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C.T.I.A. A.C.A.A.
TRURO, N.S. - 1987

CANADIAN
TREE IMPROVEMENT
ASSOCIATION

PROCEEDINGS
TWENTY-FIRST MEETING
PART 2

TRURO, NOVA SCOTIA
AUGUST 17 - 21, 1987

ASSOCIATION
CANADIENNE
POUR L'AMÉLIORATION
DES ARBRES

COMPTES RENDUES
VINGT-ET-UNIÈME CONFÉRENCE
2e 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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DE LA

VINGT ET UNIÈME CONFÉRENCE

DE

L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

PARTIE 2

COLLOQUE SUR

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TENUE À

TRURO (N.-É.)

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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 22e conférence, veuillez les adresser au: Canadian Forestry Service, Northern Forestry Centre, 5320-122 St., Edmonton, AB T6H 3S5.

À: *T.J.B. Boyle, rédacteur, A.C.A.A./C.T.I.A.*
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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

CONTENTS/TABLES DES MATIÈRES

INVITED PAPERS/CONFÉRENCIERS INVITÉS

VOLUNTARY PAPERS - ABSTRACTS/CONFÉRENCIERS VOLONTAIRES - RÉSUMÉS

APPENDICES/ANNEXES

CONFÉRENCIERS INVITÉS

INVITED PAPERS

THE STATUS OF PROVENANCE RESEARCH IN CANADA

Cheng C. Ying[1] and E.K. Morgenstern[2]

[1] Research Branch, Ministry of Forests and Lands, Victoria, B.C.
V8W 3E7

[2] Faculty of Forestry, University of New Brunswick, Fredericton, N.B.
E3B 6C2

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 (Heimburger 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 hierarchal 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).

Englemann 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) genecological 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). Hierarchal 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:

Geographic region	Features
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 Heimburger 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 Heimburger 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 m^3 per ha per year on good sites, which could be further augmented by a selection program (Park and Fowler 1983, Vallée and Stipanicic 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 tends 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
Abies *amabilis* (Dougl.) Forbes	1.5	1980
balsamea (L.) Mill	1.9	1960-78
grandis (Dougl.) Lindl.	7.5	1980-84
procera Rehd.	11.0	1981
Larix *decidua*	6.0	1977-80
laricina (Du Roi) K. Koch.	32.2	1974-92
leptolepis	10.0	1973-82
sibirica Ledeb.	2.5	1972-86
Picea *abies*	78.7	1941-91
glauca	49.1	1958-87
mariana	49.1	1957-87
rubens	8.2	1958-86
sitchensis	30.5	1969-85
Pinus *banksiana*	58.4	1954-92
contorta	165.3	1972-91
monticola	7.0	1974-75
nigra Arn.	8.0	1973-83
resinosa	29.2	1954-83
strobus	4.7	1943-86
sylvestris	35.6	1940-86
Pseudotsuga *menziesii*	101.8	1957-91
Tsuga *heterophylla* (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
Acer saccharum Marsh.	1.0	1978
Alnus cordata Lois.	1.0	1989
glutinosa (L.) Gaertn.	19.1	1963-89
incana (L.) Moench	2.5	1986-89
rubra Bong.	4.5	1985-89
Betula alleghaniensis	1.3	1965
Fraxinus americana L.	0.7	1977
pennsylvanica Marsh.	2.0	1974
Populus trichocarpa 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
Larix spp.	4.0	1953-86
Picea spp.	53.0	1942-86
Pinus spp.	19.1	1940-87
Pinus mugo Turra	1.0	1985
Pinus ponderosa	2.0	1975-90
Picea engelmannii	0.4	1953
Picea pungens Engelm.	0.7	1985
Thuja occidentalis	1.0	1980-86
Populus nigra L.	1.0	1992
Prunus serotina Ehrh.	0.4	1969
Tilia cordata Mill.	0.3	1982
TOTAL	82.9	

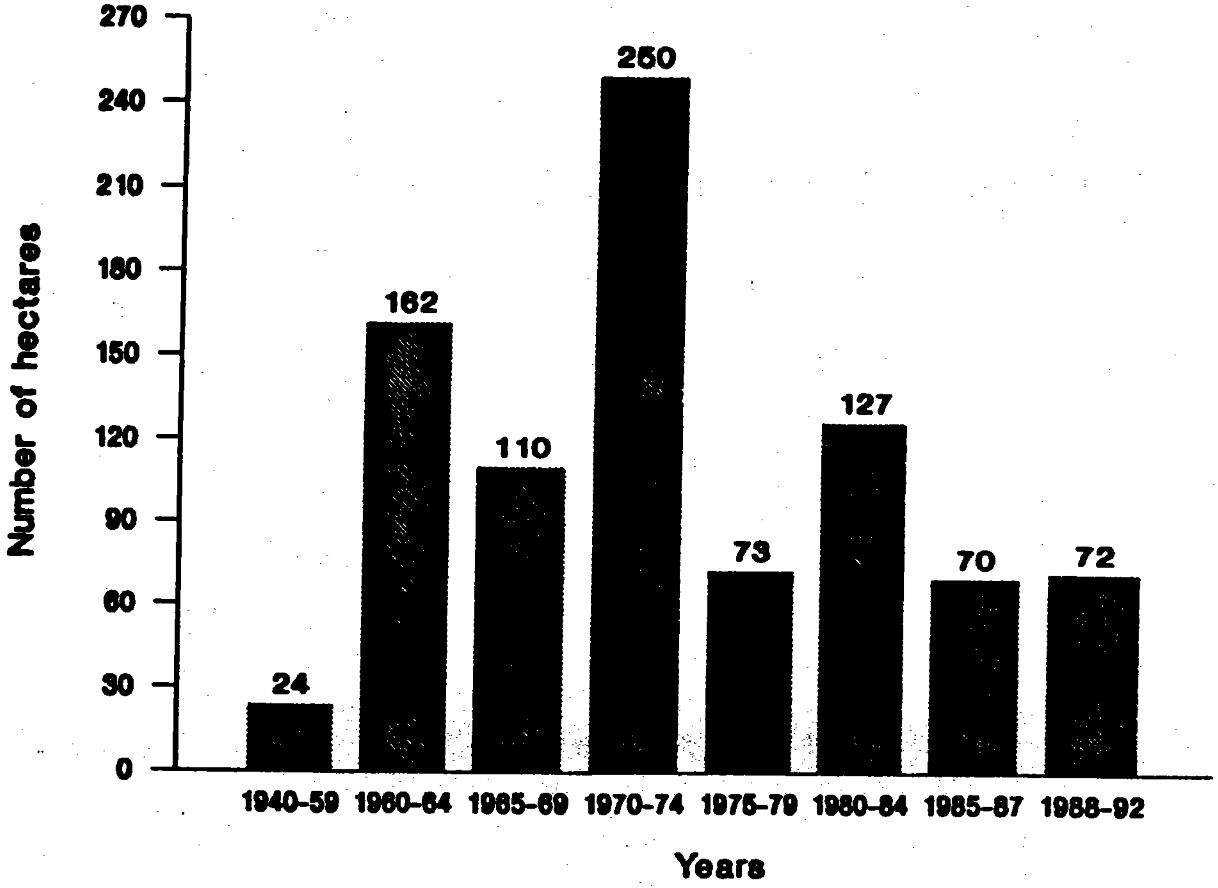

FIGURE 1. Chronological distribution of provenance tests (period 1940-1992).

