront. La tendance de la survie est particulièrement intéressante puisqu'elle permettra peut-être de modifier davantage les graines et les zones de reproduction. On dispose maintenant d'informations très utiles sur les principales espèces, mais très peu sur les résultats d'expériences effectuées sur des espèces de moindre importance. Certaines de ces espèces deviennent maintenant plus importantes dans le reboisement qu'elles ne l'ont été dans le passé.

On conclut que les tests de provenance apportent une contribution importante à la sylviculture et à l'amélioration des arbres et nous permet de mieux comprendre la biologie fondamentale des espèces.

INTRODUCTION

The importance of the geographic origin of seed to reforestation has long been recognized in Canada. Sixty years ago A.C. Thrupp pointed out the need for "scientific seed collection" in an article in the Forestry Chronicle. He referred to the tremendous range of environments occupied by such species as Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), Ponderosa pine (Pinus ponderosa Laws.), and red oak (Quercus rubra L.), and reported large differences in hardiness and growth of various seed sources when observed in the nursery. Adaptation to environment should be recognized (Thrupp 1927). Between 1942 and 1945 Dr. Carl Heimburger established the first provenance test plantations of Norway spruce (Picea abies L. Karst.) and white spruce (Picea glauca (Moench) Voss) at the Petawawa Forest Experiment Station (now Petawawa National Forestry Institute) in Ontario. The first graph showing a relationship between height growth of white spruce on the one hand and latitude and summer temperature at the place of origin on the other, was developed from data obtained from one of these plantations (Holst 1955). Following an intensive period of provenance research undertaken by Mark Holst and his associates at Petawawa, Yeatman and Morgenstern (1979) stressed the importance of seed source variation in silviculture and tree breeding. Fowler (1979b) surveyed accomplishments and the need for additional research during the next 10 years.

This paper will review the present state of provenance research, discuss the pattern of geographic variation in native and exotic species as recognized today, and the impact of results on silviculture and tree breeding.

PRESENT STATUS OF PROVENANCE RESEARCH

Survey Results

A questionnaire was sent to each province to gather the following statistics: number of provenances tested, number of tests and hectares (ha) established, and year of establishment. Results received indicated that tabulation of accurate numbers of provenances was not possible because identification of the relevant variables was not uniform. However, we found that the area of experiments established is a good indication of the status of provenance research. This information is recorded in Figures 1 and 2, and Tables 1, 2 and 3.

Provenance research in Canada started in the late 1930's and gained momentum in the mid-1950s (Fowler 1979b). Mark Holst was the driving force for about 20 years following his arrival at Petawawa in 1950. Establishment of long-term field tests increased steadily, peaked in the mid-1970s, and still continues but at a reduced rate (Figure 1). According to the survey, only Alberta and Quebec plan to establish a substantial number of tests in the next five years (1988-1992). British Columbia, Ontario and Quebec are the leading provinces (Figure 2). Many of the tests east of the Rocky Mountains were organized by Petawawa National Forestry Institute in cooperation with the provinces and regional research centres of the Canadian Forestry Service.

As expected, provenance research has been focused on the major native conifers, black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce, and Sitka spruce (*P. sitchensis* (Bong.) Carr.), Douglas-fir, jack pine (*Pinus banksiana* Lamb.), and lodgepole pine (*P. contorta* Dougl.) (Table 1). Considerable attention was devoted to some exotics: Norway spruce, Scots pine (*Pinus sylvestris* L.), and European larch (*Larix decidua* Mill.) (Table 1). Experiments with deciduous species are a recent event and limited in scope (Table 2). Across Canada, the total area of experiments already established and planned for the next five years adds up to about 900 ha.

Variation Patterns by Species

Black spruce. The pattern of geographic variation is predominantly clinal in seedling phenology and growth behavior (Morgenstern 1969a, 1969b, Pollard et al. 1975), a response to natural selection along the gradients of daylength and temperature (Morgenstern 1978). The expression of this pattern was much weaker in field tests at ages 9 to 15 years from seed (Boyle 1985, Fowler and Park 1982, Khalil 1984). Boyle (1985) attributed these differences to growth behavior: free growth is the predominant component in leader elongation of young seedlings whereas in older trees, growth potential is largely predetermined. Khalil (1975b) found that geographic variation in Newfoundland is related to regional temperature, a pattern which he described as ecotypic. An overall evaluation of the range-wide study at age 15 based on 29 experiments in the United States and Canada is currently underway. Survival differences are beginning to emerge in some experiments but need to be followed over a longer time period (Morgenstern and Villeneuve 1987).

Over much of its range and particularly in boreal climates, black spruce occupies a broad range of sites from bogs to uplands. Many of the pure stands on the fertile upland sites originated after fire and are replaced by more tolerant species or become mixed stands in the course of succession (Heinselman 1957). The question whether edaphic ecotypes exist, therefore must take species ecology into account and this has not always been done. Results of a variety of studies provide little evidence for edaphic ecotypes although stand-to-stand differences exist (Morgenstern 1969b, Fowler and Mullin 1977, O'Reilly et al. 1985).

Red spruce. Red spruce (*Picea rubens* Sarg.) received very early attention in investigation of geographic variation with the expectation that populations in unglaciated areas would be of high genetic diversity and would offer the potential to expand the climatic and edaphic range of the species (Heimbürger and Holst 1955). Results from field tests based on samples from the whole range indicated weak population differentiation, inferiority to black spruce or their hybrids in growth and survival, and, therefore, a very low potential of success in silviculture based on clear-cutting and planting (Morgenstern et al. 1981). Another test series with samples from Maine and New Brunswick demonstrated a random pattern of variation independent of geoclimatic variables (Dr. D.P. Fowler, pers. comm.). In Newfoundland the growth of red spruce is inferior to native species and introduction is not warranted (Hall 1986a).

White spruce. The early provenance tests established from late 1950s to mid 1960s identified very productive seed sources in southeastern Ontario, e.g., Beachburg (Lat. 45.7°N, Long. 76.8°W) and Peterborough (Lat. 44.1°N, Long. 78.0°W) (Nienstaedt 1969, Teich 1973, Khalil 1974, Teich et al. 1975). However, in Newfoundland the fast growing Ontario sources had below average survival at 25 years from seed (Hall 1986b). Provenance samples involved in these early studies were usually small and concentrated in Ontario and Quebec. The pattern of geographic variation across the whole range of this species is not yet fully understood (Nienstaedt and Teich 1971). The recently established regional and range-wide experiments with systematic sampling will fill this information gap (Ying 1980, Murray and Cheliak 1985).

Evidence from studies of chemosystematics, cone morphology and seedling phenology points to a major division of the range at approximately 95°W. This hypothesis is supported by the assumption of glacial refugia in the Yukon Valley and Appalachian Mountains. Within each division variation is clinal along latitudinal gradients (Nienstaedt and Teich 1971). Studies using hierarchical sampling within limited geographic areas showed high tree-to-tree variation and low stand-to-stand variation in growth (Dhir 1976), seedling phenology (Pollard and Ying 1979a, 1979b), and cone morphology (Khalil 1975a).

In contrast to black spruce, there is evidence for edaphic ecotypes in white spruce in eastern Canada (Teich and Holst 1974, Murray and Skeates 1985).

Sitka spruce. A clinal pattern of variation south-to-north and coast-to-inland Sitka spruce has been well established. Provenances of northern and inland origins flushed earlier, had a shorter duration of shoot elongation and less volume growth, but were more winter hardy than southern and coastal provenances (Pollard et al. 1975, Falkenhagen 1977, Illingworth 1978b). Seed sources of the Oregon-Washington coast planted on northern Vancouver Island outgrew the local source by 40% in total height in 10 years (Ying, unpublished data). This pattern of clinal variation is most obvious along the Pacific 'fog belt', but become complicated from outer coast to inland by increasing environmental complexity and introgressive hybridization with white and Engelmann spruce (*Picea engelmannii* Parry) (Fowler and Roche 1975). Introgression of white spruce increased the winter hardiness but decreased the growth potential of Sitka spruce (Ying and Morgenstern 1982).

Sitka spruce seems to offer little promise in other regions of Canada (Khalil 1977) although it is an important plantation species in many other countries, Britain in particular (Roche and Fowler 1975).

Engelmann spruce and spruce complex in interior British Columbia. Engelmann spruce hybridizes freely with white spruce wherever they are sympatric (Roche 1969, Fowler and Roche 1975). In British Columbia, in silviculture they are treated as a single species complex - interior spruce. Our understanding of the geographic variation of interior spruce is largely derived from Roche's (1969) geneecological study at the nursery stage and in subsequent field tests of the same material. Elevation of seed source was the dominant geographic variable determining genetic

differentiation; high elevation sources completed shoot elongation and entered dormancy earlier, and produced less dry matter than low elevation sources. Clinal variation with latitude was weak although growth behavior of northern sources was similar to that of high elevations. Provenance performance in field tests after 15 years showed a similar trend associated with elevation (Jaquish et al. 1984).

Jack pine and lodgepole pine. Both are closely related genetically and natural hybridization occurs where their ranges overlap, i.e. in north-central Alberta and between the Peace and MacKenzie Rivers in the Northwest Territories (Rudolph and Yeatman 1982). The natural range of lodgepole pine is limited to Alberta and British Columbia whereas jack pine is distributed from the Atlantic Coast to the Northwest Territories. Both species received the most comprehensive study among our native pine species (Table 1).

Geographic variation in jack pine is characterized predominantly by a clinal pattern across its range (Rudolph and Yeatman 1982). This pattern is associated with latitude (photoperiod) and length of growing season. Heat sum (degree days) at seed origin is the most important environmental variable explaining the response among seed sources (Yeatman 1974). Adaptive traits such as growth behavior, phenological events and winter hardiness showed stronger clinal trends than branching habit, foliage color, seed yield, and tolerance of insects and diseases (Rudolph and Yeatman 1982). Hierarchical analysis revealed little stand-to-stand genetic differentiation and much variation among families within climatic regimes (Yeatman 1975).

Lodgepole pine provenance studies have been concentrated in British Columbia and a comprehensive provenance test program was organized in 1960. Sharp geographic differentiation occurred along the coast-interior division resulting in distinct differences between the coastal and interior forms of the species, which were classified by Critchfield (1980) as P. contorta ssp. contorta (shore pine) and P. contorta ssp. latifolia (Rocky Mountain pine). They differ not only in gross morphology, but also in less obvious features such as leaf anatomy. Coastal provenances can be readily distinguished in the nursery and field tests by their shorter, thicker and darker green foliage and susceptibility to frost (Illingworth 1971, 1975, Ying and Illingworth 1986). A high number of stomata per square millimetre of needle surface is an important adaptive trait associated with coastal sources. A strong north-south clinal pattern along the Pacific Coast was evident in growth, autumn frost damage and other traits, but became less discernable in the interior where the complex interplay of mountains, the maritime air from the Pacific and cold air from the Arctic tend to obscure north-south environmental gradients and the associated pattern of variation (Illingworth 1975).

Long-term field tests exist mainly in interior British Columbia where lodgepole pine is a major commercial species. Provenance response to environments throughout the interior of the Province indicated broad regional patterns, a strong influence of elevation of seed origin, and weak provenance x site interaction (Illingworth 1978c, Ying et al. 1985, O'Reilly 1986, Yanchuk 1986). The main distinguishing features of sources from different geographic regions are:

<u>Geographic region</u>	<u>Features</u>
Pacific coast	Least hardy (repeated winter damage)
Coast-interior transition	Susceptible to frost and disease
Yukon and northern B.C.	Susceptible to disease, slow growing
Central and southern B.C.	Hardy, large variation in growth and tolerance of disease (high elevation sources slow growing and susceptible to disease).

Red pine. Red pine (*Pinus resinosa* Ait.) is known for its genetic homogeneity (Fowler and Heimbürger 1969a). Field tests confirmed this low genetic variability, but a latitudinal pattern of geographic variation can still be detected (Park and Fowler 1981) and use of wrong sources can result in substantial loss in productivity (Roller 1968, Klein 1976).

White pine. Both eastern white pine (*P. strobus* L.) and western white pine (*P. monticola* Dougl.) have been very valuable timber species in Canada for a long time. More work has been done in Canada with eastern than with western white pine. Even so, only very few samples of eastern white pine have been taken in Ontario and Quebec where the range extends into boreal regions. Both species exhibited less genetic variability than other species with a similar ecological range and they were marked by extreme phenotypic plasticity. Provenance samples of eastern white pine covering 13 degrees of latitude, 30 degrees of longitude and 2300 m of elevation showed little differences in winter hardiness, although a broad latitudinal cline was detected in growth (Fowler and Heimbürger 1969b). Similarly, sampling of parts of the range of western white pine failed to detect geographic, ecological, or elevational patterns of differentiation (Rehfeldt and Steinhoff 1970, Steinhoff 1981). Three broad latitudinal zones could be delineated only from samples representing the entire range, approximately 20° latitude (Rehfeldt et al. 1984).

Coastal Douglas-fir. Douglas-fir, the most important timber species in the coastal region, has been planted in British Columbia since 1930. Systematic provenance research in British Columbia began and a network of field tests was established between 1969 and 1975. Populations exhibited a broad pattern of geographic variation parallel to major climatic gradients from maritime to subcontinental areas. Beyond these major trends, provenance variation was only weakly correlated with environmental and ecological factors indicating no discernible pattern of local adaptation (Illingworth 1978a, Ying, unpublished report). Provenances from northeastern Washington were fast growing and showed remarkable stability over a wide range of environments.

Other native species. We know very little about geographic variation of the true firs, the larch species and most of the deciduous species. Systematic provenance testing of some of these species has been initiated in recent years, e.g. the true firs, tamarack, and some of the deciduous species (Tables 1 and 2). No doubt we will learn more about them as time goes on. Because of limited resources, we cannot expect to develop comprehensive field experiments for species with low commercial value.

Exotic species. Provenance testing of exotic species has been undertaken mostly in eastern and central Canada. Norway spruce is the most widely planted exotic conifer and has been most extensively tested, but none of the tests sample the entire elevational and latitudinal range in systematic fashion. For descriptions of the variation pattern we must therefore go to European investigators (Schmidt-Vogt 1977). Fowler (1979a) points out that this species encounters biotic agents in North America not found within its natural range, particularly the white pine weevil (Pissodes strobi Peck) and spruce budworm (Choristoneura fumiferana Clemens). In the colder continental climates of central Canada winter drying of provenances from milder areas (low-elevation areas of western Europe) is also a serious problem. In spite of these problems, experiments indicate a potential of certain provenances to outgrow white spruce on carefully selected, slightly acid sites, particularly in the Maritime provinces which are within the temperate zone (Fowler 1979a). In the boreal and extreme continental areas of Canada, Norway spruce introduction is probably not justifiable (Klein 1977).

Scots pine from the Soviet Union was tested in Canada and results showed that the northern Ukraine and southern Russia is a region of potential seed sources (Teich and Holst 1970). In the Prairie Provinces there was extensive insect and disease damage resulting in poor form in later ages (Klein 1979) but in Ontario and Quebec a number of good stands exist which regenerate naturally.

Japanese larch (Larix leptolepis (Sieb. et Zucc.) Gord.) and European larch and their hybrids were tested extensively. Japanese larch showed high variation among provenances but there was no geographic pattern related to latitude, longitude or elevation (Park and Fowler 1983). Assessments of plantations of both species and hybrids in eastern Canada indicated yields of 7 to 14 m³ per ha per year on good sites, which could be further augmented by a selection program (Park and Fowler 1983, Vallée and Stipanovic 1983). Two recent workshops in Ontario and New Brunswick have summarized the information on variation in the genus Larix Mill. and of prospects for breeding (Graham et al. 1983, New Brunswick Forest Research Advisory Committee 1986).

DISCUSSION

This review has shown that geographic variation received very early attention in tree improvement but that early tests were exploratory in nature involving small numbers of provenances, typically 30 or less, and often were not replicated.

Our information on geographic variation is largely derived from the newer test established since about 1955 in eastern Canada and after 1960 in western Canada. These newer tests were carefully designed with extensive and systematic provenance samples, e.g. the cooperative range-wide tests of jack pine, black spruce and white pine organized by Petawawa National Forestry Institute, and the lodgepole pine, coastal Douglas-fir and Sitka spruce provenance tests established by the B.C. Forest Service.

The degree of geographic differentiation varies with species: red pine and both western and eastern white pine showed little geographic differentiation even though ecological magnitude within their natural range is comparable to that of many other species. Most species, however, exhibited clinal patterns parallel to environmental gradients. Clinal differentiation is expected from natural selection, but the degree of parallelism in clinal trend varies from species to species, and also from one part of the species range to another. For example, Sitka spruce and shore pine exhibited a high degree of parallelism following the north-south gradient along the Pacific Coast. This north-south parallelism was not as obvious in coastal Douglas-fir, although the type occupies a similar range of environments. From coast to inland, the increasing complexity of environmental gradients tended to obscure this parallelism in clinal trends in both Sitka spruce and lodgepole pine (Illingworth 1987 b, c, Ying et al. 1985). Provenance test results seem to indicate low parallelism in clinal trends (broad regional variation) in most conifers. Predictability of provenance performance would be high with species showing a high degree of parallelism, which would render the task of seed zone delineation and control of seed transfer much simpler.

Despite the fact that the newly established tests usually involve large provenance samples, often over 100, the samples are far from adequate to unravel variation patterns within regions. Different adaptive patterns of variation could have evolved within a limited geographic area (Campbell 1987). Although we now have an idea of major geographic trends there is still a long way to go before we fully understand the underlying evolutionary processes and adaptive mechanism in relation to regional and local environments.