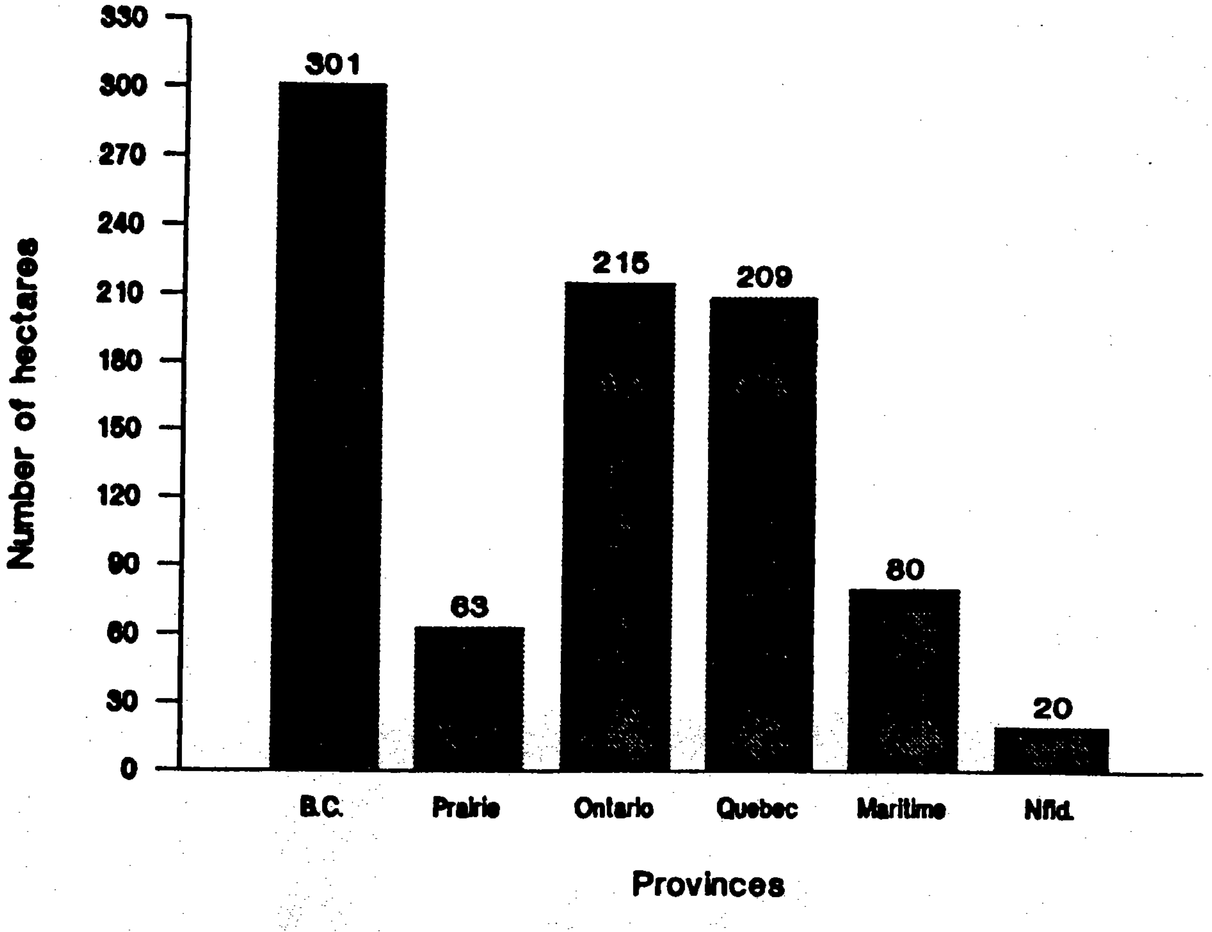

FIGURE 2. Regional distribution of provenance tests (period 1940-1992).

WITHIN-POPULATION GENETIC VARIATION - IMPLICATIONS FOR SELECTION AND BREEDING

T.J.B. Boyle[1] and F.C. Yeh[2]

[1] Canadian Forestry Service, Petawawa National Forestry Institute, Chalk River, Ont. K0J 1J0.

[2] Dept. of Forest Science, University of Alberta, Edmonton, Albert, T6G 2H1.

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 Edmon 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)/engelmann 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-compatability 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 multilocus 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 analagous 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 multilocus 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 epistatis 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
Pinus contorta	58.7	0.160		0.041	Yeh & Layton (1980
		0.119			Wheeler & Guries (1982)
	51.4	0.184	2.50	0.018	Dancik & Yeh (1983)
	64.0	0.165			Yeh et al. (1985)
P. banksiana	74.0	0.146	2.70	0.052	Danzmann & Buchert (1983)
		0.286			Hamrick et al. (1981)
	46.0	0.115	2.10	0.024	Dancik & Yeh (1983)
		0.146-0.169			Knowles (1985)
P. resinosa	0.0	0.000	1.00	-	Fowler & Morris (1977)
P. rigida		0.146			Guries & Ledig (1981)
	78.8				Hamrick et al. (1981)
P. monticola	65.0	0.177	1.70		Steinhoff et al. (1983)
	64.0	0.180	1.79		El-Kassaby et al. (1987)
Picea mariana	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)
P. glauca	85.7	0.183			Cheliak et al. (1984)
	83.4	0.174			Yeh & Arnott (1986)
P. sitchensis	51.3	0.147		0.079	Yeh & El-Kassaby (1981)
	75.9	0.199			Yeh & Arnott (1986)
P. abies		0.32-0.41			Lundkvist & Rudin (1977)
	91.7				Lundkvist (1979)
	71.0-100.0		2.14-3.00		Bergmann & Gregorius (1979)
Pseudotsuga menziesii					
Coastal	68.8	0.155	2.19	0.026	Yeh & O'Malley (1980)
		0.388			Yang et al. (1977)
	54.0	0.171	1.79	0.068	El-Kassaby & Sziklai (1982)
Interior	68.0	0.175		0.043	Yeh (1979)
Abies balsamifera	64.0				Neale (1978)

Table 2. Estimates of outcrossing rates for Canadian conifers.

Species	Biochemical		Morphological	Reference
	Single locus	Multilocus		
Pinus contorta	0.94-1.06	0.95-1.03		Epperson & Allard (1984)
P. banksiana	0.82-0.92	0.91		Cheliak et al. (1985)
	0.80-1.03			Danzmann & Buchert (1983)
P. monticola	0.95	0.98		El-Kassaby et al. (1987)
Picea mariana	0.89-0.96	0.89-0.98		Boyle (1985)
			0.85-0.94*	Morgenstern (1972)
P. glauca	0.98			Cheliak et al. (1984)
Pseutodsuga menziessii	0.66-0.97	0.91-0.93		Shaw & Allard (1982)
	0.90			El-Kassaby et al. (1981)
	0.40-0.94	0.89		Yeh and Morgan (1987)
Larix laricina	0.58-0.91			Knowles & Perry (1987)

* Assumes inbreeding equilibrium

SHORTENING THE BREEDING CYCLE OF SOME NORTHEASTERN CONIFERS

Michael S. Greenwood[1], Greg W. Adams[2] and Michelle Gillespie[2]

[1] College of Forest Resources, University of Maine, Orono, ME, U.S.A., 04469

[2] J.D. Irving Ltd., Sussex, N.B., CANADA E0E 1P0

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 abréger 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 shadeframe 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 ℓ 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 shadeframe 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.

	♀ Strobili		♂ Strobili	
Clones	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 shadeframe for the last half of the GA application period.

		$GA_{4/7}$	Control
Outdoor mid-May to mid-June,	♀	23 (70%)	1 (10%)
then greenhouse thereafter	♂	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.

$GA_{4/7}$ 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 $GA_{4/7}$, 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 $GA_{4/7}$ 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 $GA_{4/7}$ across several years.