Furthermore, most tests are too young to determine survival trends over a whole rotation period. Studies of Douglas-fir pursued over a 60-year period indicate that decimation of non-adapted sources began after age 30 (Silen 1978). Long-term studies of several species in Europe underline the critical role of survival. At greater age, stocking may control volume production more strongly than height (Campbell 1974, Oleksyn and Giertych 1984). It is therefore important to maintain existing experiments for several more decades so that survival can be followed and seed zone boundaries be adjusted as new information becomes available.

SILVICULTURAL IMPACT

The results of provenance research are being used in several ways in silviculture. In British Columbia, the existing seed zones based on ecological classifications have been revised according to the geographic variation patterns derived from provenance tests of lodgepole pine and interior spruce (Anon. 1987). These new zones and guidelines for seed transfer provide the framework for decision on selection of seed sources, parent-tree selection and seed orchard planning (Lester et al. 1988). Provenance test results are also used to guide seed collection and distribution in the Maritime Provinces and in Ontario, and to develop breeding zones (Murray and Skeates 1985, Boyle 1985, Fowler 1986). In Quebec, stands designated for cone collection are chosen on the basis of

available provenance test results, and regulations are frequently updated (Y. Lamontagne, pers. comm.).

In reforestation, after a choice of species has been made, selection of the best available seed sources comes next. The most tangible impact of provenance testing is the identification of productive seed sources. A few of the better known examples are the Ottawa Valley white spruce, coastal Douglas-fir from northwestern Washington, and Sitka spruce from the Oregon-Washington coast. By planting these productive sources on the right sites, increases in wood production can be substantial, e.g. 40% in volume over a local source by planting Oregon-Washington Sitka spruce on Vancouver Island (Ying, unpublished data).

Time is the most valuable ingredient in research with tree species. Despite many compromises in design and sampling of the older provenance tests (Fowler 1979b), they are very valuable. Genetic variation in certain traits takes many years to develop. Tremendous variation among provenances of Sitka spruce was observed in the ability to deter and recover from weevil (Pissodes strobi Peck) attack (Ying, 1986), of Sitka and coastal Douglas fir to recover from deer browsing, and of lodgepole pine to overcome frost injury. Much of this information will be lost when assessment is limited to growth and survival. Such old tests are also invaluable for interdisciplinary research, e.g., assessment of site specific productivity, study of plantation ecology, and impact of intensive silviculture on wood density.

Other, more intangible benefits of provenance research are the experience gained in seed collection and raising of nursery stock, which can be used to improve silvicultural practices, particularly of lesser known species.

CONCLUSIONS

This review has presented the major facets of variation in our most important species. After 45 years of work a much better understanding exists of basic species biology, and this knowledge has been applied to improve methods of silviculture and tree improvement. At the same time, the development of more comprehensive reforestation programs involving a greater variety of species is calling for investigation of additional species, e.g. yellow cedar (Chamaecyparis nootkatensis (D. Don) Spach) and red cedar (Thuja plicata Donn) on the west coast and some hardwood (deciduous) species in eastern Canada. As Tables 1 to 3 indicate, experiments with species of the genus Abies L. and several hardwoods have been recently established and early results are available.

Beyond this, it is necessary to recognize the fact that we have few results that allow final conclusions. We are too optimistic if we rely on field experiments that are only 10 to 20 years old and if we limit our attention to tree height. Particularly when seed sources have been moved considerable distances, changes in ranking are still taking place and slowly declining survival can be expected to influence volume production. The maintenance and continued observation of the major experiments is therefore required.

In the final analysis, the study of variation in relation to the complexities of local environments is a matter of combining studies of population structure with long-term field tests. Isoenzyme methods, greenhouse and nursery tests of open- and control-pollinated material allow the handling of large samples at a reasonable cost and often lead to results that complement those from field tests or other more traditional methods (Park et al. 1984, Rehfeldt 1984, Yeh and Arnott 1986). The integration of results from such diverse methods is promising. A detailed knowledge of the genetic structure of our forests is needed, not the least to support breeding programs that are now beginning to make an impact in all regions of the country.

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Table 1. Area of conifer provenance tests established and planned (period 1940-1992).

Species	ha	Establishment years
<u>Abies</u> <u>amabilis</u> (Dougl.) Forbes	1.5	1980
<u>balsamea</u> (L.) Mill	1.9	1960-78
<u>grandis</u> (Dougl.) Lindl.	7.5	1980-84
<u>procera</u> Rehd.	11.0	1981
<u>Larix</u> <u>decidua</u>	6.0	1977-80
<u>laricina</u> (Du Roi) K. Koch.	32.2	1974-92
<u>leptolepis</u>	10.0	1973-82
<u>sibirica</u> Ledeb.	2.5	1972-86
<u>Picea</u> <u>abies</u>	78.7	1941-91
<u>glauca</u>	49.1	1958-87
<u>mariana</u>	49.1	1957-87
<u>rubens</u>	8.2	1958-86
<u>sitchensis</u>	30.5	1969-85
<u>Pinus</u> <u>banksiana</u>	58.4	1954-92
<u>contorta</u>	165.3	1972-91
<u>monticola</u>	7.0	1974-75
<u>nigra</u> Arn.	8.0	1973-83
<u>resinosa</u>	29.2	1954-83
<u>strobus</u>	4.7	1943-86
<u>sylvestris</u>	35.6	1940-86
<u>Pseudotsuga</u> <u>menziesii</u>	101.8	1957-91
<u>Tsuga</u> <u>heterophylla</u> (Raf.) Sarg.	47.0	1971
TOTAL	745.2	

Table 2. Area of provenance tests of deciduous species established and planned (period 1940-1942).

Species	ha	Establishment years
<u>Acer saccharum</u> Marsh.	1.0	1978
<u>Alnus cordata</u> Lois.	1.0	1989
<u>glutinosa</u> (L.) Gaertn.	19.1	1963-89
<u>incana</u> (L.) Moench	2.5	1986-89
<u>rubra</u> Bong.	4.5	1985-89
<u>Betula alleghaniensis</u>	1.3	1965
<u>Fraxinus americana</u> L.	0.7	1977
<u>pennsylvanica</u> Marsh.	2.0	1974
<u>Populus trichocarpa</u> Torr. & Gray	28.0	1985
TOTAL	60.1	

Table 3. Area of provenance tests of lesser species (area less than one ha) including combined provenance/species trials (period 1940-1992).

Species	ha	Establishment years
<u>Larix</u> spp.	4.0	1953-86
<u>Picea</u> spp.	53.0	1942-86
<u>Pinus</u> spp.	19.1	1940-87
<u>Pinus mugo</u> Turra	1.0	1985
<u>Pinus ponderosa</u>	2.0	1975-90
<u>Picea engelmannii</u>	0.4	1953
<u>Picea pungens</u> Engelm.	0.7	1985
<u>Thuja occidentalis</u>	1.0	1980-86
<u>Populus nigra</u> L.	1.0	1992
<u>Prunus serotina</u> Ehrh.	0.4	1969
<u>Tilia cordata</u> Mill.	0.3	1982
TOTAL	82.9	

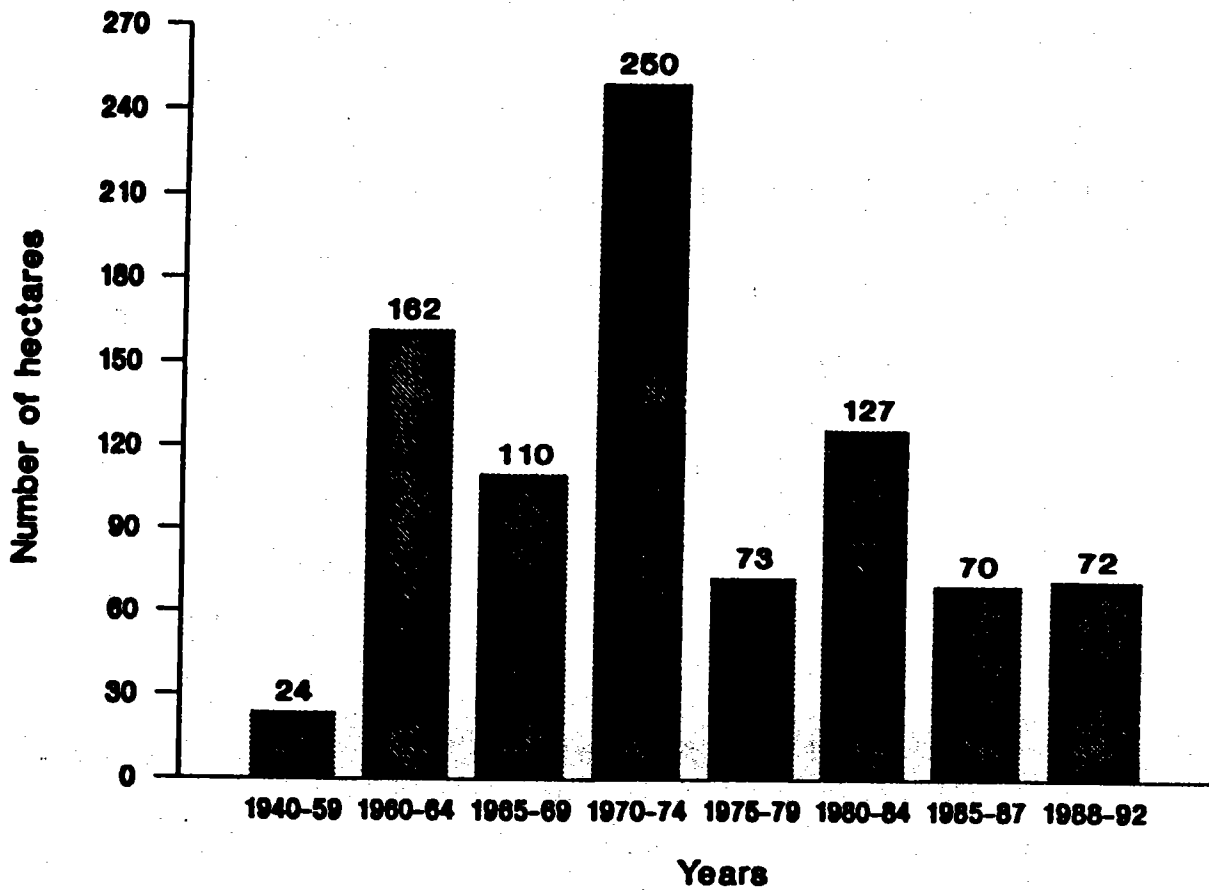


FIGURE 1. Chronological distribution of provenance tests (period 1940-1992).

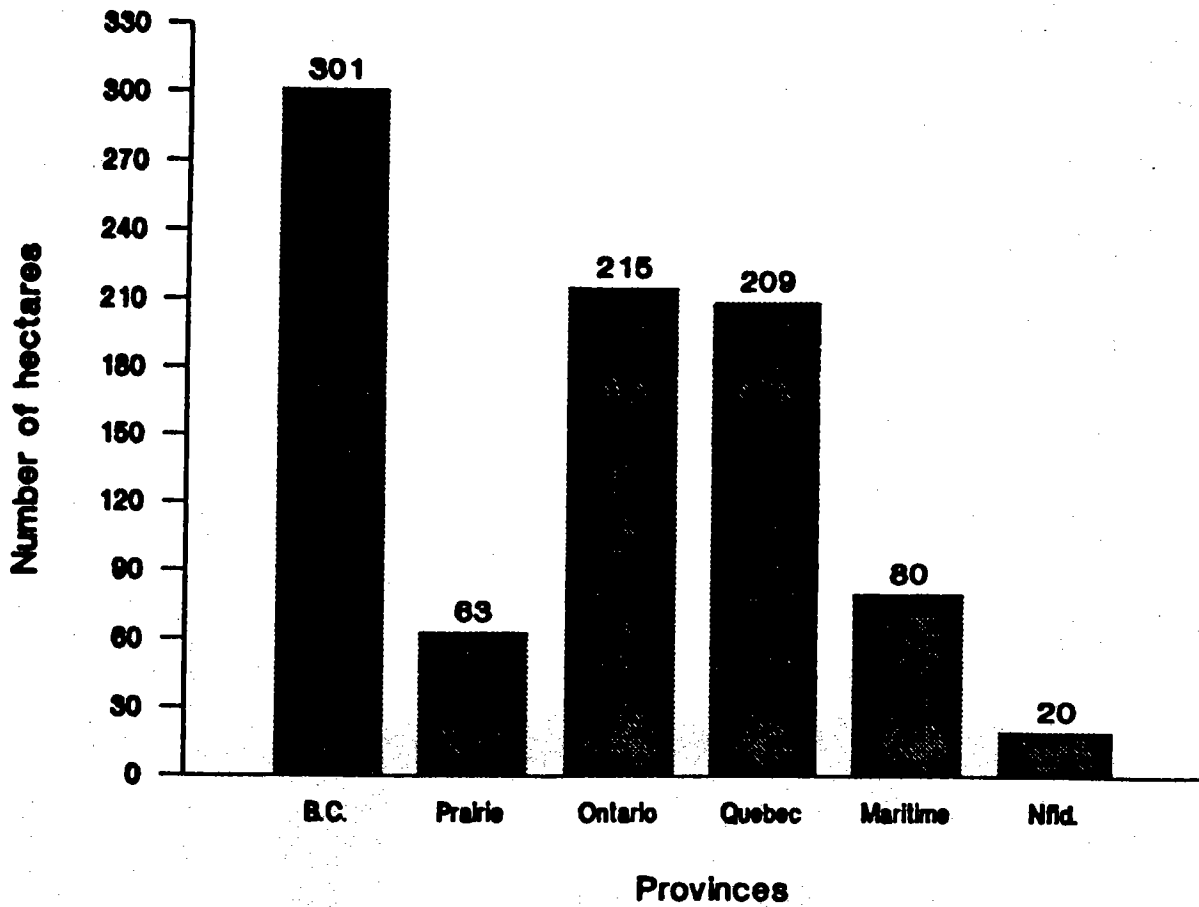


FIGURE 2. Regional distribution of provenance tests (period 1940-1992).

WITHIN-POPULATION GENETIC VARIATION -
IMPLICATIONS FOR SELECTION AND BREEDING

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ABSTRACT

Methods for measuring genetic variation within populations are described and results summarized from studies on Canadian forest trees. Most species demonstrate high levels of variation, with generally more than 90% of the total variation contained within populations. The implications of variation in allele frequencies and mating system parameters are discussed in relation to selection, breeding, and conservation. It is argued that knowledge of within-population variation is required, not only for efficient selection in natural populations, but also in order to design optimum breeding and production populations and tree improvement strategies. Some major gaps in current knowledge are also listed.

RÉSUMÉ

On décrit les méthodes pour mesurer la variation génétique à l'intérieur des populations, et on résume les résultats d'études effectuées sur des arbres forestiers du Canada. La plupart des espèces présentait des variations considérables; généralement plus de 90 % de la variation totale se manifestait à l'intérieur des populations. On expose les répercussions de la variation de la fréquence des allèles et des paramètres du système de croisement en relation avec la sélection, l'amélioration génétique et la conservation. Il faut connaître les variations au sein des populations, non seulement pour la sélection efficace chez les populations naturelles, mais aussi pour mettre au point des stratégies optimales d'amélioration des arbres ainsi que des populations optimales de production et d'amélioration génétique. On énumère également certaines lacunes dans nos connaissances actuelles.

INTRODUCTION

The fact that genes vary in their effects, and that individuals inherit different combinations of these genes, provide the basis for genetic improvement of forest trees. The contribution of forest tree breeders towards the optimization of silvicultural practice, therefore, lies in the creation of progressively improved breeding populations so that successive generations of forest regeneration on different sites can

yield cumulatively better forest products. This is achieved through cycles of selection, in which genetic variabilities of economic traits are measured, manipulated, and delivered so as to improve the population average of the traits over time. An additional consideration is that the rate of genetic progress should be as rapid as possible. Genetic gain (ΔG) per unit time (T) can be formulated as:

$$\Delta G = i \sigma_p h^2 / T$$

where i is the intensity of selection, σ_p is the phenotypic standard deviation, and h^2 is the heritability of the trait. Maximising ΔG therefore requires efficient control of all four factors.

Developing an efficient improvement strategy requires genetic knowledge pertaining to the breeding population, its selection methods, criteria, and sizes (Comstock 1977). Indeed, as Guries and Ledig (1977) noted, a breeding strategy inappropriate to the actual population structure of a species will reduce the genetic gain achieved. Thus the sum total of the ecological and genetic relationships among individuals and the population they comprise (i.e. population structure) (Jain 1975) must be known. The term "population" requires further elaboration before discussion on within-population genetic variation can proceed. For forest trees, a "population" has usually been considered to be synonymous with a stand. However, in population genetics, a "population" is a panmictic group of individuals among which the probability of a mating event occurring between any two individuals is equal.

An individual's genotype at loci controlling an economic trait determines its breeding value. Thus the frequency distribution of genotypes in a population at any given time is of major interest to geneticists and breeders. The mating system of a population determines the pattern in which gametes unite (Allard et al. 1975), and consequently exerts primary control on the genotypic frequency distribution of subsequent generations. The mating system can be defined by two parameters, (i) the outcrossing rate (t), and (ii) allele frequencies in the outcrossed pollen pool (p_i). Important questions about variation in the mating system are: whether t is constant for all genotypes, and whether t and p_i are changing through time and space (Allard et al. 1975).

Extent and pattern of gene flow, through the transfer of pollen and seed, further determine the spatial arrangement of genotypes in forest trees. Restricted gene flow and within-population differentiation due to microsite selection pressure cause the formation of neighbourhoods, or family clusters, among which allele frequencies will differ. Such population subdivision brings about an excess of homozygotes when trees from the neighbourhoods are pooled, a phenomenon known as Wahlund's effect (Wahlund 1928). Restricted gene flow also reduces effective population size. This will increase genetic drift (Kimura and Crow 1963) and also the level of inbreeding due to mating among relatives.

Differential self-compatibility among genotypes causes heterozygote deficiencies (Brown 1975). In contrast, differential sexuality among genotypes, a situation often observed in forest trees (e.g. Schoen et al.

1986; Schoen and Stewart 1987; Cheliak et al. 1987), will result in an excess of observed heterozygotes, whilst differential fertility can cause either effect, depending on whether heterozygotes or homozygotes are the more fertile. Polyembryony and post-zygotic selection can also cause an excess of observed heterozygotes (Park and Fowler 1983). Thus the mating system and the major evolutionary forces of selection, migration, and drift combine to produce the observed allele and genotypic frequencies in populations, their spatial distributions, effective sizes, inbreeding coefficients, and gene flow, all of which may vary within and among populations and over time.

Measuring the amount and organization of genetic variation in economic traits of forest trees has been problematical in the past. In addition to the long duration of the tests, the identification of the effect of individual genes is formidable because most morphological characters are environmentally sensitive and are subject to polygenic inheritance and some degree of non-additive gene effects. In other words, the observed phenotype (P) is the sum of the genotype (G), the environment (E), and the interaction of genotype and environment (GE):

$$P = G + E + GE$$

The genotype can be further partitioned into additive (A) and non-additive (NA) variation, so:

$$P = A + NA + E + AE + NAE$$

Since the breeding value for a trait depends solely on A, if NA and/or E are non-zero and cannot be separated from A, observation of the phenotype gives no information on genetic variation.

In the past several decades, the use of morphological characters for studying the genetic structure of forest populations has been supplemented by biochemical methods. One such method is enzyme electrophoresis, which enables the detection of molecular differences in enzyme proteins (Lewontin and Hubby 1966). Although not shown to be of direct interest for breeding, enzymes offer a number of advantages over morphological or many other biochemical characters: (1) genetic inheritance and linkage of enzyme variants (allozymes and isozymes) can be easily demonstrated; (2), expression of allozymes is generally codominant so that homozygous and heterozygous genotypes can be differentiated from each other without the necessity of genetic crosses; (3) genotypes are scored directly without any epistatic interaction; (4) the estimate of genetic variation is directly quantifiable and can be compared between populations or species; and, (5) investigation of multilocus genetic organization in maternal trees and pollen gametes is possible (Yeh and Morgan 1987).

The advent of enzyme electrophoresis has led to the development of various measures of gene diversity within populations. These include expected and observed percentage of heterozygous loci per individual, percentage of polymorphic loci, average number of alleles per locus (e.g. Yeh and Layton 1979), and effective number of alleles per locus (Brown and Moran 1979). It should be noted that because only about 27% of the total

enzyme variability is detectable by electrophoresis (Lewontin 1974), the observed percentage of heterozygous loci should be adjusted accordingly.

Several measures have been derived to quantify the distribution of this genetic diversity in forest populations. The chi-square test of Workman and Niswander (1970) measures the significance of heterogeneity in allele frequencies among populations. The F -statistics of Wright (1965) or, when genotypic frequencies are not available, the G -statistics of Nei (1973) can be used to partition the genetic variation among different hierarchical levels of the population structure. The extent of dissimilarity in allele frequencies among populations or among individuals within a population can be measured with the genetic distance (D) of Nei (1975).

Enzyme electrophoresis has three major drawbacks despite enjoying a number of distinct advantages over morphological markers for surveys of genetic variation in forest populations. Firstly, about 27% of amino acid substitutions will result in surface charge alteration, as noted above. Thus a large percentage of amino acid substitutions in forest populations remain as "hidden" variation undetectable by enzyme electrophoresis. Secondly, there is reason to suspect that electrophoretically detectable variations do not constitute a truly random sample of the genome because they represent primarily structural genes. Thirdly, the biological significance of enzyme variation observed in natural populations has been an issue of strong debate (Lewontin 1974).

Molecular techniques now exist that allow the isolation of DNA. These provide the opportunity to conduct a complete inventory of all nucleotide sequences in the DNA of each population member (Sanger et al. 1977), approaching the ideal measure of genetic variation proposed by Brown (1978). However, molecular techniques are costly so, except in laboratory organisms, the technique has to date been largely confined to accessible and comparatively small DNA molecules, such as those found in mitochondria and chloroplasts. An alternative to DNA sequencing is protein sequencing by such methods as the Edman degradation (Stryer 1981). Because of redundancy in the genetic code, protein sequencing is not as sensitive as DNA sequencing, and is subject to the same disadvantages as isoenzymes.

POPULATION STUDIES OF FOREST TREES

Morphometric Studies

i. Genetic variation

Progeny tests of most forest tree species, or provenance tests in which individual tree progeny have been kept separate, have generally demonstrated high levels of within-population variation, compared with variation among populations. Such is the case for lodgepole pine (*Pinus contorta* Dougl.) in British Columbia (Ying et al. 1985), the white spruce (*Picea glauca* [Moench] Voss)/englemann spruce (*P. engelmannii* Parry) complex (Kiss 1986), white spruce in Quebec (Corriveau et al. 1986), jack pine (*Pinus banksiana* Lamb.) in Ontario (Yeatman 1975), and for many other cases. A notable exception is red pine (*Pinus resinosa* Ait.), for which

variation in economic traits is very low (Fowler 1964). On the other hand, population repeatabilities tend to be several times larger than within-population heritabilities, so levels of variation do not need to be as great in order to achieve similar genetic gain.

ii. Mating system parameters

Morphometric studies of inbreeding and mating system parameters are scarce due to the lack of marker genes. Morgenstern (1972) estimated inbreeding coefficients for black spruce (Picea mariana [Mill.] B.S.P.) in six regions of Ontario from analysis of a hierarchical structure of variance components in terms of an additive genetic model. Under the assumptions that non-additive genetic variation was absent and inbreeding alone caused population differentiation, inbreeding estimates ranged from 0.08 for small, isolated stands in the south, to 0.03 for the large populations of the north. Coles and Fowler (1976), Fowler and Park (1983), Park and Fowler (1983), and Park et al. (1983) used controlled pollinations to estimate natural inbreeding and selfing rates in white and black spruce. They found self-compatibility varied widely among individuals and white spruce, in particular, appeared to form distinct neighbourhood structures.

Biochemical Studies

i. Genetic variation

Information on gene diversity within and among populations of many northern temperate conifer species, based on isozyme data, is summarized in Table 1. With the single exception in red pine, which had no apparent genetic variation due to a historical, hypothesized population bottleneck (Fowler and Morris 1977), levels of allozyme variation were moderate to high for most species. The percentage of polymorphic loci ranges from 51% for Sitka spruce (Picea sitchensis [Bong.] Carr.) (Yeh and El-Kassaby 1980) to almost 92% for both black spruce (Boyle 1985) and Norway spruce (P. abies Karst) (Lundkvist 1979). The extremes of heterozygosity per locus are 0.119 for lodgepole pine (Wheeler and Guries 1982) and 0.41 for Norway spruce (Lundkvist and Rudin 1977). All these figures must be treated with caution, as the sample of enzyme loci included in the analysis will affect the results. Nevertheless, levels of allozyme variation noted in coniferous trees, at least at the total species level, are generally consistent with estimates of genetic variation from provenance-progeny tests. Those species considered quite variable in morphological and physiological traits such as lodgepole pine (Critchfield 1957) and black spruce (Morgenstern 1978) also show moderate to high levels of allozyme polymorphisms (Wheeler and Guries 1982; Boyle 1985). More importantly, morphologically uniform species such as red pine (Wright et al. 1972) and western red cedar (Thuja plicata Don) (Minore 1969) have been found to be essentially monomorphic at all or most allozyme loci (Fowler and Morris 1977, Copes 1981).

Levels of allozyme differentiation among populations, compared to within populations, as judged by Nei's (1975) G_{ST} values, range from 1% for black spruce (Boyle 1985) to 7.9% for Sitka spruce (Yeh and El-Kassaby 1980). Again, both the sampling of loci and populations can

affect the values obtained, but there is nevertheless clear evidence that a large proportion of the genetic variation of forest tree species is contained within populations. The extent of population differentiation at isozyme loci is, however, less than the differentiation seen in morphological traits from provenance-progeny tests. Nevertheless, Wheeler and Guries (1982) in a coordinated allozyme and morphological study of lodgepole pine concluded that the two sets of data assigned most populations to the same group, despite greater morphological differences among hierarchical units.

Several recent reviews of genetic diversity in plants have compared the values obtained for species with different life history characteristics and among taxa (e.g. Brown 1979, Hamrick et al. 1979, Hamrick et al. 1981, Hamrick 1983). Pooled data from a large number of studies suggest that long-lived, wind-pollinated, and predominantly outcrossing perennials exhibit higher levels of genetic diversity and have a greater proportion of this genetic diversity resident within populations than plants with different life histories.

ii. Mating system parameters

Studies on the mating system of northern temperate conifers have generally confirmed the expected high levels of outcrossing (Table 2), with selfing rates in excess of 0.2 being unusual. Estimates from different populations of the same species indicate that apparent outcrossing rates vary only slightly within a region in any given year (e.g. Shaw and Allard 1981, 1982, Boyle and Morgenstern 1986). Cheliak et al. (1985) investigated the variation in apparent outcrossing rates within a single population of jack pine over several consecutive years. Estimates ranged from 0.82 to 0.92, although the deterioration of seed stored in serotinous cones at different rates (depending on the type of mating event) could account for some of this variation. Studies in clonal seed orchards have indicated much larger variation in outcrossing rates among trees. For example, Shaw and Allard (1982) estimated that the rate ranged from 58% to complete outcrossing for clones in a Douglas-fir (*Pseudotsuga menziessii* [Mirb.] Franco) seed orchard. Ritland and El-Kassaby (1985) also found significant variability in outcrossing rates among families in an open-pollinated Douglas-fir orchard.

A consistent feature of many mating system studies is the apparent inter-locus heterogeneity in estimated outcrossing rates (e.g. Brown et al. 1975, El-Kassaby 1981, Mitton et al. 1981, Shaw and Allard 1982). As Ellstrand and Foster (1983) point out, the population structure of a species can significantly affect apparent outcrossing rates. Wahlund's effect or family clustering will bias outcrossing estimates down, while gametic or post-zygotic selection will increase the apparent rates (Shaw et al. 1981). Because multilocus estimates are less sensitive to such failures in the assumptions of the mixed mating model, a comparison of single-locus and multilocus estimates can yield information on those factors causing the heterogeneity in single-locus estimates (Shaw and Allard 1981). In this way, Shaw and Allard (1981) concluded that there was some evidence of neighbourhood structure for Douglas-fir in Oregon. On the basis of significant heterogeneity in pollen pool allele frequencies, Cheliak et al. (1984) also detected a neighbourhood

structure in a white spruce stand in Ontario and estimated the radius of these neighbourhoods to be considerably less than 100 m. However, Boyle and Morgenstern (1985) concluded that family clusters were not apparent in six upland stands of black spruce in New Brunswick because there was limited heterogeneity in single-locus outcrossing estimates, the average of the single-locus estimates of outcrossing rates exceeded the multi-locus estimate, and pollen pool allele frequencies were homogeneous within populations.

Occasionally, localized differences in allele frequencies are so significant that genetically distinct sub-populations can be recognized directly. Such is the case for high elevation ponderosa pine (Pinus ponderosa Laws.), for which genetically distinct sub-populations could be separated by distances as small as 8 m (Linhart et al. 1981). Possible reasons for this microgeographical differentiation include extremely restricted gene flow and differences in microsite selection coefficients.

Limited effective population sizes provide evidence for neighbourhood structure. Cheliak et al. (1984) estimate an effective population size of 47 females and 19 males for a stand of white spruce. For a jack pine stand, Cheliak (1983) detected effective population sizes of the same magnitude, but of opposite sex ratio. Given the ecological characteristics of these two species, small effective population sizes are to be expected. White spruce usually forms stands in mixture with other species, especially in eastern Canada, so even with effective gene flow, population size would be restricted. The serotinous nature of jack pine cones results in seed dispersal under uniform conditions following fire or, alternatively, from cones lying on the ground after heating by the sun. Seed dispersal will, therefore, be limited. In contrast to white spruce and jack pine, Boyle (1985) estimated large effective population sizes for black spruce of 100 or more. In the particular stands sampled, there was no evidence of serotiny, but black spruce has very small, light seeds that are liable to be widely dispersed. On the other hand, Barrett et al. (1987) recorded effective population sizes as low as 17 (13 females and four males) in a clonal black spruce seed orchard.

IMPLICATIONS FOR SELECTION

Most tree improvement strategies involve repeated cycles of testing, selection, and breeding. During the initial stage of an improvement programme, genetic material is secured by the selection of parent trees in natural populations. There are several basic methods of effecting this selection (Morgenstern et al. 1975, Morgenstern 1983). A common approach is the comparison tree selection method, in which measurements are carried out on a candidate tree and compared with measurements of several nearby trees. Ledig (1974) examined various theoretical considerations and showed that the comparison tree method is most effective for traits with high heritability or in even-aged populations with low environmental variation and where the coefficient of relationship among the candidate and comparison trees is low. If these conditions do not apply, more sophisticated selection methods may be preferred. Thus, species that tend to develop family clusters are not suited for the comparison tree method

of selection, especially when based on environmentally sensitive and low heritability traits such as height and diameter.

Whatever the selection method, the inbreeding depression associated with, for most economic traits, consanguineous mating requires that efforts be made to avoid the inclusion of relatives in the same breeding population. Based on the assumption that widely separated trees are less likely to be related than neighbours, most parent-tree selection programmes require a certain minimum distance between selections. This distance will also be affected by non-genetic considerations such as accessibility and total available resource in relation to the required numbers of plus-tree selections. In the absence of genetic information, minimum separation distances have tended to be conservative. A knowledge of the population structure of a species allows a far more realistic estimate of the required separation. Thus Cheliak et al. (1984) recommend a separation of 65 m for white spruce in eastern Ontario. On the other hand, the lack of any apparent family clustering of upland black spruce in New Brunswick (Boyle and Morgenstern 1987) suggests that separation distances less than 65 m among select trees would be tolerable. Again, the optimum separation distance will depend on other factors such as stand density and species composition.

Seed source studies can outline the regions and/or stands to ensure the best available source of genetic material for tree improvement. Similarly, the breeding value of selected trees from natural stands must be evaluated in well-replicated progeny tests before inclusion in a breeding programme. The bases for these tests is that allele frequency differences among samples of populations or families will be manifested as differences in trait mean values when grown in uniform environmental conditions. However, as noted above, the mating system and existing genetic structure can seriously affect levels of inbreeding and the genetic relationships among populations or family members. In addition, these factors are neither temporally nor spatially constant, either among or within populations. Thus provenance tests may reflect real variation in mean breeding value of populations, differing levels of inbreeding, or a combination of these factors.

Similarly, open-pollinated family tests may reflect real variation in mean breeding values of families or may be biased by differing levels of inbreeding and selfing among the families. Inbreeding and selfing also increase the genetic correlation among open-pollinated family members, which are assumed to be half-sibs. This results in upward bias in the estimation of additive genetic variance (Squillace 1974) and heritability and genetic gain predictions will be correspondingly overestimated. Consider the extreme example of an open-pollinated progeny test in which the parents are assumed to be unrelated but, in fact, due to layering or other forms of asexual reproduction, are all members of one clone. Covariances among the progeny will then equal 0.667 (Squillace 1974), rather than the assumed 0.25. Family performance is also confounded with non-additive genetic variance. Finally, meaningful extrapolation of genetic parameters for the population or species are difficult to obtain when based on estimates derived from open-pollinated tests if variation in the spatial arrangement of relatives and mating system parameters is significant.

As information is accumulated on the performance of populations and families within populations in different test environments, the opportunity for inclusion of this information in subsequent selections increases. This is analogous to considering the performance of several traits in the selection of individuals or families. Various approaches exist for exploiting the information from more than one source or on more than one trait (Lerner 1958), but the most efficient method is the construction of a selection index (Baker 1986). Land et al. (1987) give examples of analytical approaches for the construction of selection indices incorporating information on individual, family, population, plot, and replication performances in a provenance-progeny test. Information on inbreeding coefficients of the maternal populations and the outcrossing rates of the mating event that produced the experimental material could also be included in such an index to improve the accuracy of selection. However, before such index weightings can be assigned with any confidence to these effects, more research on the magnitude of inbreeding depression in economic traits with increasing levels of inbreeding for different species is required.

A further decision concerns the allocation of effort to population and within-population sampling. Again, this decision may be subject to non-genetic constraints such as accessibility, but the relative magnitude of genetic variation at the two levels of sampling should influence the appropriate allocation of effort. As previously noted, for most conifers, more than 90% of the total genetic variation appears to be contained within populations as judged from the average apportionment of the total genic diversity. This implies that appreciable genetic gain can result from exploitation of within-population variation, provided the parent trees sampled are reasonably suited to the proposed planting sites.

IMPLICATIONS FOR BREEDING

Knowledge pertaining to the within-population genetic variation for a given species has a major influence on its breeding and deployment strategies. A number of questions require consideration in this respect, including the appropriate breeding system and the optimum level and arrangement of genetic variation in the artificial populations produced as a result of genetic improvement (breeding, production, and commercial populations).