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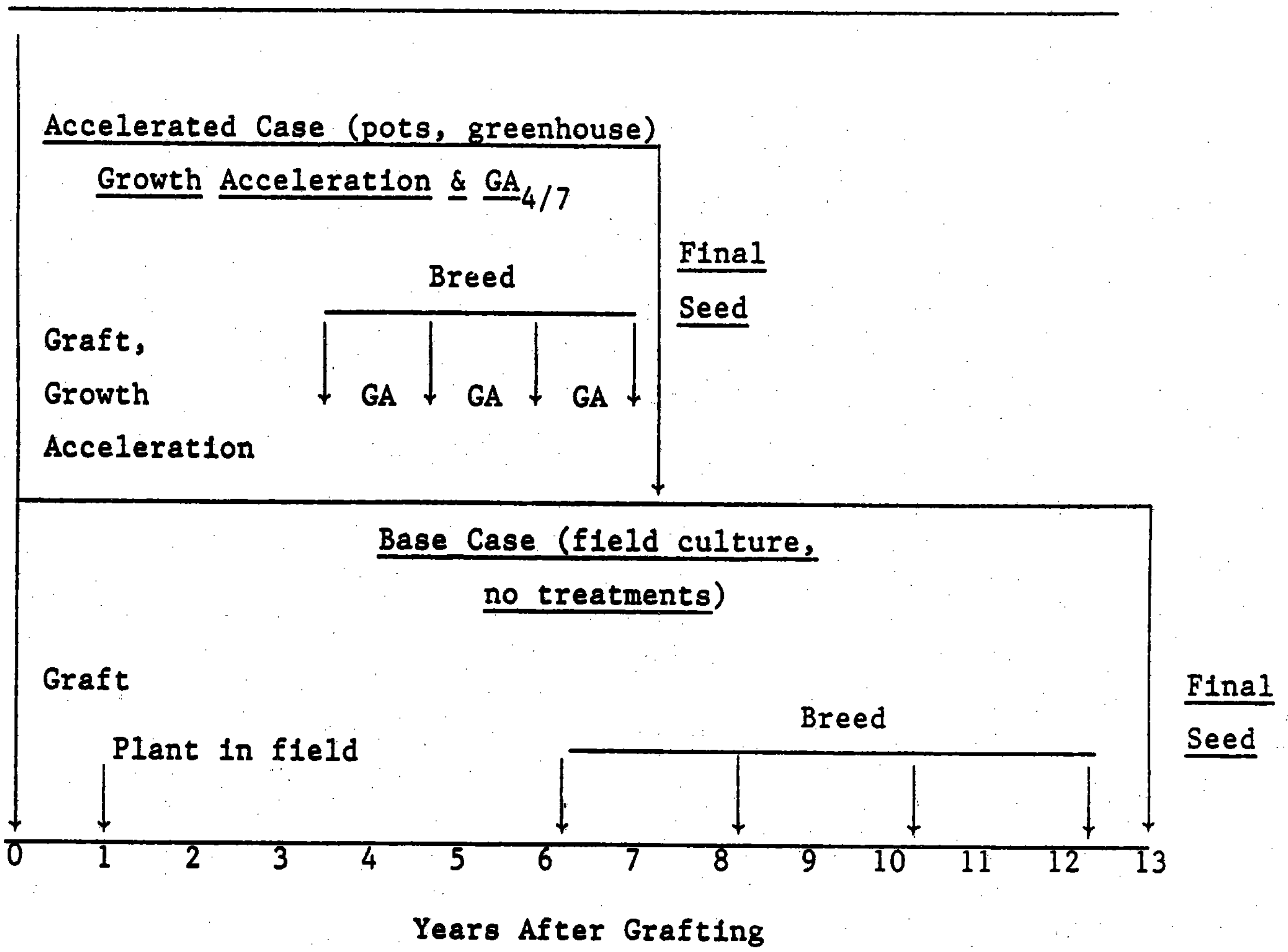

Figure 1. Breeding interval (years between grafting and final seed) with and without accelerated growth, $GA_{4/7}$ applications, for white spruce.

THEORETICAL BASIS FOR EARLY TESTING IN GENETIC IMPROVEMENT PROGRAMS

S. Magnussen and C.W. Yeatman

Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario
K0J 1J0

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] $G(m/j) = h_j \cdot h_m \cdot r_{jm} \cdot i \cdot \sigma_m$ 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] $G(m) = h_m^2 \cdot i \cdot \sigma_m$

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] $G(m/j) = CGP(j,m) \cdot i \cdot \sigma_m$ where

CGP = coefficient of genetic prediction = $\dfrac{COV(A_j, A_m)}{\sigma_j \cdot \sigma_m}$ 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:

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

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 $0.64 \cdot 0.6 = 0.39$ ($\frac{1}{2}\tau^2=1.4$). 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] $r_{jm}' = r_{jm} \sqrt{\frac{1-i(i-x')}{i-r_{jm}^2 \cdot i(i-x')}}$, 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] $CV_R = (CV_j^2 + CV_m^2 - 2 \cdot r_{jm} \cdot CV_j \cdot CV_m)^{\frac{1}{2}}$

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

Similarly, we obtain for the mature selection

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

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

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: p = 2%		p = 6%		p = 10%	
(Yrs.)	(Yrs.)		Tj	GR(m/j)	Tj	GR(m/j)	Tj	GR(m/j)	Tj	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	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	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	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)

$\frac{1}{2}\tau^2 = \frac{r_{jm}^2 (n-p-1)}{2(1-r_{jm}^2)}$	p = Number of x-Variates			
	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:

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

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

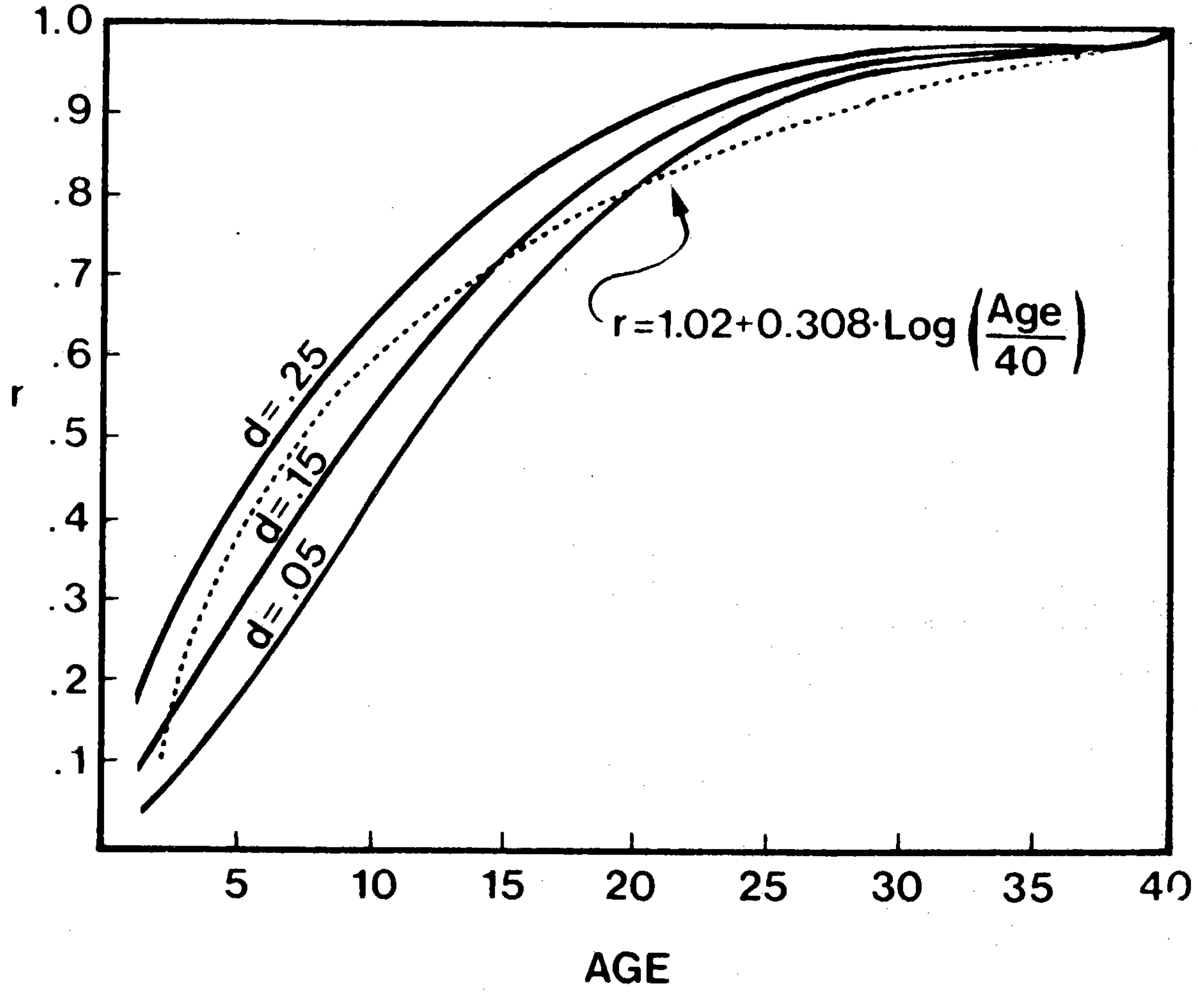

Fig. 1. Juvenile-mature correlation over age for three levels of d. Lambeth's equation for correlation predictions are displayed for comparison (dashed line).