Applied breeding programs in northern conifers have emphasized the additive genetic effects in random mating populations, usually within seed orchards. Such an approach assumes that other forms of the genetic variation are not of such a magnitude to warrant exploitation. There is evidence for black spruce (Boyle 1987, and unpublished data) and coastal Douglas-fir (Yeh and Heaman 1987) that such an assumption may not be justified. Significant levels of dominance deviation or specific combining ability will result in suboptimal genetic gains from purely additive strategies. Thus there is the need to exploit non-additive genetic strategies if we choose to maximize gains from tree improvement and to capitalize on recent and future advances in micropropagation and other forms of asexual reproduction.

The existence of complex forms of genetic variation, involving multi-locus associations, could further complicate breeding strategy. Such multilocus associations, or linkage disequilibria, are expected to be far more common in inbreeding species (Allard et al. 1975, Brown 1975) as the selection intensities required to maintain such disequilibria are much lower than in outbreeding species. Multilocus disequilibria can arise for many reasons, but large sample sizes are required to demonstrate their existence (Muona 1982). Strong disequilibria have been found in barley (Hordeum vulgare L.) and oats (Avena barbata) (Clegg et al. 1972, Weir et al. 1972, Brown et al. 1977), indicating the existence of "co-adapted gene complexes". Although disequilibria can only be maintained as a result of epistatic selection, new equilibria can be generated every generation (Muona 1982). One of the primary causes of such unstable disequilibria is the pooling of samples from populations having differing allele frequencies, i.e. such disequilibria are the multilocus equivalent of Wahlund's effect (Muona 1982). This is important because it suggests that if co-adapted gene complexes are to be expected in forest trees, the pooling of trees from populations with differing allele frequencies will disrupt the disequilibrium which has resulted from the co-adaptation, thereby reducing the breeding value of the progenies and increasing the rate of decay of additive genetic variance in the breeding population (Karlin 1978). In such a situation, there is a selective advantage in subdividing the breeding population, with repeated cycles of within- and between-line (sub-division) selection (Katz and Enfield 1977) to exploit "multiple peak epistasis" (Wright 1963). Subdividing the breeding population also increases the problem of genetic drift, so some suitable compromise is required.

Another problem with the pooling of select trees from a number of populations into a single breeding population is that calculation of the genetic covariances among relatives for the construction of selection indices requires the assumption of linkage equilibrium (Baker 1986). Thus even if linkage equilibrium exists in natural populations, conventional selection indices cannot be constructed for progeny from crosses among individuals from populations having different allele frequencies.

Even without multiple peak epistasis, Katz and Enfield (1977) and Madalena and Robertson (1974) showed that although an undivided breeding population will result in a greater ultimate genetic gain, the best of the subdivided populations will generate a higher rate of gain in the early generations, peaking at around generations three to four. The choice as to whether to subdivide breeding populations of forest trees must therefore not only consider whether multiple peak epistasis is likely, but also whether the phenotypic selection criteria are likely to remain constant for more than a few generations.

These principles have been demonstrated in some experimental organisms, for example Drosophila, for which the better subdivided breeding populations did provide greater genetic gain than an undivided population in early generations (Madalena and Robertson 1974). This same study also provided corroboration of Robertson's (1960) theoretical prediction of greater response to lower selection intensities. Robertson showed that for purely additive inheritance, maximum gain at the selection

limit would be obtained by selecting 50% of the population in every generation and that linkage slightly increased the required proportion.

Muona (1982) notes that epistatic selection is expected to be common, especially among functionally related loci such as those coding for polygenically inherited traits. This emphasizes the importance that Brown (1978) attaches to the investigation of co-adapted gene complexes. Because evidence of multilocus disequilibrium has most often been obtained in selfing plants, the prediction for outcrossing plants such as forest trees has been that any initial disequilibrium will rapidly decay in the absence of strong epistatic selection. This is a prediction, however, of long-term behaviour. In the reestablishment of a forest tree population after a fire, for example, there could be disequilibrium initially, which will slowly decay for closely linked genes and if there is nonrandom mating. Thus, in studies designed to study multilocus associations in natural populations of forest trees, evidence of disequilibria have been found. Multilocus associations are apparent, particularly among pollen gametes in coastal Douglas-fir (Yeh and Morgan 1987), yellow poplar (Liriodendron tulipifera L.) (Roberds and Brotchol 1985), lodgepole pine (Epperson and Allard 1987, Yeh et al. 1985), and black spruce (Boyle 1985).

Brown (1978), Lundkvist (1982), and Namkoong (1986) have all drawn attention to the need to consider the relationship between within-population structure in natural populations, compared with that in artificial populations. Natural populations of forest trees have evolved to maximize the net population fitness (Lundkvist 1982). This involves the maintenance of high levels of genetic variation (high homeostasis) as a buffer against temporal and spatial variations in selection intensities. Namkoong (1986) particularly stressed the importance of high levels of genetic variation as a safeguard against co-evolving biotic factors such as pests and diseases.

Although breeding populations retain some degree of variability, genetic homeostasis will inevitably be reduced. This trend is greatly accelerated in production populations, where a high degree of genetic uniformity is desired, especially for clonal forestry programmes such as are being proposed or implemented for several species. If economic factors dictate larger management units, so the risk of establishing a host-pathogen relationship increases (Namkoong 1986).

Brown (1979) suggests that high levels of genetic homeostasis may also result in greater total yields and greater yield stability over time. Selecting genetic resources on the basis of performance over a very limited time scale may therefore incorporate the risk of long-term yield instability. As Lundkvist (1982) points out, the optimum genetic structure of artificial populations is unlikely to be identical to that of natural populations, but a study of natural population structure is essential in establishing the relationship between population structure and yield stability. Without an understanding of the causal relationship between the two, the development of more advanced breeding methods will be greatly impaired.

Unlike natural populations, the structure of an artificial population can be controlled. Despite this, artificial populations are still subject to within-population variation in mating system parameters and effective sexuality, the variation of which is most critical in seed orchards. Much research has, therefore, been carried out on pollen pool heterogeneity and outcrossing rates in seed orchards (e.g. Rudin et al. 1977, Rudin and Lindgren 1977, Muller-Starck 1979, Shen et al. 1981, Rudin and Ekberg 1982, Schoen et al. 1986, Barrett et al. 1987). In general, high levels of pollen pool heterogeneity, elevated selfing rates, and effective population sizes considerably smaller than the actual population size are common features. For example, Shen et al. (1981) found that the pollination pattern in a Swedish Scots pine (Pinus silvestris L.) seed orchard was strongly dependent on coincidence of flowering time and wind direction at that time. Selfing rates of 16% were observed in another Swedish Scots pine seed orchard (Rudin and Ekberg 1982) and of up to 24% in a widely-spaced Scots pine seed tree stand (Rudin et al. 1977). In a clonal white spruce seed orchard in Ontario as few as two of the 33 clones contributed about 50% of the pollen in two consecutive years (Schoen et al. 1986).

IMPLICATIONS FOR GENETIC CONSERVATION

The population structure of forest trees is an underlying consideration in the conservation of genetic resources. Specific objectives of genetic conservation should include the maximum procurement of variation at gene loci and the maintenance of specific adaptive gene complexes (Brown 1979). The relative magnitude of within- as compared to among-population variation would determine the allocation of effort to the two levels of the variation. Evidence for family clustering or stable disequilibria would affect the intensity of within-population sampling (Yeh et al. 1985). These considerations should include populations that are currently of little commercial interest because, as Namkoong (1985) points out, the currently tenuous understanding of evolutionary processes in forest ecosystems demands the maintenance of as wide a sample as possible.

CONCLUSIONS

For most forest trees, which are long-lived, predominantly outcrossing, wind pollinated, and generally quite widespread in distribution, within-population variation accounts for most of the genetic variation. Thus tree improvement programmes must place great emphasis on the exploitation of this within-population variation. Knowledge of the genetic structure of forest populations is important in determining the efficiency of different selection procedures (Katz and Enfield 1977). Despite recent advancements in elucidating the genetic structure of forest populations, the role of the evolutionary processes and the effects of interactions among these processes remain largely unresolved for both natural and artificial populations. Studies in clearly defined and controlled environmental conditions are therefore needed.

Specifically, many critical questions remain unanswered for forest trees, including:

- to what extent can genetic variability be reduced in commercial populations without jeopardizing stability?
- what is the optimum genetic structure of artificial populations, both with respect to yield and stability (Lundkvist 1982)?
- how significant are epistatic selection and coadapted gene complexes?
- what is the pattern and intensity of response of commercial traits to increasing levels of inbreeding?
- what is the range of variation in outcrossing rates from year to year, for different individuals and for different natural and artificial populations?

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Table 1. Measures of gene diversity for Canadian conifers and important exotics.

Species	Percent polymorphic loci	Heterozygosity per locus	Average # alleles locus	Nei's G_{ST}	Reference
<u>Pinus contorta</u>	58.7	0.160 0.119	1.70	0.041	Yeh & Layton (1980) Wheeler & Guries (1982)
	51.4	0.184	2.50	0.018	Dancik & Yeh (1983)
	64.0	0.165			Yeh et al. (1985)
<u>P. banksiana</u>	74.0	0.146	2.70	0.052	Danzmann & Buchert (1983)
	46.0	0.286 0.115	2.10	0.024	Hamrick et al. (1981) Dancik & Yeh (1983)
		0.146-0.169			Knowles (1985)
<u>P. resinosa</u>	0.0	0.000	1.00	-	Fowler & Morris (1977)
<u>P. rigida</u>	78.8	0.146			Guries & Ledig (1981) Hamrick et al. (1981)
<u>P. monticola</u>	65.0	0.177	1.70		Steinhoff et al. (1983)
	64.0	0.180	1.79		El-Kassaby et al. (1987)
<u>Picea mariana</u>	91.7	0.229	2.31	0.010	Boyle (1985)
	38.1	0.107	1.44	0.059	Yeh et al. (1986)
		0.294-0.336			Knowles (1985)
<u>P. glauca</u>	85.7	0.183			Cheljak et al. (1984)
	83.4	0.174			Yeh & Arnott (1986)
<u>P. sitchensis</u>	51.3	0.147		0.079	Yeh & El-Kassaby (1981)
	75.9	0.199			Yeh & Arnott (1986)
<u>P. abies</u>	91.7	0.32-0.41			Lundkvist & Rudin (1977)
	71.0-100.0		2.14-3.00		Lundkvist (1979) Bergmann & Gregorius (1979)
<u>Pseudotsuga menziesii</u>					
Coastal	68.8	0.155 0.388	2.19	0.026	Yeh & O'Malley (1980) Yang et al. (1977)
Interior	54.0	0.171	1.79	0.068	El-Kassaby & Sziklai (1982)
	68.0	0.175		0.043	Yeh (1979)
<u>Abies balsamifera</u>	64.0				Neale (1978)

Table 2. Estimates of outcrossing rates for Canadian conifers.

Species	Biochemical		Morphological	Reference
	Single locus	Multilocus		
<u>Pinus contorta</u>	0.94-1.06	0.95-1.03		Epperson & Allard (1984)
<u>P. banksiana</u>	0.82-0.92 0.80-1.03	0.91		Cheliak et al. (1985) Danzmann & Buchert (1983)
<u>P. monticola</u>	0.95	0.98		El-Kassaby et al. (1987)
<u>Picea mariana</u>	0.89-0.96	0.89-0.98	0.85-0.94*	Boyle (1985) Morgenstern (1972)
<u>P. glauca</u>	0.98			Cheliak et al. (1984)
<u>Pseudotsuga menziessii</u>	0.66-0.97 0.90	0.91-0.93		Shaw & Allard (1982) El-Kassaby et al. (1981) Yeh and Morgan (1987)
<u>Larix laricina</u>	0.58-0.91	0.89		Knowles & Perry (1987)

* Assumes inbreeding equilibrium

SHORTENING THE BREEDING CYCLE OF SOME NORTHEASTERN CONIFERS

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ABSTRACT

Methods for shortening the breeding period for white spruce (between selection and final seed production) by about 6 years are discussed. These include: growth acceleration, a mixture of Gibberellins 4 and 7 (GA_{4/7}) foliar sprays, which consistently promote strobilus production in both white and black spruce on both potted and field growth trees, and manipulation of the environment during the period of strobilus initiation and differentiation. The growth acceleration techniques, and GA_{4/7} application show the most promise for shortening the breeding cycle. The benefits and advantages of indoor, potted breeding orchards for white and black spruce are still being evaluated.

RÉSUMÉ

On expose les méthodes utilisées pour abrégé d'environ 6 années la période de reproduction de l'épinette blanche (depuis le moment de la sélection jusqu'à la production finale de graines). Ces méthodes sont: l'accélération de la croissance; un mélange des gibbérellines 4 et 7 (GA_{4/7}) que l'on utilise pour l'aspersion des feuilles et qui favorise constamment la production de strobiles chez les épinettes blanches et noires poussant dans des pots ou sur le terrain; et la manipulation du milieu pendant la période de stimulation et de différenciation des strobiles. Les techniques d'accélération de la croissance et l'application de GA_{4/7} sont les plus prometteuses pour l'abrégement du cycle de reproduction. On est encore en train d'évaluer les avantages de vergers de reproduction d'épinettes blanches et noires croissant en pot à l'intérieur.

INTRODUCTION

The value of tree improvement programs is well established in the southeastern USA, where virtually all of the one billion seedlings (mostly loblolly pine, *Pinus taeda* L.) produced in southern nurseries each year are grown from genetically improved seed (Talbert et al. 1985). The estimated gain from this material is 7 percent in height (site index), 12

percent in volume, and an estimated 32 percent in harvest value, based on data from progeny tests aged at least 12 years. Nonetheless, 35 years have elapsed between plus tree selection and full production of first generation improved seed.

Advanced generation breeding of forest trees is vital to determine the inheritance patterns of desirable traits, as well as identification of improved genotypes. Therefore, as Fowler (1986) points out, the early stages of any tree improvement program include a necessary learning period, during which future breeding strategies will be developed and tested. Consequently, genetic gain increments will increase for future generations, as pedigrees are established, and the heritability of desirable traits is estimated. Shortening generation turnover time in a breeding program is obviously of great value. Currently, delayed flowering and lack of reliable selection methods at early ages result in generation times of 20 years or more (Zobel and Talbert 1984). For northeastern species, generation times will vary considerably by species, with earlier flowering species showing shorter time periods between selection and completion of breeding. For example, the breeding period may be as little as 5 years for tamarack (Larix laricina (Du Roi) K. Koch), and as much as 12 years for white spruce (Picea glauca (Moench) Voss) (Fowler 1986). The total generation time also includes the interval between progeny test establishment and the time the trees are big enough to make selections for the next generation, which at present is at least 10 years.

Genetic gain per unit time can be increased using the following three approaches: 1) induction of earlier flowering in breeding orchards, 2) development of earlier selection methods for progeny tests (discussed elsewhere in these proceedings), and 3) earlier production of improved seed and its further multiplication using vegetative propagation (Greenwood 1983).

A strong interest in realizing genetic gains from advanced generations of conifer breeding programs, combined with the discovery that $GA_{4/7}$ promoted flowering in the Pinaceae, has resulted in significant progress in controlling flowering behavior during the last 15 years. The effects of the Gibberellins and cultural treatments on conifer flowering have been reviewed (Pharis and Kuo 1977, Pharis and Ross 1986), and include several reports of successful treatments of both white and black spruce (Picea mariana Mill. B.S.P.). In this paper we will describe the methods we have developed over the last two years for decreasing the breeding period for white and black spruce. Our approach has two components: 1) acceleration of early graft development, so that trees will reach flowering size sooner, and 2) promotion of female and male strobilus production using $GA_{4/7}$ combined with promotive cultural treatments (Pharis and Ross 1986). Similar approaches are being used successfully on an operational scale on loblolly pine (Lambeth and Greenwood 1987).

MATERIALS AND METHODS

The materials and methods for accelerated growth and flower induction phases will be discussed separately. Since experiments began in 1985, procedures have been modified as new results become available. The methods described in the following sections are those which have been most successful to date, but these too may be refined based on current and future experiments.

Accelerated Growth

Since grafts of white spruce put on relatively little growth in the 2 years after grafting, techniques for accelerating the early growth of white spruce are being developed. This work is currently being expanded to include black spruce and jack pine (*Pinus banksiana* Lamb.). The cultural regimes used to promote one additional growth cycle in one year involve manipulation of daylength and the use of cold storage to satisfy chilling requirements. Potted grafts are brought into the greenhouse in early January in a dormant condition. Temperatures are gradually raised over one week until the minimum greenhouse temperature reaches 15°C. Photoperiod is increased to 18 hours using incandescent lighting. The grafts are grown in these conditions until elongation is completed, normally by mid-March. Soluble 10-52-10 fertilizer is used at the beginning and end of the growth cycle and 20-20-20 during active growth; irrigation is used as required. When shoot elongation is completed, daylength is reduced to 8 hours using a blackout curtain. The trees set bud and are held in this condition in the greenhouse until mid-May. The optimum interval between bud set and chilling treatment is still being investigated. Grafts are moved into a cooler held at 4°C and remain there for 1000 hours. The amount of time in the cooler that is required to break dormancy has been studied in two trials and 1000 hours has been found to be the most suitable. The grafts are moved into a shade frame following cold treatment and allowed to grow normally. Fertilizer and irrigation regimes are similar to those used in the first cycle. Grafts are moved into cold frames or an unheated greenhouse in September and remain until January. Those which have developed sufficiently to begin flower induction treatments (1.0 to 1.5 m in size) may remain in a cold frame until spring. Repotting is done as required from an initial pot size of 4.5 l to a 16 l container. The soil used is a 2:1:1 mix of peatmoss, loam and aggregate.

Flower Induction

Flower induction has been conducted using 3- to 4-year-old white spruce grafts and, to a lesser extent, black spruce. The white spruce grafts averaged less than 1 m in height prior to treatment. Experiments have focused on the effect of gibberellic acid (GA_{4/7}) application in different environments on female and male strobilus production. GA_{4/7} is applied weekly beginning at bud burst, and continues for 8 applications. Although further refinement is possible, the most successful rate to date has been 500 mg/l of GA_{4/7} in a solution of 5% ethanol with Aromox C/12 used as a surfactant (0.01-.02% active ingredient). This solution is applied with a hydraulic sprayer to wet the foliage but not to run off. Experiments have been carried out on grafts

growing in the seed orchard, and on similar ramets transplanted to 59 pots and moved to the Sussex Nursery. Trees used in the nursery experiments were lifted from the orchard in early spring with a Vermeer tree spade and potted with the rootball intact in mineral soil. Some root pruning occurred during this operation. Several ramets representing a total of 24 white spruce and 10 black spruce clones were used. Environmental regimes which have been tested at the nursery are as follows:

- 1) Trees are moved into a plastic-covered cold frame or greenhouse as buds begin to swell in the spring. They remain inside for the entire growing season during which GA applications are made.
- 2) Trees are held outdoors under a shade frame until elongation is 50-70% completed. They are then moved into a greenhouse for the rest of the growing season, similar to experiments described by Ross (1985).

Moisture status has been monitored using irrometers. Pots are watered when a reading of 30 centibars is reached, so the trees probably have experienced little water stress. A combination of liquid and granular fertilizer is applied through the growing season. Temperatures in the greenhouse rise above ambient levels throughout the growing season. During the warmest periods, shade cloth is used to prevent temperatures from exceeding 35°C. The grafts are moved out of the greenhouse in the fall and held in sealed coldframes for the winter. Treatments are evaluated at budbreak the following spring. The current schedule involves treating each graft every other year. The population is split so that half receives treatment in alternating years.

RESULTS

Some of the results from the first two years of experimentation will be discussed to illustrate some of the major findings.

Accelerated Growth

One of the first accelerated growth experiments was performed on white spruce grafts made in January and February, 1985. Normally, these grafts would elongate shortly after grafting, set bud and remain in that condition until the following spring. In this experiment five clones were refrigerated for a duration of 500, 1000 and 1500 hours beginning in mid-July. A non-refrigerated control was held at the nursery. Upon completion of the cold treatment the grafts were moved into the greenhouse and maintained under 18 hours photoperiod until late October. Height increments for these treatments are shown in Table 1. The 1000 hour treatment appeared to be optimum for satisfaction of the chilling requirement and the 1500 hour treatment provided no better results. Analysis of variance showed the effect of cold treatment on height growth to be significant at the $P < 0.01$ level. Subsequent experiments have shown that with proper handling and use of blackout curtains, it is possible to put the grafts through the refrigeration phase two months earlier. This allows for the second growth cycle to be completed with enough time to satisfy dormancy requirements before January.

Table 1. Accelerating growth of white spruce grafts: effects of chilling on terminal shoot elongation by grafted white spruce.

# Chilling Hours	Elongation and # Grafts Flushing (starting date 7/11/85)					
	Wk 13		Wk 16		Wk 19	
	Elongation cm	Flushing #	Elongation cm	Flushing #	Elongation cm	Flushing #
0	0.0	0 of 15	1.5	3 of 15	2.3	6 of 15
500	0.2	2 of 15	1.2	2 of 15	1.3	3 of 15
1000	7.4	15 of 15	7.6	15 of 15	7.6	15 of 15
1500	2.7	13 of 15	5.1	13 of 15	5.1	13 of 15

Flower Induction

The use of GA_{4/7} in foliar sprays has been found to greatly enhance female strobilus production for black and white spruce. The effect on male strobilus production has been generally promotive, although somewhat inconsistent. There has been significant clonal variation in response to GA_{4/7} treatment, which persists in different environments (see results for white spruce in Table 2) at the nursery and in the clonal seed orchard. One ramet per clone was treated either at the seed orchard, or in pots indoors with a foliar spray of GA_{4/7} (200 ml/l). Treatments made at the seed orchard were more effective than those made on potted trees in 1985. It should be noted that all potted trees used in the 1985 and 1986 experiments had been recently lifted from the orchard and potted so this operation may have some residual effect in the first year of treatment. Table 3 summarizes the effects of two years of treatments on potted trees versus those growing in the clonal orchard.

Table 2. ♀, ♂ strobilus production (1986) by grafted white spruce clones in response to GA_{4/7} foliar sprays (200 mg/l in 1985) on similar grafts, in pots or left in the orchard.

Clones	♀ Strobili		♂ Strobili	
	Potted trees	Orchard	Potted trees	Orchard
1	19	34	0	0
2	10	56	0	30
3	48	0	0	0
4	0	83	20	88
5	0	6	0	2
6	0	1	1	0
7	37	50	2	0
8	25	57	1	5
9	0	1	0	4
10	11	49	0	19
\bar{X}	15.0	33.7	2.4	14.8

Table 3. ♀, ♂ per ramet (clones flowering, %) for grafted white spruce, in pots and in the field, in response to GA_{4/7} in 1986 and 1987.

		GA _{4/7}	Control
Orchard+, 1986	♀	32.4 (81%)	11.1 (44%)
	♂	9.2 (38%)	0.6 (13%)
Pots, 1986*	♀	15.0 (60%)	0 (0%)
	♂	2.4 (40%)	0 (0%)
Orchard+, 1987	♀	9.9 (56%)	0.9 (25%)
	♂	5.2 (44%)	0.3 (13%)
Pots, 1987*	♀	22.3 (90%)	4.2 (30%)
	♂	20.2 (80%)	1.4 (10%)

* Different clones in '86 and '87.

+ Same ramets treated in '86 and '87.

In 1987, better flowering occurred on the potted trees, in contrast to the results of the previous year. Although the same clones were treated in the orchard in both years, a different set of clones were treated in pots. Nonetheless, a comparison of flower production on orchard controls shows a substantial drop in female flowering between 1986 and 1987. This illustrates expected annual fluctuation in flowering by orchard grown trees, which can probably be avoided with a potted, indoor orchard.

Experiments comparing the effect of two environments during shoot elongation on flowering of potted white spruce grafts have been inconclusive. Table 4 shows the average number of strobili for all clones and the percentage of clones flowering in a 1986 trial. No statistical significance between environments was found using analysis of variance for either sex.

Table 4. Effect of greenhouse environment on number of ♀, ♂ per ramet (clones flowering, %) for white spruce in 1987. An equal number of ramets were either left in the greenhouse throughout the shoot elongation and GA_{4/7} application, or moved into the greenhouse from a shade frame for the last half of the GA application period.

		GA _{4/7}	Control
Outdoor mid-May to mid-June, then greenhouse thereafter	♀	23 (70%)	1 (10%)
	♂	28 (60%)	1 (10%)
Continuous greenhouse	♀	23 (70%)	8 (30%)
	♂	16 (50%)	3 (10%)

The GA treatment effect was significant for females ($P < 0.05$ using $\sqrt{n+1}$ data transformation) but not significant for males. A similar comparison of environments for black spruce is shown in Table 5, but also includes orchard treatments. No significant environmental effect was found for females; however, the opposite was true for males ($P < 0.01$ using $\sqrt{n+1}$ data transformation) mainly because of the orchards results.

Table 5. Effect of GA_{4/7} and environment on ♀, ♂ per ramet (clones flowering, %) for black spruce in 1987.

		GA _{4/7} *	Control
Orchard, 1987	♀	30 (80%)	2 (11%)
	♂	320 (70%)	175 (44%)
Pots, 1987 (Greenhouse)	♀	64 (80%)	1 (10%)
	♂	1 (20%)	33 (30%)
Pots, 1987 (Shadeframe)	♀	26 (80%)	3 (20%)
	♂	13 (60%)	4 (20%)

* 8 applications

No differences between nursery environments were found using Duncan's Multiple Range Test. Significant GA_{4/7} treatment effects were found for females ($P < 0.01$) but not for males.

DISCUSSION

The breeding period for both white and black spruce can be decreased both by increasing graft size by accelerating growth and stimulating flowering with GA_{4/7} foliar sprays. A somewhat similar approach applied to indoor, potted breeding orchards of loblolly pine has apparently shortened the breeding period by about 7 years, and is being used operationally in several tree improvement programs, as mentioned earlier. We project that similar results could be attained for white spruce (see Fig. 1), but continuous greenhouse culture, which promotes flowering (especially male) in loblolly pine, may not be the optimum approach for the spruces. While there is a promotive effect of greenhouse culture on some species of spruce (e.g. Philipson 1983, Ross 1985), Ross concludes that this treatment is most effective when applied during the later stages of shoot elongation. He proposes that the greenhouse promotes flowering by providing higher temperatures during a critical phase of reproductive bud differentiation. He also concludes that drought stress will also promote flowering, although independently of the temperature effect. To date, we have not demonstrated the effect of continuous greenhouse culture on flowering by the spruces, although these experiments are in progress. However, we found little difference in flowering between trees which remained in the greenhouse throughout shoot elongation, and those moved into the greenhouse during the latter phase of shoot elongation as described by Ross (1985), see Tables 4 and 5.

GA₄/7 application, by foliar spray, has consistently promoted flowering (a 3- to 30-fold increase over the untreated control) on both potted and field grown trees. We compared foliar sprays with periodic topical treatment of individual buds, and found the latter treatment differed little from the untreated control (unpublished data), although Ross (1985) reports some success with this method on Engelmann spruce (Picea engelmannii Parry). The flowering response to foliar sprays reported here compares very favorably with the results reported for other spruces. Just prior to GA treatment, the 3- to 4-year-old white spruce grafts averaged 57 cm tall, and yielded over 30 female strobili per graft for the best treatments. Marquard and Hanover (1984) report 16 females per tree for 6-year-old white spruce grown from seed, and 113 females per tree on the same trees 2 years later in response to foliar sprays of GA₄/7, which was about double that observed on the control trees. Male flowering was insignificant in Marquard and Hanover's study, whereas we observed a promotive effect for males, but the response was less than that of females. In general, less male flowering occurs in response to GA₄/7 in the Pinaceae (Pharis and Ross 1986).

Our preliminary results show that we may be able to obtain more consistent flowering on potted trees in the greenhouse than on similar trees grown in the field (see Table 3). Ho (1986) also reports inconsistent response of field-grown white spruce to foliar sprays of GA₄/7 across several years.

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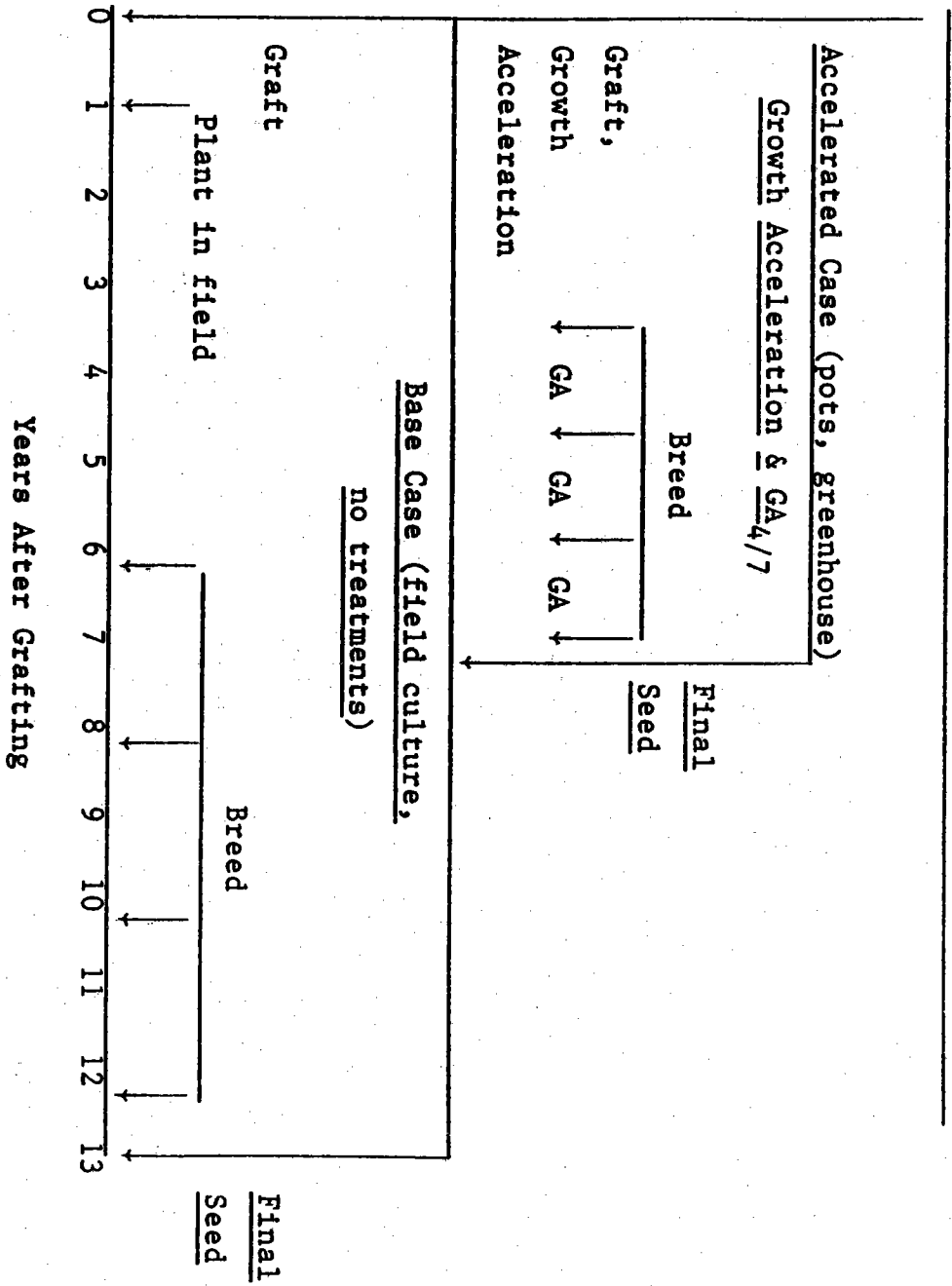


Figure 1. Breeding Interval (years between grafting and final seed) with and without accelerated growth, GA_{4/7} applications, for white spruce.

THEORETICAL BASIS FOR EARLY TESTING IN
GENETIC IMPROVEMENT PROGRAMS

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ABSTRACT

Calculations of genetic gain at rotation age in a trait arising from juvenile selection is simple in theory but hampered in practice due to insufficient knowledge about parameters in the gain equation. This paper points out the shortcoming of existing juvenile-mature correlation models and two alternative models are presented. A simulation study illustrates the impact of various temporal aspects of a breeding program on the optimum selection age; the standard error of the ensuing optimum ratio of juvenile to mature genetic gain per year is shown to be considerable. Nevertheless, juvenile selection appears to be superior to selection at maturity, even under less favourable conditions.

RÉSUMÉ

Il est simple en théorie de calculer les gains génétiques à l'âge de rotation pour une caractéristique surgissant d'une sélection d'arbres juvéniles, mais difficile à mettre cela en pratique à cause des connaissances insuffisantes sur les paramètres de l'équation des gains. Cet article souligne les lacunes des modèles actuels de corrélation juvénile-adulte, et deux modèles de rechange sont présentés. Une étude de simulation illustre les répercussions des divers aspects temporels d'un programme de reproduction sur l'âge optimum de sélection; l'erreur type du rapport optimum du gain génétique réalisé chaque année entre les arbres juvéniles et adultes est considérable. Néanmoins, il semble que la sélection juvénile soit supérieure à la sélection réalisée à la maturité, même dans des conditions moins favorables.

INTRODUCTION

The period required for testing and evaluation is the principal impediment to rapid progress in genetic improvement of forest trees. Time between generations can be reduced dramatically by environmental manipulations to accelerate growth and induce early flowering (Bongarten and Hanover 1985, Ross and Pharis 1976). However, short generation times can only be used to full advantage if characteristics determining the quality and quantity of wood at harvest can be recognized at an early age.

Genetic correlations between juvenile and mature characteristics must be sufficient to lend confidence to early selection and to set realistic

limits on the proportion that may be selected to achieve an expected gain. The purpose of this paper is to examine the genetic and mathematical basis for early selection and to suggest ways to increase efficiency of early selection relative to later gain.

Theoretical Basis

The expected gain at rotation age in a trait (m) that arises from juvenile (j) selection can be expressed (Becker 1984, Falconer 1981, Searle 1965) as:

$$[1] \quad G(m/j) = h_j \cdot h_m \cdot r_{jm} \cdot i \cdot \sigma_m \quad \text{where}$$

h_j, h_m = square root of juvenile and mature heritability,

r_{jm} = genetic correlation between juvenile and mature traits,

i = selection intensity,

σ_m = phenotypic standard deviation of mature trait.

Direct selection for the mature trait leads to the gain prediction:

$$[2] \quad G(m) = h_m^2 \cdot i \cdot \sigma_m$$

If $r_{jm} > h_m/h_j$, then juvenile selections will result in more gain than direct selection. However, this situation is likely rare for economically important traits in forest trees. The attraction of juvenile selection in forestry is therefore its potential for improving the rate of genetic improvement (= genetic gain per unit time).