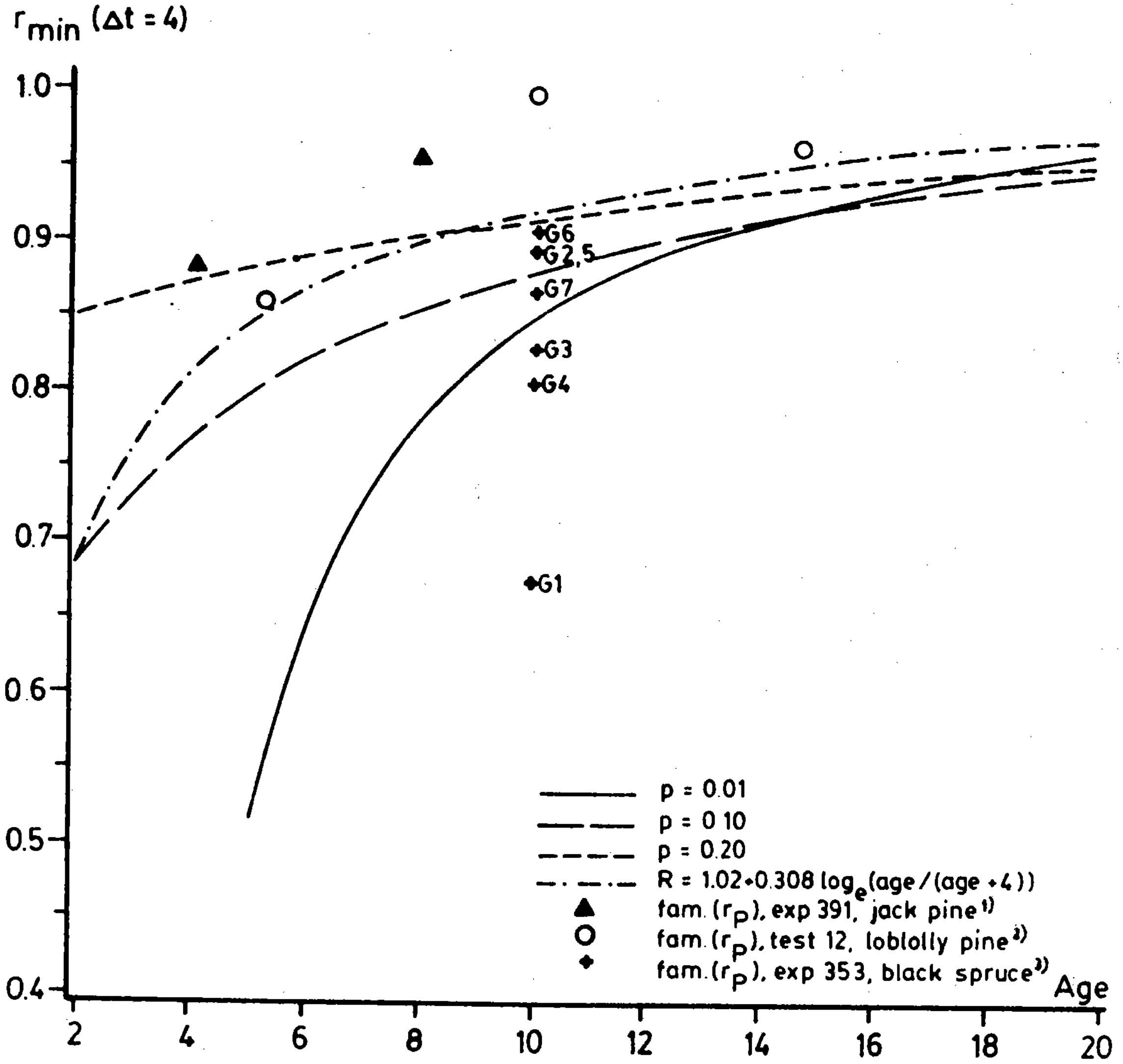

Fig. 2. Minimum age-to-age correlation for three proportions (P) of selected entries.

MICROPROPAGATION OF CONIFERS: METHODS, OPPORTUNITIES AND COSTS

Trevor A. Thorpe[1] and Sadiq Hasnain[2]

[1] Plant Physiology Research Group, Department of Biological Sciences,
University of Calgary, Calgary, AB T2N 1N4

[2] Protein Structure and Design Centre, Division of Biological Sciences
National Research Council, Ottawa, ON K1A 0R6

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 (plagiotropy) until, after varying periods of time, the terminal meristem changes to radial symmetry and vertical growth (orthotropy). 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 Erkisson'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 N^6-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 that 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 sclerroderiris 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
Araucaria cunninghamii Ait. (Hoop pine)	shoot tips
Chamaecyparis obtusa[1] Sieb. et Zucc. (Hinoki cypress)	seedling
Picea abies (Norway spruce)	embryos, dormant buds
Picea engelmannii[2] (Engelmann spruce)	embryos, seedling parts
Picea glauca (White spruce)	hypocotyls, epicotyls
Picea mariana (Black spruce)	epicotyls
Picea sitchensis (Bong.) Carr. (Sitka spruce)	shoot apices
Pinus brutia[3] Ten. (Calabrian pine)	shoot tips, fasicular buds
Pinus contorta (Lodgepole pine)	embryos, cotyledons, hypocotyls
Pinus palustris Mill. (Longleaf pine)	embryos
Pinus pinaster (Maritime pine)	cotyledons, needle fascicles
Pinus radiata (Monterey or radiata pine)	cotyledons, embryos, sec needles
Pinus monticola Dougl. (Western white pine)	embryos
Pinus rigida (Northern pitch pine)	embryos, cotyledons
Pinus rigida x *P. taeda*	embryos
Pinus strobus[4] (White pine)	cotyledon - hypocotyl
Pinus sylvestris L. (Scots pine)	brachyblast with needles, shoot tips
Pinus taeda (Loblolly pine)	cotyledons
Pseudotsuga menziesii (Douglas-fir)	cotyledons, dormant buds
Sequoia sempervirens (Redwood)	stem segments with buds, basal shoot tips
Thuja occidentalis[5] (Eastern white cedar)	embryos
Thuja plicata Donn. (Western red cedar)	cotyledons, lateral shoot tips
Tsuga heterophylla (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
Larix decidua Mill. (European larch)[1]	megagametophyte
Picea abies (Norway spruce)[2]	immature/mature embryos
Picea glauca (White spruce)[3,4]	immature embryos
Picea mariana (Black spruce)[3]	immature embryos
Pinus lambertiana Dougl. (Sugar pine)[5]	mature embryos
Pinus taeda (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)

TREE IMPROVEMENT STRATEGIES - FLEXIBILITY REQUIRED

D.P. Fowler

Canadian Forestry Service - Maritimes
P.O. Box 4000, MacKay Drive
Fredericton, N.B. E3B 5P7

ABSTRACT

Technological advances in our ability to manipulate the genetic characteristics of our forests can have a profound effect on current breeding strategies. Tree breeders are urged to keep their breeding strategies flexible to accommodate and best utilize the new technology as it becomes available.

The implication of technological advances in both vegetative propagation and early testing are discussed in some detail. Other anticipated advances in the areas of tissue culture, flower induction, rejuvenation of conifers, single-cell culture, protoplast fusion, and gene transfer are also discussed.

> "If you are going to plan ahead,
> you had better do it often."
> Chairman Mao

RÉSUMÉ

Les progrès technologiques que nous avons réalisés dans la manipulation des caractéristiques génétiques de nos forêts peuvent avoir un effet marqué sur les stratégies actuelles de reproduction. Nous incitons les généticiens forestières à faire en sorte que leurs stratégies de reproduction soient flexibles pour adopter et mieux utiliser la nouvelle technologie.

On expose en détail la répercussion des progrès technologiques réalisés dans les premiers essais et dans le domaine de la multiplication végétative. On expose également les autres progrès prévus dans les domaines de la culture de tissus, la stimulation de la floraison, le rajeunissement de conifères, la culture de cellules uniques, la fusion de protoplastes et le transfert de gènes.

INTRODUCTION

Over the past few years I have devoted a considerable part of my time to the development of strategies for the genetic improvement of tree species of importance for reforestation in the Maritimes Region. The strategies were worked out in consultation with numerous colleagues all of whom have contributed one way or another. Despite the broad input and fairly intensive review, it is evident that the proposed strategies can benefit from new innovation or change. This possibility was anticipated when we first drew up the strategies, however, we did not fully appreciate just how flexible the strategies should be.

In developing long-term strategies we look ahead several generations and design our plans based on what we consider the best genetic and biological information available. At the same time, we know that advances in such things as biotechnology and molecular genetics make it inconceivable that we will actually follow the proposed strategies for more than two or three generations. As we learn more about a species, our ability to manipulate that species changes - sometimes dramatically. Also, technological changes in our ability to manipulate the genetics of our species may make our current strategies redundant. One wonders if it is desirable to plan ahead more than a generation or two except in a broad sense, e.g., maintenance of a reasonably broad genetic base for the breeding populations. Of course it is necessary to plan ahead. However, it would make the planning more realistic if we could anticipate some of the changes what will occur and, where possible, design our strategies to accommodate those changes.

In this paper, I discuss some of the technical advances that will likely occur and point out some of the effects that the changes will have on our improvement strategies. To simplify the presentation I will concentrate on species and strategies with which I am reasonably familiar, i.e., conifer species currently of importance for reforestation in the Maritimes. I have listed the anticipated technological advances as short-, mid-, or long-term:

Short-term

- mass vegetative propagation of juvenile materials
- tissue culture of juvenile materials
- flower induction
- early testing

Mid-term

- rejuvenation of mature conifers
- regeneration of conifers from single cells

Long-term

- protoplast fusion
- gene transfer

Mass vegetative propagation of juvenile materials

I anticipate that over the next decade vegetative propagation using juvenile materials will become a viable alternative for the mass production of most species and hybrids. Currently, black spruce, Picea mariana (Mill.) B.S.P., is being propagated vegetatively on a commercial scale by one nursery in Nova Scotia at a cost of about 1.5 times the cost of comparable seedling stock. Techniques are also available to mass propagate Larix Mill. species and hybrids from stem cuttings. Norway spruce, P. abies (L) Karst., although not propagated from cuttings on a commercial scale locally, can also be propagated from stem cuttings. Vegetative propagules of white spruce, P. glauca (Moench) Voss, red spruce, P. rubens Sarg. and jack pine, Pinus banksiana Lamb. will probably be somewhat more expensive to produce commercially.

To date, vegetative propagation of northern conifers has been used primarily to increase the numbers of plants available from selected seed sources. There has been little opportunity to mass produce genetically improved trees, because proven stocks have not been available. This is rapidly changing. Many tree improvement programs in Canada are now contributing at least a small portion of the seedlings used for reforestation. These seedlings can be multiplied by vegetative propagation to increase the proportion of improved materials available for reforestation. In several programs, high general combining ability (GCA) parents have been identified, and can be crossed to produce small numbers of substantially superior seed which, when multiplied by vegetative techniques, can provide operational quantities of genetically improved trees.

The change from sexual to asexual methods of mass producing materials for reforestation will require some, although not massive, changes in our improvement strategies. Breeding gardens or breeding halls will replace seed orchards and hedges or greenhouses will be used to multiply the stock materials. For multigeneration improvement it will still be necessary to breed for high GCA using a polycross or a more sophisticated breeding design. For those species or hybrids where specific combining ability (SCA) is important, e.g. tamarack (Larix laricina (Du Roi) K. Koch) and larch hybrids, the number of pair matings will have to be increased to identify the better specific combiners.

Figure 1 is a flow chart for the black spruce improvement strategy currently being used in the NBTIC program. Details of this chart are presented elsewhere. Figure 2 outlines the changes anticipated in this strategy if vegetative propagation of juvenile materials is accepted as the method of mass producing planting materials.

The New Brunswick Tree Improvement Council (NBTIC) program has progressed to the point where the selection of the best individual, in each of the best 400 families (5) has commenced. These selections will be cloned and go to a breeding garden or preferably to a breeding hall (6). The clones will be divided into two groups, A and B. Clones in group A will be crossed with a pollen mix from group B, and visa versa (7). The resulting progenies will be progeny tested (8) and additional materials from these same crosses will be used as stock for mass vegetative propagation (9). The resulting propagules will be the equivalent of what would

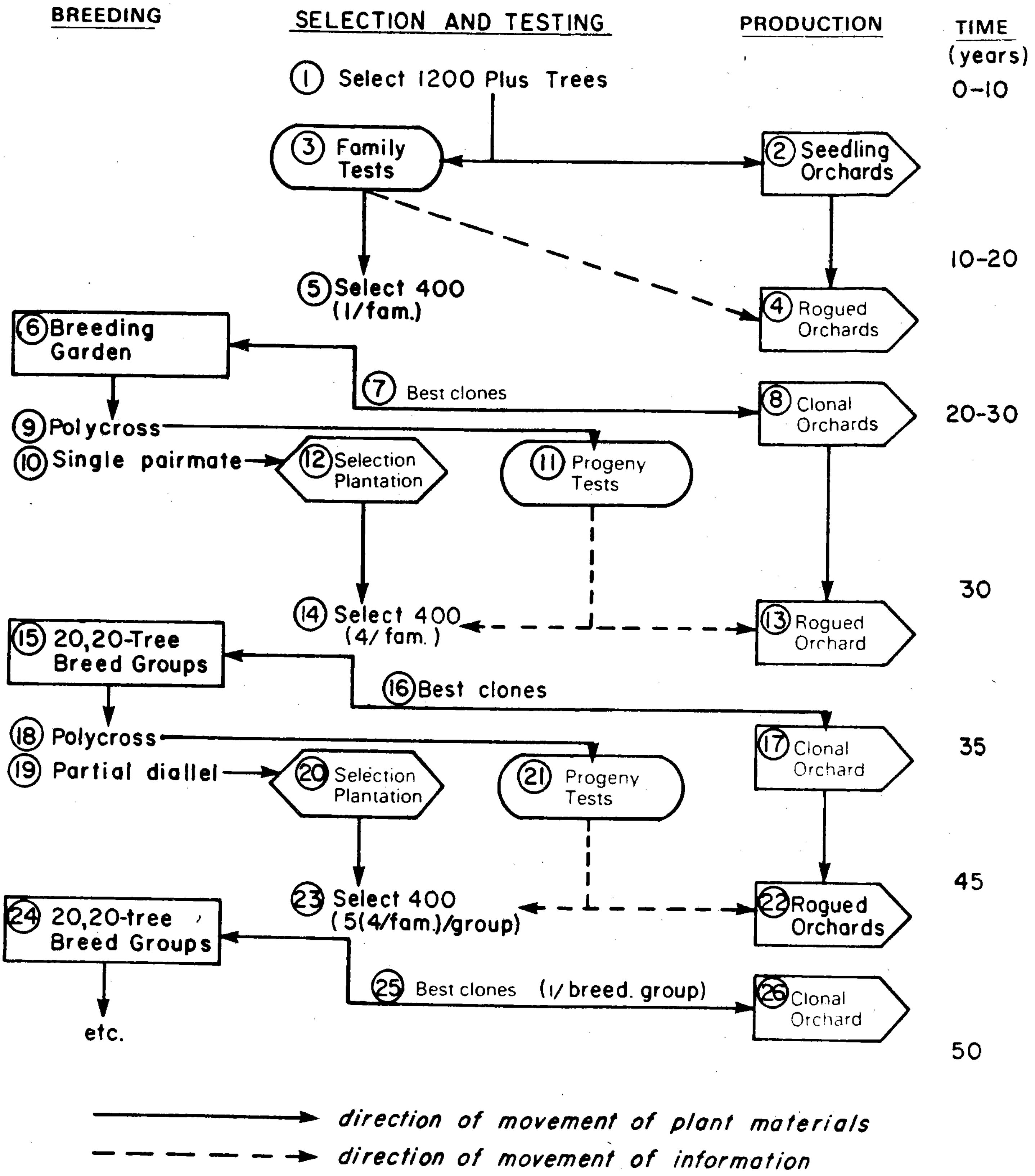

Fig. 1. Flowchart for black spruce tree improvement strategy NBTIC Program, (initiated 1976) (from Fowler 1986).