Baradat (1976) proposed an alternative formulation of the gain by indirect (juvenile) selection for a mature trait:

$$[3] \quad G(m/j) = CGP(j,m) \cdot i \cdot \sigma_m \quad \text{where}$$

$$CGP = \text{coefficient of genetic prediction} = \frac{COV(A_j, A_m)}{\sigma_j \cdot \sigma_m} \quad \text{where}$$

A_j = breeding value of juvenile trait,

A_m = breeding value of mature trait,

σ_j = juvenile phenotypic standard deviation.

The CGP is a standardized regression coefficient (or generalized correlation coefficient) of genetic value of one trait on the phenotypic value of another trait (Falconer 1981). When we shift the phenotypic mean for one trait, one standard phenotypic deviation in a given direction, the breeding value of the other trait is shifted by the CGP times phenotypic standard deviation of this other trait. Stated this way CGP is, in effect, the relative merit of juvenile selection, and it is worthwhile to

calculate this coefficient whenever possible. Currently, however, only few published results of CGP are available (Loo et al. 1984) and we shall therefore concentrate on the more familiar equations [1] and [2].

In the following we shall consider the gain realized through either roguing of a seed orchard based on juvenile evaluation of a progeny test or through cloning or breeding of selected juvenile individuals. A genetic gain is realized at each harvest of improved seed or harvest of forest stands grown from improved seed. Hence, the total gain from one generation of breeding depends on the number (N) and frequency (S) of seed harvests. In a continuous breeding program with several breeding cycles, (C) the cumulated gain produced per year can be computed as:

$$[4] \quad CG_N(m/j) = \sum_{c=1}^{C-1} \sum_{n=1}^{N_c} \frac{c \cdot G(m/j)}{c \cdot (T_j + D) + S \cdot (n-1)} + \sum_{n=0}^{N_F-1} \frac{C \cdot G(m/j)}{C \cdot (T_j + D) + S \cdot n}$$

where

T_j = age of selection, $\min(T_j) = 3$,

D = delay between selection and gain realization,

c = breeding cycle,

d = delay between selection and first seed harvest of improved seed,

C = number of breeding cycles in a given total time (TT), $C = TT / (T_j + d)$,

S = years between seed harvests,

n = number of seed harvests,

N_c = total number of seed harvest during a breeding cycle, $N_c = (T_j + d) / S$,

N_F = number of seed harvests after the last breeding cycle.

The net present value of the cumulative gain is given by:

$$[5] \quad CG_{N_0}(m/j) = \sum_{c=1}^C \sum_{n=1}^{N_c-1} c \cdot G(m/j) \cdot (1+p)^{-(c \cdot (T_j + D) + S \cdot (n-1))} + \sum_{n=0}^{N_F-1} C \cdot G(m/j) \cdot (1+p)^{-(C \cdot (T_j + D) + S \cdot n)}$$

where p = interest rate ($0 < p < 1$).

An optimum selection age T_j can be derived by, for example, maximizing the ratio of juvenile to mature rates of genetic improvement. Results of mature selection at time T_m are derived from [4] or [5] by insertion of T_m for T_j .

Although simple in theory, a multitude of practical and theoretical problems present themselves immediately. We shall discuss some of the most pertinent problems associated with individual parameters in the above equations. Aspects of growth rhythm and ontogenetic thresholds for economically important traits are intentionally omitted (Rehfeldt 1983, Williams 1987).

Time

To optimize the gain ratio we need to know (i) when and how gain is realized, (ii) how time is valued, (iii) the duration of the breeding program, and (iv) the number and frequency of seed harvests (gain produced). The way various answers to these questions affect the optimum selection age is illustrated in Table 1 for a simple model of heritability and juvenile-mature correlation. It is apparent from Table 1 that a breeding program with a long time horizon (2-3 rotations) should opt for higher selection ages than a short-lived program. Similarly, if gain is not considered realized before the actual harvest of trees from improved seed (a company or province with extensive forest lands may adopt this attitude) then the optimum selection age will be higher than in a program aimed entirely at seed or seedling production. Higher discount factors make the above mentioned trends less important.

Costs of breeding, testing, and seed management have not been considered here. However, Magnussen (1987b) has shown that the "fixed" costs of a breeding cycle will tend to increase slightly the optimum selection age on a cost/benefit basis.

Correlation

Calculation of mature gain from juvenile selections requires an estimate of the juvenile-mature correlation r_{jm} . In an ideal situation a continuously updated estimate based on a large sample is available, ready to plug into equation [1]. In reality, however, the breeder will rarely have such information, and gain calculations must proceed with hopefully reliable estimates obtained elsewhere. One of the best known estimates of juvenile-mature correlations (phenotypic) was published by Lambeth (1980). Based on eight studies with a total of eight conifer species he derived the regression for juvenile-mature correlation of tree height:

$$[6] \quad r_{jm} = 1.02 + 0.308 \cdot \log_e (T_j/T_m)$$

Although this equation is useful as a first approximation, its limitations are obvious: (i) it predicts equal correlations for identical but biologically different age ratios (for example, $(r_{10,50} = r_{4,20})$); (ii) its predictive power decreases towards younger ages for T_j (optimum selection ages derived by using equation [6] often fall outside the recommended use of the model); (iii) the logarithmic model concept is more appropriate in the exponential growth-phase (T_j) than in older plantations (T_m) where growth is declining.

As an alternative to Lambeth's equation we suggest that correlations be predicted from (Magnussen 1987a)

$$[7] \quad r_{jm} = \frac{(1+k \cdot d) \cdot \sigma_j}{\sigma_m} \quad \text{where}$$

k = intrinsic growth rate = $f(d, \sigma_j/\sigma_m)$,

d = the average relative proportion of size dependent growth ($0 < d < 1$).

This prediction model does not depend on time but on the growth rate, and variances of the actual trait in question. Estimates of σ_j and σ_m ought not to pose a problem (they are needed for heritability estimates anyway, cf. eq. [1]). For height, d values can be expected to be in the interval [0.05 - 0.30] (Magnussen 1987a). The effect of d on the correlation is illustrated in Figure 1 for a simulated development of the height variance over time (Magnussen 1987b). At this point, it is believed that d values are trait-, species- and environmentally-specific. For prediction purposes even a crude guess of the upper and lower limit of d will yield useful information about expected correlations.

Another alternative to remedy the lack of reliable juvenile-mature correlations is to compute age-to-age correlations in the actual test and compare them to specified minimum values. Juvenile selection is then deemed efficient when observed correlations exceed the minimum requirements. The rather cumbersome and lengthy procedures involved in finding those minimum correlations are described by Magnussen (1987c). An application of this principle is illustrated in Figure 2 where empirical four-years' correlations are plotted on a graph showing the required minimum correlations for various proportion (p) of selected entries. The model assumes a constant heritability and that our ability to predict future performance increases steadily over time (Magnussen 1987c). In the example, family selection at around age 10 appears efficient.

Juvenile-mature correlations can often be strengthened by use of more than one juvenile trait as predictors of mature performance, but at a price. Cochran (1951) has shown that the expected gain in the mature trait is less than indicated by the multiple correlation coefficient. Table 2 gives the approximate reduction of the correlation coefficient due to errors in the regression coefficients. To give an example, suppose that a regression on four juvenile variables is computed from an initial sample size of 10. If $r_{jm}=0.6$ then the expected correlation upon which gain is to be calculated is $0.64 \cdot 0.6 = 0.39$ ($\frac{1}{2} r^2=1.4$). The loss in correlation increases rapidly with the number of variables and with decreasing r_{jm} . With large sample sizes ($n>100$) the loss is unimportant (<5%) for correlations above 0.5.

With repeated selection cycles the juvenile-mature correlation will likely decrease. For example, the correlation between selected individuals and the mature trait is expected to be

$$[8] \quad r_{jm}' = r_{jm} \sqrt{\frac{1-i(i-x')}{i-r_{jm}^2 \cdot i(i-x')}} \quad \text{Cochran (1951), where}$$

x = truncation point of selection ($E(x)=0$, $\sigma_x^2=1$)

The question remains as to what extent a recovery of r_{jm} can be expected after breeding. A potentially promising approach to increasing the juvenile-mature correlation is to include information on relatives in a juvenile index as the breeding work advances (Burdon 1982).

Heritability

The efficiency of juvenile selection is proportional to the square root of the ratio of juvenile to mature heritability (cf. eg. [1]). Knowledge of this ratio is therefore crucial to early selection. The usual procedure of calculating heritability as the ratio of genetic to phenotypic variance (Becker 1984, Crow 1986, Falconer 1981) makes heritabilities sensitive to experimental design, plant material (Ditrichson and Kierulf 1983, Jackson 1983, Jacquard 1983, Matheson and Raymond 1984, Nanson 1976), and methods of measurements (Kung 1977, Lee 1981). It is, therefore, not surprising that published heritability estimates show no consistency over time, environments, or traits (Cotterill and Dean 1987, Foster 1986, Namkoong and Conkle 1976, Nienstaedt 1984). Although Franklin (1979) succeeded in grouping heritabilities into phases of growth development (maximum juvenile genetic expression, mature genetic culmination, and a suppression phase), it has done little to improve our ability to predict mature heritability. With little published data on heritabilities at the time of actual tree harvest we must continue to rely on crude extrapolations when judging the efficiency of juvenile selection.

Selection intensity

The optimum selection ages listed in Table 1 assume equal selection intensities in both juvenile and mature selections. However, mortality and possible thinnings of a progeny test leads frequently to a decrease in selection intensity with age. Ignoring any truncation and thinning effects on the juvenile-mature correlation, the estimated ratio of juvenile to mature genetic gain tends to be conservative.

To counter the negative effects of repeated juvenile selection cycles on the effective population size and associated loss in effective selection intensity (Falconer 1981, Becker 1984) less intensive selections are preferable in juvenile selections. Even a reduction of the juvenile selection intensity by 30-50% will not alter the conclusion reached for equal intensities. This strategy for juvenile selection appears also advantageous in light of the small test sizes and low costs of establishing juvenile trials as compared to long-lived field trials.

Standard errors

Calculations of the optimum selection ages presented in Table 1 were based on the gain ratio of juvenile to mature selection. Sample estimates of juvenile-mature correlations, heritabilities, and variances are used for this purpose, all of which are more or less accurate and have considerable sampling errors associated with them (Becker 1984, Falconer 1981). If we just consider the ratio of genetic gains per unit selection differential then the only relevant sampling errors are those of heritability and correlation. A good approximation of the relative error (CV) on a ratio ($R=j/m$) can be written as (Kendall et al. 1983, p. 238):

$$[9] \quad CV_R = (CV_j^2 + CV_m^2 - 2 \cdot r_{jm} \cdot CV_j \cdot CV_m)^{\frac{1}{2}}$$

where CV = coefficient of variation ($CV_x = x/S_x$)

Ignoring the covariance between h_j and r_{jm} , and between h_m and r_{jm} , the coefficient of variation of the juvenile gain can be approximated by:

$$[10] \quad CV_j^2 = CV_{h_j}^2 + CV_{h_m}^2 + 2 \cdot r_{h_j h_m} \cdot CV_{h_j} \cdot CV_{h_m}$$

Similarly, we obtain for the mature selection

$$[11] \quad CV_m^2 = CV_{h_m}^2$$

Inserting [10] and [11] in [9] one obtains the final expression for the relative error on the gain ratio. Juvenile selection is only significantly superior when the gain ratio minus twice its standard error is larger than 1.0, i.e.

$$[12] \quad GR(m/j) > 1/(1-2 \cdot CV_{GR})$$

To give an example, assume that:

(i) $h_j = h_m$,

(ii) $r_{h_j h_m} = 0.7$,

(iii) $CV(h_m^2) = 40\%$ (Cotterill and Dean 1987, Dean et al. 1983, Foster

1986, Loo et al. 1984, Magnussen and Yeatman 1987),

(iv) $CV_h = 0.5 \cdot CV_h^2$, and

(v) $r_{jm} = 1.02 + 0.308 \cdot \log_e(T_j/40)$, then the relative error on GR(m/j)

is:

$$[13] \quad CV_{GR} = (-0.0049 - 0.0909 \times \log_e(T_j/40))^{\frac{1}{2}}$$

For $T_j=2, 4, 6, 8,$ and 10 the critical minimum values of GR(m/j) are: 18.0, 5.0, 3.4, 3.0, 2.7, and 2.2 respectively. Most of the GR(m/j) values in Table 1 satisfy the significance requirements. The standard error of GR(m/j) decreases rapidly with the juvenile-mature correlation and, of course, with decreasing sampling errors on the heritabilities.

Errors in juvenile selection can also arise from predictions of genotype performance in an environment different from the one upon which the estimates of heritability and correlation are based. Formulae for calculating the reduction in expected gain due to limited testing are given by Lindgren (1984), Matheson and Raymond (1986), and Owino (1977). Whether this reduction is the same for juvenile and mature material is largely unknown. Before we can answer this we need to know whether genotype x environment x age interactions are important or not, and how the relative importance of genotype x environment interactions changes with time.

CONCLUSION

Juvenile selection has been treated here as an independent breeding activity, and its merits have been derived from a simple theoretical model for a single trait only. Economic and ecological factors, management, and technical aspects have been ignored in order to achieve simplicity. More complex schemes, such as the optimized two-stage selections with restrictions, hold promise in forestry trials (Cotterill and James 1981). Considering the general lack of mature data, it appears that applied juvenile selection is still ahead of the theory. Against these shortcomings it appears that selection before one half the economic rotation age will always result in considerable more genetic gain per unit time than selection at maturity. Under favourable conditions optimum selection age is less than one-tenth of the rotation age.

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Table 1. Optimum selection ages (T_j) and ratio of juvenile to mature gain per unit selection differential in a continuous breeding programme with a tree species which reaches economic maturity at age 40.
 (Assumptions: constant heritabilities, $r_{jm}=1.02+0.308 \cdot \log_e(T_j/40)$, delay (D) = 3 yrs., frequency of seed harvest (S) = one every three years).

Duration of breeding programme (TT)	Gain delay (d)	No. of final harvests of improved seed (N _F)	Maximizing objective -----					
			Avg. rate of impr.	Net present value of gain at interest rate p	T _j	GR(m/j)	T _j	GR(m/j)
1(40+3)=43	0+3	5	4 (5.9)	4 (4.8)	6 (9.8)	6 (23.5)		
		10	4 (4.2)	7 (3.6)	6 (7.7)	6 (19.6)		
		30	7 (3.0)	7 (2.9)	6 (6.8)	6 (18.7)		
40+3	0+3	5	11 (5.9)	18 (2.3)	15 (3.6)	9 (9.8)		
		10	11 (1.8)	19 (1.6)	18 (2.7)	9 (7.9)		
		30	18 (1.5)	19 (1.2)	18 (2.7)	9 (7.4)		
2(40+3)=86	0+3	5	6 (3.8)	6 (3.6)	6 (7.4)	6 (19.2)		
		10	6 (3.3)	6 (3.3)	6 (7.2)	6 (19.1)		
		30	6 (2.9)	6 (3.0)	6 (7.1)	6 (19.0)		
40+3	0+3	5	18 (1.6)	24 (1.2)	15 (2.3)	9 (7.6)		
		10	18 (1.5)	24 (1.2)	15 (2.3)	9 (7.6)		
		30	18 (1.3)	27 (1.1)	15 (2.3)	9 (7.6)		
3(40+3)=129	0+3	5	6 (3.3)	6 (3.3)	6 (7.1)	6 (19.0)		
		10	6 (3.1)	6 (3.2)	6 (7.1)	6 (19.0)		
		30	6 (2.8)	6 (3.1)	6 (7.1)	6 (19.0)		
40+3	0+3	5	15 (1.3)	27 (1.1)	15 (2.3)	9 (7.6)		
		10	15 (1.3)	27 (1.1)	15 (2.3)	9 (7.6)		
		30	15 (1.2)	30 (1.1)	15 (2.3)	9 (7.6)		

Table 2. Values of $E(r_{jm})/r_{jm}$
 (After Cochran (1951), page 465)

$\frac{1}{2}\tau^2 =$ $\frac{r_{jm}^2 (n-p-1)}{2(1-r_{jm}^2)}$	<u>p = Number of x-Variates</u>			
	2	3	4	5
0.2	.377	.324	.288	.262
0.4	.510	.441	.394	.360
0.6	.597	.521	.469	.430
0.8	.661	.581	.526	.484
1.0	.710	.629	.572	.528
1.5	.793	.714	.656	.611
2.0	.844	.770	.714	.670
3.0	.900	.838	.788	.747
4.0	.928	.876	.833	.796
5.0	.944	.900	.863	.830

* For values of $\frac{1}{2}\tau^2$ outside these limits, use the following approximations:

$$(\frac{1}{2}\tau^2 > 5): \frac{E(r_{jm})}{r_{jm}} = \frac{1}{\sqrt{1 + \frac{(p-1)}{\tau^2}}}$$

$$(\frac{1}{2}\tau^2 < 0.2): \frac{E(r_{jm})}{r_{jm}} = \frac{(\frac{p-1}{2})!}{(\frac{p}{2})!} \frac{\tau}{\sqrt{2}}$$