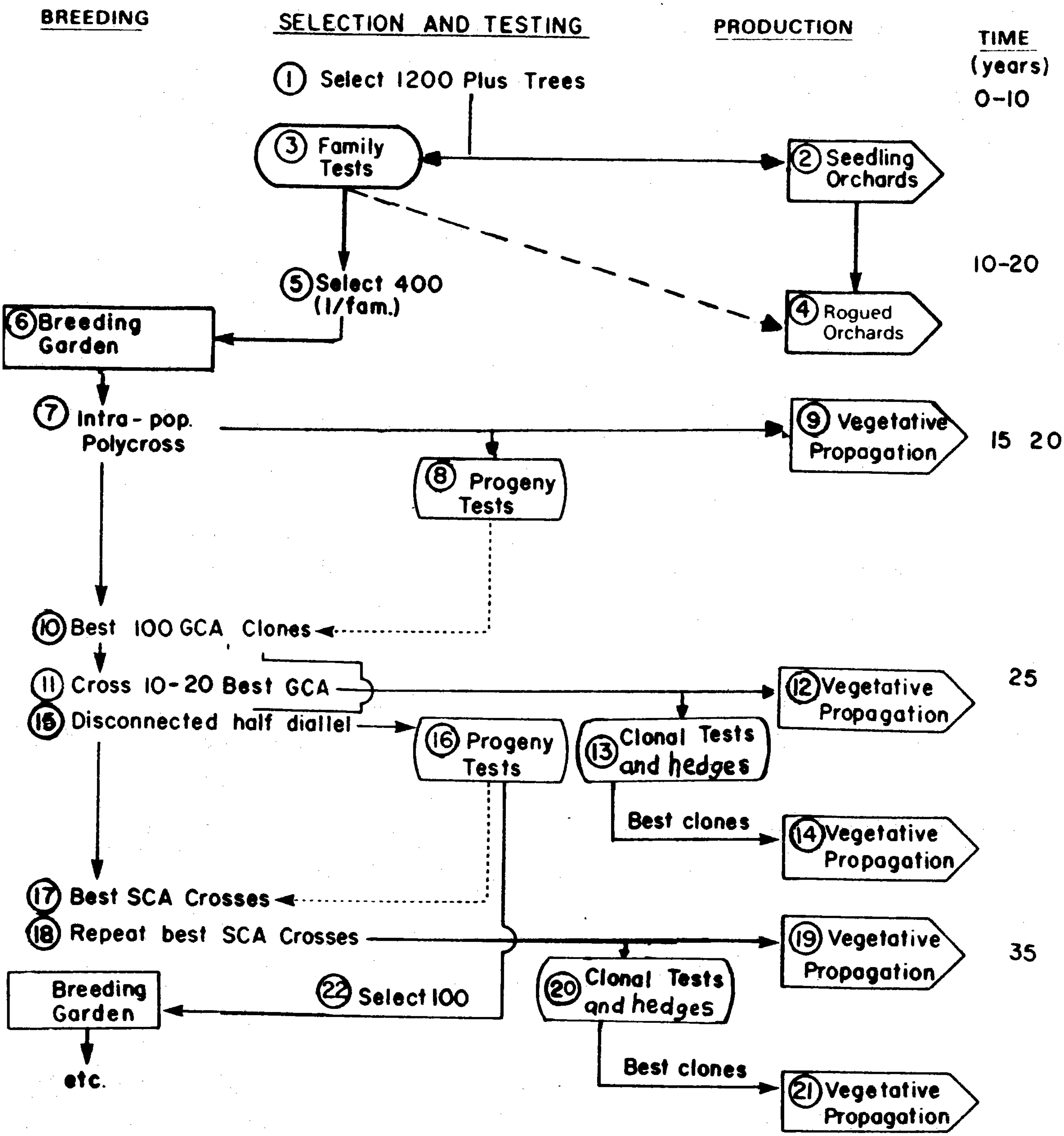

Fig. 2. Flowchart for black spruce tree improvement strategy when vegetative propagation is method of mass production.

be produced in a panmictic second generation clonal orchard free of outside pollen contamination and selfing, i.e. a perfect orchard. These crosses can be repeated as required to service the production program and as early information from the progeny tests becomes available, poor clones can be dropped from the program.

On the basis of 10-year data from the progeny test the best 100 clones can be identified (10). The 10-20 very best GCA clones will be crossed (11) again to provide improved materials for mass vegetative propagation (12). Several individuals from each of these families will be cloned, with ramets of each clone going to clonal tests and to hedges (13) where they can be maintained in a juvenile condition. The number of individuals/family that should be cloned to identify superior individuals and the number of ramets/clone required to identify best clones is the subject of current studies. Once the best clones have been identified the hedged ramets will be used as a source of materials for mass vegetative propagation (14).

The best 100 clones will be crossed in a disconnected half diallel design and progeny tested (16). Information from these tests will identify high SCA parents (17). The high SCA crosses will be repeated (18) as stock for vegetative propagation (19), and a number of individuals will be cloned, tested and hedged (20). Again based on the clonal tests the best clones will be mass produced (21). The best individuals in the progeny test will be selected (22) to provide materials for the next cycle of breeding, testing, and selection.

Tissue culture of juvenile materials

Over the past few years we have seen the development of operational tissue culture techniques for the mass propagation of numerous angiosperm species, however, conifers have proven much more recalcitrant. Culture of juvenile tissues of northern conifers, as an alternative to more conventional vegetative propagation methods, is currently possible only with very juvenile materials, i.e., embryos and very young seedling parts. Recent developments in somatic embryogenesis indicate that successful, operational techniques will be worked out over the next few years. Only when tissue culture proves to be less expensive than conventional rooting methods will it be widely used. The use of tissue culture techniques to mass produce planting materials will provide essentially the same opportunities and require the same strategy changes as other methods of vegetative propagation.

Tissue culture has advantages over conventional rooting methods. For example, it is theoretically possible to build up the number of ramets/clone much more rapidly, thus making it possible to produce the required numbers for planting, essentially "on demand". It is also possible to introduce new families or genotypes into a program more quickly. Tissue culture may also provide a means of long term storage of juvenile materials as an alternative to hedging or continuous growth.

Flower induction

The major impact of early flower induction is that it makes it possible to reduce the time between generations. However, unless early testing protocols are also developed, early flowering per se will have little direct effect on overall strategies. Techniques to induce or increase flowering in mature conifers have been developed for many species. Flower induction in juvenile materials has been less successful for most conifer species (except western hemlock, Tsuga heterophylla (Raf.) Sarg.). However, it can be assumed that within the next few years techniques will be developed to reduce substantially the age-to-flowering in most conifers.

Techniques to induce flowering in mature materials will lead to the greater use of breeding houses for controlled pollination work and, in some cases, breeding houses will replace seed orchards.

Early testing

Of the anticipated changes in technology that will occur in the near future, the development of effective early testing methods will be most important. Currently, most breeding programs, including those in the Maritimes, plan to carry-out controlled crosses to determine GCA and simultaneously to produce materials for the next generation of selection and breeding. The breeding designs being used (polycross, disconnected half diallel, or factorial) are fairly efficient for identifying high GCA parents or progenies with high GCA parents. They are not efficient for the production of materials for next generation selection and breeding. For example, starting with a 400 tree breeding population, a single pair mating design would produce 200 (0.3%) of the possible 79,800 crosses (excluding selfs and reciprocals). A 5-tree disconnected half diallel would produce 800 (1.0%), while an expensive 8-tree disconnected half diallel would produce only 1.8% of the possible crosses. Obviously, none of these designs are effective for producing a large proportion of the best crosses.

If effective early testing procedures can be worked out it should be possible to breed for next generation materials after GCA information on the parents is available, without decreasing the genetic gain per generation. In most of our northern conifers, female flowering occurs at least 3-4 years before there is a reliable supply of pollen. If these "first flowers" are used to produce polycross progenies, three-year GCA data would be available about the time that it would be possible to do the single pair matings, the generation time would not be increased at all. Low GCA parents could be eliminated from the program and the proportion of crosses between good GCA parents would increase.

Again taking the example of the NBTIC black spruce improvement strategy where 1200 plus trees have been selected and are being progeny tested. The 400 best GCA trees will be identified and the best individual in each open-pollinated family will be selected for the second generation breeding population. The GCA distribution for the best individual in each of the 1200 open-pollinated families should be about normal (Fig. 3A).

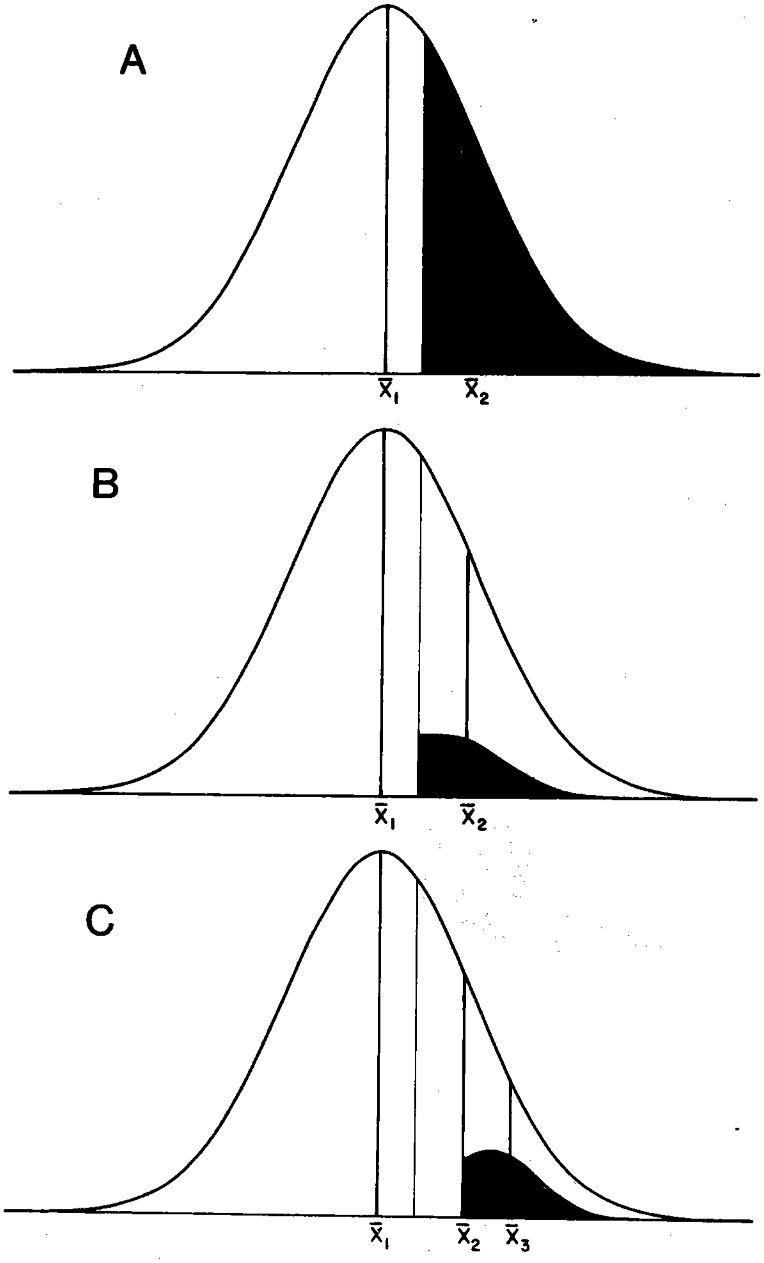

Fig. 3.
A. GCS distribution for the best individual from each of 1200 families from open pollination (mean = $\bar{X}_1$); GCA distribution for best individual in best 400 families (mean = $\bar{X}_2$).
B. Mean parental GCA of progenies derived from single pair matings made without the benefit of GCA information on parents. (mean = $\bar{X}_2$).
C. Mean parental GCA of progenies derived from single pair matings after the poorest 50% of the parents have been rogued from the breeding population (mean = $\bar{X}_3$).

The mean GCA of the 400 selections will be approximately 1 standard deviation above the mean of the base population. When we intercross these 400 selected trees in a single pair mating or disconnected half diallel design, the mean parental GCA of the progenies will not change (Fig. 3B). In the NBTIC program, where we plan to select the four best individuals in each of the 100 best families, we will in effect be selecting the best tree in every family with average or above average mean parental GCA. If on the other hand, we are successful in identifying best GCA selections on the basis of early tests, and then do our crosses for next generation selection and breeding, we can concentrate on making crosses between only high GCA parents. For example, if we could eliminate the 50% of the selections with below average GCA, all the progenies we produce would have above average GCA parents (Fig. 3C).

The efficiency of early testing is dependent on the time required for testing and the correlation of early and mature attributes. The correlation does not have to be strong to make it possible to increase the proportion of crosses between better GCA parents. Any positive correlation should make it possible to increase the proportion of crosses between above average GCA parents. Our data, based on unsophisticated field tests, suggest that the correlation between 4-year nursery performance and field performance is reasonably high. For example, in our provenance trials correlations for black spruce (ages 4 and 14) and red spruce (4 and 23) are 0.70 and 0.54, respectively.

The implementation of effective early testing methods, such as farm-field, growth chamber, biological, chemical, or more conventional field testing, will not require major changes in improvement strategies. Such tests will, however, make the existing strategies more effective.

Rejuvenation of mature conifers

Of the anticipated technological advances that will occur over the next two decades, rejuvenation of mature conifers will have the greatest impact on current breeding strategies. When we can rejuvenate mature conifers, we will be in a position to effectively utilize vegetative propagation (rooting of cuttings or tissue culture) as a mass propagation technique for superior genotypes. It will then be possible to screen large numbers of promising genotypes without the constraint of having to maintain each clone in a juvenile condition by hedging, continuous growth, or long-term cold storage of tissue. Conifer breeding will become much more like breeding for improvement in Populus and Eucalyptus.

To take advantage of this opportunity, emphasis will change from the production of uniformly good populations, to the production of large, highly variable populations or even provenance or species hybrids. The importance of expensive pedigreed breeding will diminish substantially.

Regeneration of conifers from single cells

It is now possible to regenerate some angiosperms from single cells. It is not possible to do the same thing with conifers and it may be several decades before the appropriate techniques developed. The development of this technology is essential to the utilization of other innovative technologies such as protoplast fusion and gene transfer.

It is doubtful if single cell culture will be developed as an alternative means of mass vegetative propagation because of the lack of genetic fidelity inherent to the system. Single cell culture provides a strong tool for selection of cells that are superior in respect of specific attributes such a disease resistance, chemical tolerance, etc. When it becomes possible to regenerate trees from single cells this technology will be important in resistance breeding programs. It is doubtful, however, if the ability to regenerate conifers from single cell cultures, per se, will have a serious impact on current improvement strategies as it will still be essential to develop genotypes that are capable of meeting other requirements, e.g., well adapted, superior growth, etc.

Protoplast fusion

Protoplast fusion provides an opportunity to bring together diverse genotypes that are not available in nature or that cannot be produced using species hybridization. When linked with a functional single cell culture system it offers considerable potential. The major impasse to using protoplast fusion in conifers is our inability to regenerate from single cells. It is doubtful if it actually offers as much promise as we have sometimes been led to believe. The resulting genotypes must be able to live and function, and consequently will require an extended testing period before we will be confident enough to use them. I look at protoplast fusion as a technique to extend the opportunities of species hybridization. It could be a useful as a tool for increasing the genetic variability of a species such as red pine, *Pinus resinosa* Ait., a species that is genetically uniform and does not cross with other *Pinus* species. The development of effective methods for protoplast fusion of conifers will not have a major effect on tree improvement strategies.

Gene transfer

It will eventually be technically possible to transfer specific genes or groups of genes in conifers in much the same way it is now possible in some angiosperm species. However, before this technology will be of practical importance much research will be required to identify and map genes with specific functions. I suggest, and I do not think I am pessimistic, that it will be at least 40 years before the technology for gene transfer in conifers becomes operational. When it does, it will have a substantial effect on improvement strategies. We will still require the good-growing, well adapted genotypes being developed in our existing improvement programs, but we will be in a much better position to modify those genotypes to better fulfill our needs.

In the preceding I have briefly touched on some of the technological changes that I anticipate will have an influence on our developing tree improvement strategies. These are only some of the possibilities. There are others that I have not considered but more important there will be many other advances in our ability to manipulate the genetics of trees that have not even been conceived. The "new technology" will have a profound effect on how we produce our new forests. However it is a giant step from the laboratory into the real world. We as tree breeders have an opportunity to see that the new technology is used to its full potential. We must be ready and willing to modify our strategies to accommodate and best use this technology. We must plan well ahead using the best biological and genetic information that is available to us now, but we must insure that our strategies do not lead us into a cul-de-sac where we cannot extricate ourselves. We must plan a high degree of flexibility into our strategies so that they can be changed, tomorrow, or 20, 30 or 100 years from now.

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TREE IMPROVEMENT COOPERATIVES IN CANADA: AN OVERVIEW

J.F. Coles[1] and J.D. Simpson[2]

[1] Ontario Tree Improvement Council, Johnston Hall, University of Guelph, Guelph, Ontario N1G 2W1.

[2] New Brunswick Tree Improvement Council, Canadian Forestry Service - Maritimes, P.O. Box 4000, Fredericton, N.B. E3B 5P7.

ABSTRACT

Seven cooperative tree improvement programs involving industry, governments, and universities in Nova Scotia, New Brunswick, Ontario, Alberta and British Columbia are reviewed with respect to composition, structure, objectives, role and function in relation to provincial tree improvement programs, funding, seed distribution, and research. Although each cooperative is unique in its own right, all have been successful in coordinating and conducting applied tree breeding programs. In three cooporatives, research is a vital component and in the other four ranges from no program to a developing one.

RÉSUMÉ

On passe en revue sept programmes d'amélioration des arbres réalisés conjointement par l'industrie, les gouvernements et les universités des provinces suivantes: Nouvelle-Écosse, Nouveau-Brunswick, Ontario, Alberta et Colombie-Britannique. On étudie la composition, la structure, les objectifs, le rôle et la fonction en relation avec les programmes provinciaux d'amélioration des arbres, le financement, la distribution des graines et la recherche. Même si chacune des coopératives est unique en son genre, toutes ont connu du succès dans la coordination et l'application des programmes d'amélioration des arbres. La recherche est un élément vital de trois coopératives et les quatre autres élaborent actuellement un programme.