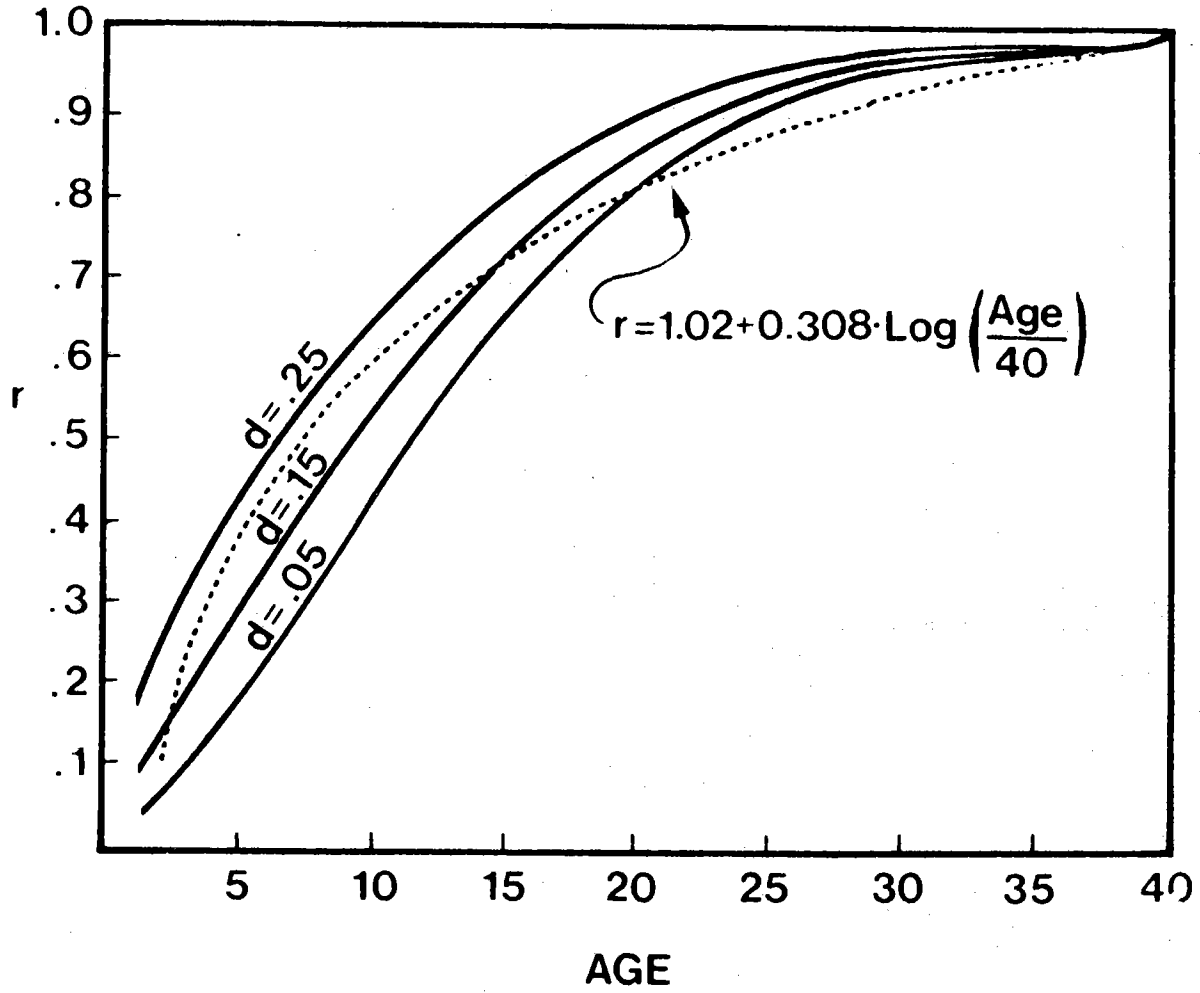


Fig. 1. Juvenile-mature correlation over age for three levels of d . Lambeth's equation for correlation predictions are displayed for comparison (dashed line).

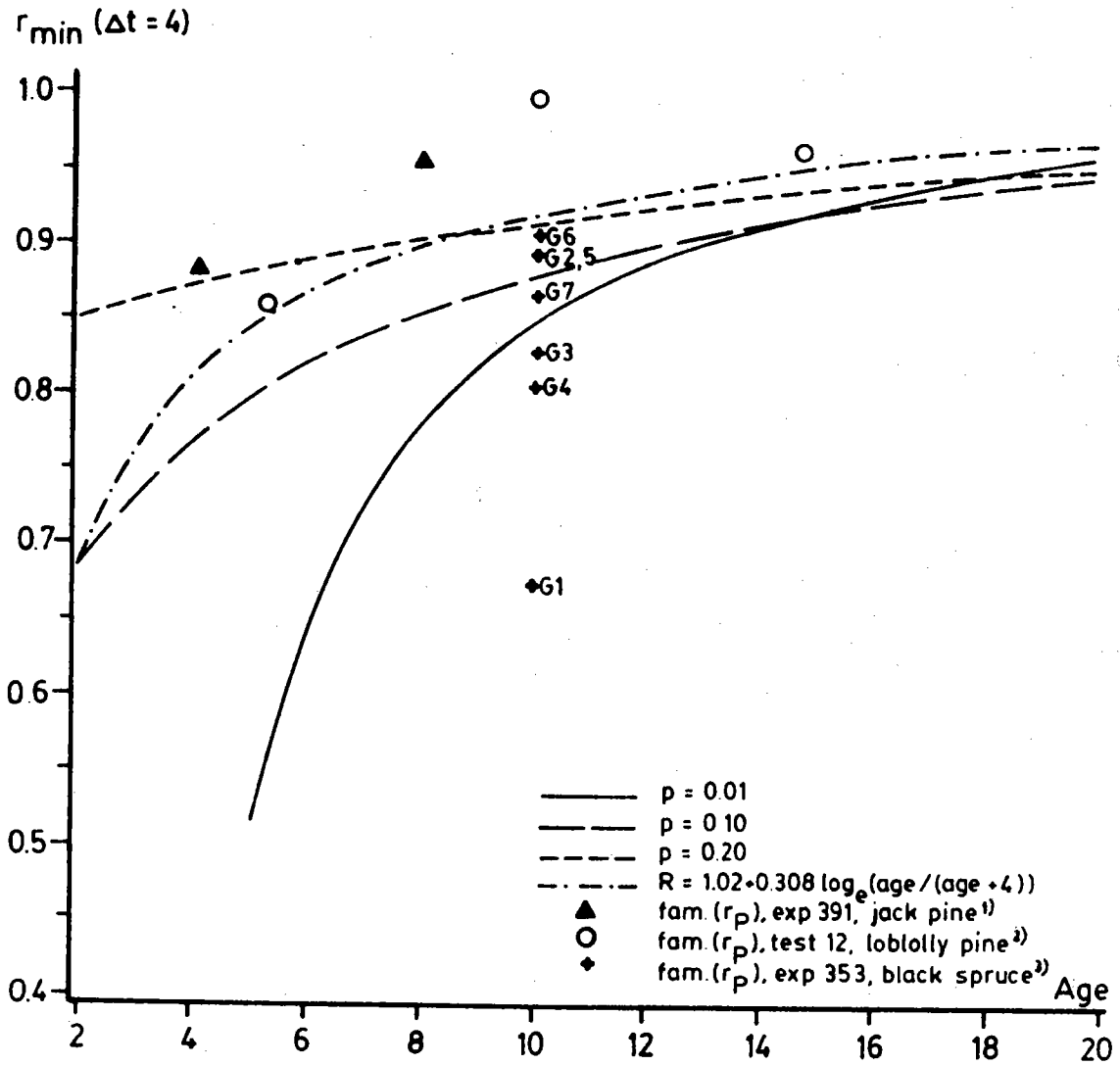


Fig. 2. Minimum age-to-age correlation for three proportions (P) of selected entries.

MICROPROPAGATION OF CONIFERS:
METHODS, OPPORTUNITIES AND COSTS

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ABSTRACT

Clonal propagation is an integral part of any tree improvement programme. During the last 10 years lab-scale protocols have been developed for the micropropagation of nearly 25 conifers via the multi-staged organogenic route; and in the last two years, somatic embryogenesis has also been achieved in six species. Success has mainly been with embryonic and juvenile tissues, but progress is being made with tissues from mature trees. Several opportunities exist for the application of presently available micropropagation technology to specific forestry problems, but some limitations exist. Based on certain assumptions, it appears that micropropagation is economically feasible in Canada, and can play a role in producing the superior planting stock required for reforestation. However, the technologies and the infrastructure for delivery needs to be developed.

RÉSUMÉ

La multiplication clonale fait partie intégrante de tout programme d'amélioration des arbres. Au cours des 10 dernières années, on a élaboré des protocoles dans des laboratoires pour la micropropagation de près de 25 conifères par l'intermédiaire de la route organogénique à étapes multiples; et au cours des deux dernières années, on a réalisé l'embryogénèse somatique chez six espèces. On a obtenu du succès principalement avec les tissus embryonnaires et juvéniles, mais on fait du progrès avec les tissus d'arbres matures. Il est possible, à plusieurs égards, d'appliquer la technique de micropropagation actuellement disponible à des problèmes précis en foresterie, mais il existe certaines limites. D'après certaines hypothèses, il semble que la micropropagation est économiquement faisable au Canada et peut jouer un rôle dans la production du stock de plantation supérieur nécessaire au reboisement. Toutefois, il est nécessaire de mettre au point les technologies et les infrastructures pour la réalisation.

INTRODUCTION

It has long been recognized that there are potential benefits to the use of clonal planting stock in reforestation programs. Kleinschmit (1974) has indicated that at least a 10% increase in gain can be expected from planting selected clonal propagules rather than selected seed families. Work carried out with western hemlock (Tsuga heterophylla (Raf.) Sarg.) has supported this view (Rediske 1979). However, to achieve the maximum possible genetic gain for forest improvement both sexual reproduction and vegetative multiplication must be used (Hasnain and Cheliak 1986). Sexual reproduction is important for both introducing new genes to prevent inbreeding and for achieving genetic gain for those characteristics controlled by additive gene effects. Asexual reproduction allows the multiplication of elite full-sib families, or individuals in a family, that exhibit significant gain due to non-additive gene effects.

The traditional methods for vegetative propagation are rooted cuttings or rooted needle fascicles (also known as brachyblasts, short shoots, dwarf spurs) for the pine species, and grafting. As far as forest tree species are concerned, Cryptomeria japonica D. Don. has been propagated by cuttings for centuries in Japan. More recently, Norway spruce (Picea abies (L.) Karst.) has been propagated in a similar manner on a large scale (2.5 million cuttings annually in Sweden). However, for the majority of conifers, propagation by rooted cuttings is in various stages of development and is often characterized by a rapid loss of rooting capacity of the ramet (cutting) with increasing age of the ortet (parent plant) (Thorpe and Biondi 1984). This tends to defeat one of the main aims of vegetative propagation, which is to multiply trees old enough to have demonstrated their superior characteristics. Percent rooting, speed of rooting, root length and number, survival and growth in and after the year of rooting, all decline, particularly when the parent plant is more than 10 years old (Girouard 1974). Furthermore, in many species, rooted branch cuttings tend to continue to grow with a horizontal orientation and bilateral symmetry (plagiotropism) until, after varying periods of time, the terminal meristem changes to radial symmetry and vertical growth (orthotropism). This reversal to normal growth frequently displays intra- and interclonal variations and thus is erratic, so that the evaluation of genotypes in a selection experiment becomes almost impossible (Libby 1974).

The main advantage of using cell culture as a tool in breeding programs and mass production is its potential for enormous (potentially unlimited) multiplication rates. Cell culture provides a much greater control over plant development and can force expression of totipotency. Thus, while a rooted cutting can produce a single plant from which, several years later, further cuttings are available, even the most limited cell culture systems - that of resting buds - with today's techniques can produce several axillary as well as adventitious shoots. Both these types of shoots can in turn be induced to form additional axillary and/or adventitious shoots, often within a matter of weeks or, at most, months. The intent of clonal propagation, therefore, is to reproduce large quantities of uniform plants of selected qualities in the shortest possible time. Since woody plants tend to be outbreeders, uniformity can only be guaranteed through clonal multiplication.

MICROPROPAGATION METHODS

Asexual multiplication of plants via tissue culture approaches can be achieved by (1) enhancing axillary bud breaking, (2) production of adventitious buds, and (3) somatic embryogenesis. The potential for forming large numbers of plantlets in vitro increases in the above order, but unfortunately, so does the difficulty in producing plantlets. Adventitious shoots or roots can be induced to form on tissues which normally do not produce these organs and is the main route presently used in the clonal propagation of conifers. The governing principles for micropropagation have been outlined recently by Thorpe and Patel (1984) and Dunstan and Thorpe (1986).

Plantlet formation via adventitious budding

The production of plantlets by this route is a multi-staged one, consisting of at least four distinct phases, namely (1) induction of shoot buds on the explant, (2) development of these buds into shoots and their multiplication, (3) rooting of the developed shoots, and (4) the hardening of the plantlets (Biondi and Thorpe 1982, Thorpe 1985). The optimum requirements of each stage and for each species must be experimentally determined. This is time consuming but can lead to rates of multiplication not available by any other method of vegetative propagation.

The formation of adventitious buds involves an interplay between the inoculum, the medium and the culture conditions. In conifers, buds are generally induced directly on the explant and the callus stage is bypassed. In general, the more juvenile the tissue, the better it will respond to in vitro treatments leading to de novo organogenesis. Thus, mature isolated embryos are frequently the explant of choice. In addition, other juvenile explants such as cotyledons, and epicotyls are also frequently used; as well as explants from adolescent and mature trees (Table 1).

Many factors influence the behavior of explants in culture (Murashige 1974). These include (1) the organ that is serving as the tissue source, (2) the physiological and ontogenetic age of the organ, (3) the season in which the explants are obtained, (4) the size of the explant, and (5) the overall quality of the plant from which the explant is taken. In some cases pretreatment of the explant source, e.g. spraying of trees with cytokinins, may also be a requirement for successful organ formation in culture. Successful sterilization of explants is a requirement for in vitro culture, and often involves several steps (Thorpe and Patel 1984).

Various mineral salt formulations have been used for the culture of conifers. In Calgary, Schenk and Hildebrandt's (1972), Quoirin and Le Poivre's (1977), von Arnold and Erkiesson's (AE) (1981), and Bornman's MCM (1983) formulations have proven to be most useful. Full strength mineral salts are not always optimum, and even different formulations work better at different stages (Thorpe and Patel 1984). In addition, a carbon-energy source (usually sucrose), vitamins, reduced nitrogen (normally amino acids) and phytohormones are needed. In general, conifers produce buds in

response to exogenous cytokinin alone, with N⁶-benzyladenine being the most commonly used cytokinin. Many factors of the culture environment influence growth and differentiation. These include (1) the physical form of the medium (2) pH, (3) humidity and gas atmosphere, (4) light, and (5) temperature. Most success has been achieved with agar-solidified medium.

A few cases of shoot formation in conifer callus cultures have been reported since the original report of Ball (1950). However, only recently has regeneration from long-term subcultured conifer callus been possible (Gladfelter and Phillips 1987). This is one prerequisite for the application of genetic engineering approaches to conifer modification and improvement (Thorpe 1983).

The second phase of plantlet formation in conifers involves the development of the nodular tissue formed during the bud induction phase into shoots with primary needles. These shoots are then multiplied, so that the potential for large scale clonal propagation can be realized. The approaches used are designed to achieve the above and at the same time lead to minimum callus formation, which has been shown to increase the chances of producing abnormal plants (Patel and Berlyn 1982). Generally, the formation of true shoot apices with juvenile leaf primordia requires transfer onto a medium with altered nutritional and/or phytohormonal levels, often with the inclusion of activated charcoal (Biondi and Thorpe 1982, Thorpe and Biondi 1984), but several factors have to be tested (Thorpe and Patel 1984).

Rooting of angiosperm trees in culture has been relatively easy while gymnosperms have presented more problems (Sommer and Caldas 1981). Some spontaneous rooting does occur, but in general an auxin treatment, usually indole butyric acid, is required for successful root formation. Here again, other factors must be tested. Quite often the level of mineral salts and sucrose in the medium must be reduced (Thorpe and Biondi 1984). One major concern is to minimize the formation of callus at the base of the shoot. Where much callus is formed, roots often arise in the callus and a functional root/shoot junction may not be obtained. Lastly, rooting is carried out under non-sterile (greenhouse or growth cabinet) conditions.

The final phase of any micropropagation protocol is the hardening of the plantlets, so that they will survive transfer to the greenhouse and finally to the field. When rooting is carried out under non-sterile conditions, acclimatization of the plantlets is relatively easy. In contrast, sterile rooting often produces plantlets with inadequate or inoperative waxy cuticles and stomata. In addition, the high sucrose and salt medium used, limits the photoautotrophic capacity of the leafy shoots. Thus, where possible, rooting and acclimatization should be combined (Dunstan and Thorpe 1984). Several factors should be considered in preparing plantlets for transfer to the soil (Sommer and Caldas 1981), but most of these considerations are horticultural in nature and are identical to those needed for any vegetatively propagated material, e.g., for rooted cuttings.

By using the above procedures empirically, it has been possible to produce plantlets for over 20 conifers via adventitious budding. As indicated in Table 1, many of these are grown in Canada. Examples of micropropagation protocols arising from the work in Calgary include those for western red cedar (Thuja plicata Donn) (Coleman and Thorpe 1977), black and white spruce (Picea mariana (Mill.) B.S.P. and P. glauca (Moench) Voss) (Rumary and Thorpe 1984), lodgepole pine (Pinus contorta Dougl.) (Patel and Thorpe 1984), pitch pine (Pinus rigida Mill.) (Patel et al. 1986), Engelmann spruce (Picea engelmannii Parry) (Patel and Thorpe 1986) and eastern white cedar (Thuja occidentalis L.) (Harry et al. 1987).

Plantlet formation via somatic embryogenesis

Plantlet regeneration via this route is preferred, whenever possible, because (1) of the difficulty and time expended with rooting of shoots derived from adventitious budding, (2) somatic embryogenesis provides an effective method for rapid propagation of large numbers of plants, and (3) embryogenic suspensions obtained from embryogenic callus can serve as a source of embryogenic protoplasts which can be used for genetic engineering of trees. However, somatic embryogenesis is achieved less frequently than other methods of regeneration, particularly in woody species.

In conifers, somatic embryo formation and subsequent plantlet regeneration has been reported for only a few species (Table 2). Most success has been with embryos. To date, only a small percentage of the somatic embryos formed develop into complete plantlets, and most of these regenerates become dormant and cease growing, when transferred to the greenhouse. However, considering that success by this method was first achieved in 1985, good progress is being made.

Micropropagation of mature conifers

As indicated above, most success in conifer micropropagation has been with embryonic and juvenile explants. Ideally, one would like to be able to select explants from phenotypically selected superior trees. A major question is what is the earliest age that such selections can be safely made? This age naturally varies with the species, but many tree breeders and silviculturalists believe that judicious selections can be made during the adolescent phase. This age varies, but for most Canadian conifers is about 10-15 years (Hasnain and Cheliak, 1986). Thus it may not be necessary to develop micropropagation protocols for mature species. Nevertheless, efforts are going on with explants from mature trees, and so far a few plantlets have been produced (see David 1982, Bonga 1987).

A major success story in conifer micropropagation is the redwood tree. Here, success resulted from the use of current year basal sprouts (Boulay 1979, Poissionier et al. 1980). These basal sprouts are juvenile and thus easier to manipulate in culture than shoots taken from the upper bole of the tree or even two-year-old basal sprouts. Perhaps the best

example of micropropagation from mature explants was achieved using fascicle shoots of 11-year-old Pinus pinaster Sol. (David et al. 1979, Franclet et al. 1980, David 1982). For white spruce, the mineral salt formulation was found to be very important in the culture of buds from 15-18 year-old trees (Mohammed et al. 1987). Orchard trees also responded more favourably than natural stand trees, and the position of the buds on the tree was also important, with terminal buds, the nearest lateral buds of a main lateral branch or of its sublateral branches of highest hierarchy being the most responsive in culture (Dunstan et al. 1987a). Dormant excised buds from 17 to 20 year-old Douglas fir trees also produced adventitious buds (Dunstan et al. 1987b). The buds arising from both white spruce and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) elongate into shoots and rooting studies are now underway.

The difficulty of micropropagating mature species is perhaps the major problem preventing a wider application of tissue culture technology to woody plants. To overcome this problem, several different approaches are being tried with some success (Brown and Sommer 1982, Thorpe and Biondi 1984, Bonga 1987, Franclet et al. 1987). These include (1) severe pruning or hedging, (2) grafting of shoot buds or root suckers, (3) use of older shoot tip cultures to initiate successive cycles of enhanced shoot production, and (4) spraying of mature branches with cytokinins prior to explant selection.

OPPORTUNITIES FOR THE USE OF MICROPROPAGATION

Due to under investment in forest management in Canada, there is now a shortage of wood of the right quality at the right price for both domestic and export markets (Hasnain and Cheliak 1986). This has led in part to the decline in Canada's share of the U.S. newsprint market. It is felt that if the Canadian forest industry is to maintain its market position, forest improvement and renewal programs must be aggressively pursued in conjunction with fibre-use integration and mill modernization.

The normal tree improvement strategy is dependent on successive cycles of sexual reproduction by controlled mating and selection (Hasnain and Cheliak 1986). However, only part of the additive portion of the genetic variation is exploited and delivered to the commercial forest by planting seedlings derived from rogued seed orchards. Tissue culture potentially provides one practical way for delivering greater genetic gain to the commercial forest in a much shorter time than conventional methods. It is estimated that with one micropropagation strategy, plants with superior traits could be available after about seven years, which includes five years for progeny testing and two years for plantlet production. The same quality of plants produced by sexual methods could take at least 20 to 25 years, if not longer.

There are also several other advantages of using micropropagation (Thorpe and Biondi 1984, Hasnain and Cheliak 1986). As indicated earlier, one can produce large numbers of plants from a few unique seeds or from a specific tree that may have some special traits.

Important characteristics could also be selected for inbreeding programs and quickly introduced into commercial operations via micropropagation. These include selection for high specific gravity, eg. in Douglas fir; disease resistance, for example to blister rust in white pine (*Pinus strobus* L.) or scleroderma in jack pine (*P. banksiana* Lamb.); for drought and frost tolerance; also the form and degree of branching of a tree, fibre length and lignin content, and chemicals and chemical feedstocks from wood. All of these could have significant commercial impact.

A further advantage of tissue culture is that clones can be matched to the site more precisely than plants produced from open pollinated seed. Because of the large genetic variation for growth response, foresters cannot make precise estimates of the suitability of open pollinated seed for a specific location. Clones, on the other hand, because of their exact genetic make-up, will give reliable measurements for environmental influences on growth. Therefore, clones could be precisely matched to geographic and climatic conditions (Hasnain and Cheliak 1986). The importance of having clones for silvicultural and other research in forestry improvement cannot be underestimated. In New Zealand, tissue culture derived material is being used in various types of silvicultural experiments (Thorpe et al. 1986).

While there may be many potential benefits, to date there are some clear limitations to the exploitation of vegetative propagation in forestry (Timmis et al. 1987). One of these is the difficulty of propagating mature tissue, but, as indicated earlier, progress is being made in overcoming this problem. A second disadvantage of vegetative propagation is the current uncertainty about the long-term growth behavior of clonal material. These include, for example, plagiotropic growth of plantlets derived from cotyledons of Douglas fir (Timmis and Ritchie 1984), and the failure of some loblolly pine plantlets derived from six-year-old trees to become fully cold-hardy under normal inductive conditions in the field (Timmis et al. 1987). However, early studies revealed that the relative growth rates of plantlets and seedlings of Douglas-fir were similar (Timmis 1985). The third major limitation may be the cost of the technology. This aspect will be discussed later.

Worldwide, thousands of tissue culture-derived plantlets of maritime pine (*Pinus pinaster* Sol.), loblolly pine (*P. taeda* L., Douglas fir, redwood (*Sequoia semperviens* (D. Don) Endl.) and radiata pine (*P. radiata* D. Don) are in the field in France, U.S.A. and New Zealand (Thorpe and Biondi 1984). Nevertheless, the major role of conifer micropropagation in the immediate future is unlikely to be that of directly providing planting stock for reforestation, afforestation, agroforestry or bioenergy plantations, although this remains the long-term strategic goal. Instead its greatest use will probably be as an intermediate phase, a way of rapidly establishing a clonal orchard from selected trees or control-pollinated seed, from which further propagules will be produced by rooted cuttings (Biondi and Thorpe 1982, Thorpe and Biondi 1984).

COST ANALYSIS OF CONIFER MICROPROPAGATION

The most advanced micropropagation technology to date is based on the multistaged organogenic process - an operation which requires a large number of discrete, labour-intensive steps. One requirement for the application of this approach is the ability to scale up the lab-level procedures currently available and maintain quality control. A major difficulty is that there are certain points at which process inefficiencies or loss of plant material can markedly influence the cost of the planting stock (Smith 1986). In radiata pine, the rate of shoot separation by workers was found to vary almost threefold, from 35 to 90 shoots per hour. Similarly, although rooting is normally over 80%, there are occasions when it drops to less than 50%. Thus the final price per plantlet can vary.

Three cost analyses have been done on the application of tissue culture in forestry, one in New Zealand (NZ) by the Forest Research Institute (FRI) (Smith 1986) the second in the United States by Weyerhaeuser Corporation in Tacoma, Washington (Timmis 1985), and the third in Canada by the National Research Council (Hasnain et al. 19867). The FRI analysis indicated that, although there has been an active tree improvement programme carried out with radiata pine in New Zealand since the 1930s (see Thorpe et al. 1986), micropropagation and clonal forestry had the potential to contribute to further improvement of their plantation forestry, even at the estimated 10-fold higher price of producing the micropropagated stock in comparison to seedlings (Smith 1986). The Weyerhaeuser study likewise concluded that micropropagation could pay off, and that one could pay five to six times more per year for planting stock if it led to a doubling of genetic gain (Timmis 1985).

In their analysis, Hasnain et al. (1986) made certain physical and financial assumptions on factors such as the cost of the facility, payback period, consumables, wages, labour, greenhouse costs, etc., and also on the current technology and the multiplication rates obtainable under large-scale micropropagation conditions. Based on these assumptions, it was estimated that the cost of producing micropropagated conifer plantlets in Canada would have been \$491 (per 1000) in 1984, of which \$384 was attributed to micropropagation and about \$107 to the final greenhouse stage, where rooting and hardening were to take place. They concluded that micropropagation could be competitive with seedlings costing \$200 per 1000, if the micropropagated stock had increased growth rates of 30%, but only if this led to a reduction of rotation age by 9 years.

One of the chief limitations to the use of presently available micropropagation procedures is the cost of labour, which accounted for 79.2% of the cost in the NZ study and was estimated to be 68.4% in the Canadian one. Thus any activity which reduces this component will enhance the potential economic competitiveness of the technology. Two major approaches are being taken at present, namely improving the technology itself and automation. In the former, research aimed at increasing shoot multiplication rates, whether by axillary and/or adventitious budding is

being pursued, as is research on somatic embryogenesis and the production of artificial seed. A novel approach in this respect is the finding that cytokinin-induced meristematic tissue in radiata pine can be made to continuously produce meristematic tissue for 2.5 years to date in the presence of cytokinin (Aitken-Christie et al. 1987). Up to 5,480 pieces of meristematic tissue, each yielding ca. 68.4 shoots were obtained from 1 embryo in 13.5 months. A method involving automated pattern recognition is being developed in Australia with a design productivity of 3600 shoots/h (Vic Hartney, personal comm.). Another development is the use of flat microporous rafts to support growth of plant tissues on liquid medium, thus facilitating easy medium change (Hamilton et al. 1985). Other approaches are being tried with some success in commercial enterprises, e.g., P.B. Industries of Israel (Arie Back, personal comm.). These improvements have the potential for cost reduction of \$100-200/1000 plantlets (Hasnain et al. 1986).

CONCLUDING THOUGHTS

Lab-scale protocols are available for several Canadian-grown conifers. Research and demonstration are needed to transform these protocols to the commercial scale. Although the present methods are being utilized elsewhere, the cost per plantlet must be reduced so that micropropagation can be seriously considered as more than a potential tool in reforestation and afforestation in Canada. Studies have shown that the potential is great enough to justify the additional research and development funds that are required to adapt the laboratory scale techniques to the commercial demonstration phase. However, we have a unique problem in this country, namely that most of the forest land is owned by the Crown and under present arrangements there is no incentive for the leaseholder to plant superior stock. However, there is no reason why the technology should not be incorporated immediately into the various tree improvement programmes being undertaken. Most of these programmes are in their first generation of selection and incorporation of in vitro propagation can go a long way to aid in capturing both additive and non-additive genetic traits.

Although the technology is clearly feasible under a number of specific conditions, as indicated earlier, other factors can play an equal or greater role in the decision to use the technology industrially (Timmis 1985). First, capital must be available for investment, and other things being equal, a lower-capital option will be preferred. Second, there must be a demonstration of the technology in the form of small, but clearly superior, block plantings. A third, and perhaps the most important consideration is whether or not the recommendation feels right to the non-technical executive. These same factors will influence the application of the technology in Canada, even though the non-technical executive is unlikely to be from the private sector.

In addition to the use of tissue culture technology in micropropagation of forest tree species, the technology is also of tremendous potential value to forest tree improvement (Thorpe 1983). It is envisaged

as playing a complementary role to more traditional methods through exploiting spontaneous or induced genetic and epigenetic variability in culture, by use of haploidy and by the use of protoplasts. Both haploids and protoplasts can aid in shortening breeding cycles and allow for unconventional crosses. Micropropagation will assume a major supporting role in forest tree biotechnology. With time it will become separated from other tissue culture technologies and become much more integrated with commercial forest nursery operations (Haissig et al. 1987).

To fully exploit the opportunities of micropropagation and other tissue culture methods for forestry, the technologies and the infrastructure for delivering them must be systematically developed. The following is a list of priority areas for research, development and demonstration required to reach the commercialization of these technologies (Hasnain et al. 1986).

1. Development of micropropagation protocols from juvenile and mature tissue of commercially important conifers.
2. Increased effort in conifer breeding with the selection of improved full-sib families and the development of early testing methods for desirable traits.
3. Within family selection for superior individuals and clone testing with plantlets from mature or adolescent tissues.
4. Research on the production of haploid plants through tissue culture to assist conifer improvement and genetic work.
5. Development of methods for cold storage of clones during clonal trials.
6. Scale-up of micropropagation methods for demonstrating commercial feasibility and for producing clones for large scale field trials.
7. Development of improved protocols for micropropagation and somatic embryogenesis, including research towards a better understanding of the biochemical and developmental basis of plant regeneration from cell and tissue culture.
8. Automation of tissue culture facilities.
9. Research on the use of tissue and callus culture for inducing somaclonal variation for the development and selection of traits such as disease resistance.
10. Development of advanced molecular genetics methods for manipulating specific genes and for the study of conifer genetics.

To tackle the above will require an infusion of money and people over a sustained period of time. Considering the importance of forestry earnings to the well-being of Canada, and taking into account the continuing decline in our share of the export market, can we afford to do less?

Worldwide, demands for wood and wood products are expected to increase during the next few decades, and shortages have been forecasted for the end of this century (Keays 1974). Therefore, there is an urgent requirement for large numbers of improved, fast-growing trees with shortened rotation cycles to meet this demand (Thorpe and Biondi 1984). In the future, forest management will be viewed as another form of intensive cropping, differing from agriculture mainly in the length of the rotation and the nature of the economic end products. To fully exploit the potential for intensive cropping systems only superior trees should be utilized for afforestation or reforestation. Micropropagation of conifers, and other aspects of forest biotechnology will play an important role in producing the required superior planting stock for the forests of the future.

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Table 1. Conifers and parts used as explants for micropropagation via adventitious budding
(Updated from Dunstan and Thorpe 1987).

Species	Explant
<u>Araucaria cunninghamii</u> Ait. (Hoop pine)	shoot tips
<u>Chamaecyparis obtusa</u> ¹ Sieb. et Zucc. (Hinoki cypress)	seedling
<u>Picea abies</u> (Norway spruce)	embryos, dormant buds
<u>Picea engelmannii</u> ² (Engelmann spruce)	embryos, seedling parts
<u>Picea glauca</u> (White spruce)	hypocotyls, epicotyls
<u>Picea mariana</u> (Black spruce)	epicotyls
<u>Picea sitchensis</u> (Bong.) Carr. (Sitka spruce)	shoot apices
<u>Pinus brutia</u> ³ Ten. (Calabrian pine)	shoot tips, fascicular buds
<u>Pinus contorta</u> (Lodgepole pine)	embryos, cotyledons, hypocotyls
<u>Pinus palustris</u> Mill. (Longleaf pine)	embryos
<u>Pinus pinaster</u> (Maritime pine)	cotyledons, needle fascicles
<u>Pinus radiata</u> (Monterey or radiata pine)	cotyledons, embryos, sec needles
<u>Pinus monticola</u> Dougl. (Western white pine)	embryos
<u>Pinus rigida</u> (Northern pitch pine)	embryos, cotyledons
<u>Pinus rigida</u> x <u>P. taeda</u>	embryos
<u>Pinus strobus</u> ⁴ (White pine)	cotyledon - hypocotyl
<u>Pinus sylvestris</u> L. (Scots pine)	brachyblast with needles, shoot tips
<u>Pinus taeda</u> (Loblolly pine)	cotyledons
<u>Pseudotsuga menziesii</u> (Douglas-fir)	cotyledons, dormant buds
<u>Sequoia sempervirens</u> (Redwood)	stem segments with buds, basal shoot tips
<u>Thuja occidentalis</u> ⁵ (Eastern white cedar)	embryos
<u>Thuja plicata</u> Donn. (Western red cedar)	cotyledons, lateral shoot tips
<u>Tsuga heterophylla</u> (Western hemlock)	cotyledons

¹ Ishii (1986); ² Patel and Thorpe (1986); ³ Abdullah et al. (1987); ⁴ Kaul (1987);
⁵ Harry et al. (1987).

Table 2. Conifer species and explants producing somatic embryos in vitro.

Species	Explant
<u>Larix decidua</u> Mill. (European larch) ¹	megagametophyte
<u>Picea abies</u> (Norway spruce) ²	immature/mature embryos
<u>Picea glauca</u> (White spruce) ^{3, 4}	immature embryos
<u>Picea mariana</u> (Black spruce) ³	immature embryos
<u>Pinus lambertiana</u> Dougl. (Sugar pine) ⁵	mature embryos
<u>Pinus taeda</u> (Loblolly pine) ⁶	mature embryos

¹ Nagmani and Bonga (1985)

² Hakman et al. (1985)

³ Hakman and Fowke (1987)

⁴ Lu and Thorpe (1987)

⁵ Gupta and Durzan (1986)

⁶ Gupta and Durzan (1987)