INTRODUCTION

Cooperation between government and industry to implement tree improvement programs has proliferated in Canada in the last 10 years, which in turn has resulted in the formation of formal and informal tree improvement cooperatives. The impetus for this increased cooperation has varied but two reasons seem to be foremost:

i) industry, through the signing of Forest Management Agreements (or variations thereof), has become responsible for forest management on a large portion of Crown land in Canada

ii) a realization that a shortfall in long-term wood supply may exist unless every effort is made to improve yields and shorten rotations.

This paper provides an overview of the structure, role, funding, seed distribution, and approach to research of the various cooperative tree improvement programs in Canada.

Tree improvement programs require:

i) the availability of a large land base from which to collect genetic material and reap future benefits,

ii) the expenditure of large sums of money and the availability of trained people for on-the-ground work,

iii) the availability of suitable greenhouse, headerhouse, and laboratory facilities (more so for advanced generation breeding), and

iv) the availability of the latest genetic information to design efficient breeding strategies.

Because of these requirements, it is usually only feasible or economically viable for large agencies, such as provincial governments, to undertake tree improvement programs. However, by participating in and contributing to a cooperative tree improvement program, small organizations can share genetic material, costs and facilities, expertise, and the benefits. The resulting material will be outplanted on a large land base thus enhancing the overall payback.

TREE IMPROVEMENT COOPERATIVES IN CANADA

The existing active Canadian cooperatives from each to west (Fig. 1), their composition, structure, and objectives are summarized.

Nova Scotia

The Nova Scotia Tree Improvement Working Group (NSTIWG) was formed in early 1977 and is composed of the three major pulp and paper companies and the provincial Department of Lands and Forests. The Canadian Forestry Service - Maritimes (CFS-M) provides technical assistance. NSTIWG has no formal agreement among its members but is based on the personal commitment of senior personnel in each agency.

A Management Committee composed of senior staff from each agency directs the program, reviews progress, assigns targets, and discusses problems. A Program Coordinator supported by the N.S. Department of Lands and Forests coordinates the planning and execution of the day-to-day operations. In addition, a Technical Advisory Committee provides technical guidance, monitors effectiveness, and suggests improvements. Corresponding members are kept informed of NSTIWG activities. The Program Coordinator reports progress to the Nova Scotia Forest Research Working Committee.

The objectives of NSTIWG are:

i) To supply sufficient genetically improved seed for improvable softwood and hardwood species to meet nursery requirements.

ii) To supply a sufficient quantity of good quality local seed for species with low improvement potential or planted on a smaller scale.

New Brunswick

The New Brunswick Tree Improvement Council (NBTIC) was formed in early 1977 and is composed of all the major forest industries in the Province as well as the N.B. Department of Natural Resources and Energy, the CFS-M, and the Faculty of Forestry, University of New Brunswick (UNB). NBTIC is not incorporated nor does it have formal agreements with members. The Council is organized and coordinated by a Management Committee chaired by a representative from the N.B. Department of Natural Resources and Energy. Each participating member has one voting representative on this committee usually at the silviculture or tree improvement forester level. In addition, non-voting members from the other Maritime provinces and State of Maine participate. The CFS-M provides the day-to-day technical and planning assistance through the services of a Technical Coordinator. The Chairman of NBTIC reports progress to the N.B. Forest Research Advisory Committee.

The NBTIC was formed to foster and undertake the genetic improvement of planting stock. The objectives are:

i) To provide adequate quantities of well adapted, good quality seed, until improved seed becomes available.

ii) To supply sufficient quantities of genetically improved seed.

Quebec

The Province of Quebec has announced the formation and tentative structure of a tree improvement cooperative involving industry and the provincial government but is awaiting the outcome of new legislation before proceeding.

Ontario

The Ontario Tree Improvement Council (OTIC) was formed in early 1985 and is composed of five major forest industries in the Province and five Regions of the Ontario Ministry of Natural Resources (OMNR). OTIC is incorporated with a full-time independent Director headquartered at the University of Guelph.

The Council has three levels of committees. The Parent Committee is composed of a senior management representative from each of the 10 agencies as voting members and three non-voting members - the Provincial

Forester, the Provincial Tree Improvement Specialist, and the Director. The Parent Committee reviews progress, sets policy, provides guidance to the Director, and establishes a yearly budget.

The Technical Committee is composed of a voting tree improvement representative from each of the 10 agencies along with non-voting scientific advisors from the universities and federal and provincial governments. The Technical Committee's function is to review progress, recommend improvements, solve short-term problems, and recommend approaches to solve long-term problems.

Regional Working Committees are in place for each project and are composed of tree improvement specialists from industry and district and regional OMNR offices. The Working Committees plan, budget, coordinate, and distribute the work load for each species project. The committee and its members are responsible for the day-to-day operation and implementation of the project.

The objective of OTIC is to increase the supply of industrial roundwood for the forest industries of Ontario by fostering the cooperative approach to an accelerated tree improvement program that will shorten rotations, increase yields, and improve wood quality. The objective will be met by a) ensuring that all seed used in reforestation is well adapted and the source identified, and b) providing genetically improved seed.

The North Shore Tree Improvement Cooperative (NSTIC) (north shore of Lake Superior) was formed in 1986 by the Northeastern region of OMNR, a pulp and paper company and three sawmills. This cooperative is not incorporated. A Steering Committee composed of senior management from the participating agencies sets policy, approves work plans, and reviews progress. A Technical Committee of tree improvement foresters from the member organizations devises projects, distributes work loads, and plans budgets. Ontario Ministry of Natural Resources supports the Technical Coordinator who oversees the day-to-day operation.

Alberta

The Alberta Cooperative Tree Improvement Program (ACTIP) was initiated in 1979 and is presently composed of five major forest industries and the Alberta Forest Service (AFS). Although not an incorporated organization, there are formal agreements between some individual companies and the AFS to share tree improvement work on Crown land. The ACTIP committee meets to establish projects, develop general work loads and budgets, and monitor progress. Work load details for specific breeding region projects are resolved directly between the companies involved and AFS. The AFS provides the technical direction and the services of a Coordinator for day-to-day operation.

The objectives of the ACTIP are:

i) To achieve genetic improvement of white spruce and lodgepole pine.

ii) To conduct genetics and tree improvement research directly applicable to applied breeding and mass propagation of improved genotypes.

British Columbia

The Coastal Tree Improvement Council (CTIC) held its inaugural meeting in January 1979. Prior to this time, industry and the B.C. Ministry of Forests had established 25 seed orchards and there was a need to coordinate the entire coastal program. The CTIC is composed of Chief Foresters and their designates from member companies, branch and regional representatives from the B.C. Ministry of Forests, and the Canadian Forestry Service - Pacific Forestry Centre (CFS). The CTIC objectives are:

i) To provide enough seed, incorporating the first level of genetic improvement, for all reforestation projects where site quality and accessibility will allow intensive forest management to be practiced.

ii) To establish programs to increase these levels of gain through testing and breeding.

The Council acts in an advisory capacity, with responsibility for making recommendations to the Provincial Chief Forester on the following:

a) program objectives, strategies and priorities,

b) plans, programs, and budgets for individual orchards along with standardizing plans and reporting,

c) identification of an agency responsible for establishment and operation of an orchard, and

d) identification of research needs.

A Technical Planning Committee with representation from government, industry, universities, and research agencies is responsible for the technical aspects of program planning. The program coordinator at the technical level is also secretary of the Council and is supported by the B.C. Ministry of Forests. The CTIC has seed production officers, supported by B.C. Ministry of Forests, to provide extension services to council members and to audit progress.

The Interior Tree Improvement Council (ITIC) was formed in 1981 and has limited its membership to senior management representatives from the B.C. Ministry of Forests, industrial cooperators, and the CFS. The Council is coordinated by the Ministry of Forests and acts in an advisory capacity to the Provincial Chief Forester. It has two objectives:

i) To produce a sufficient amount of seed, incorporating the first level of genetic improvement, for the major commercial species in the interior of British Columbia.

ii) To establish programs to increase the levels of genetic gain through provenance testing and tree breeding.

The terms of reference of the Council are to:

a) identify program objectives, strategies and priorities,

b) identify agencies responsible to establish and operate orchards,

c) plan programs and budgets for orchards,

d) standardize orchard working plan format, progress reports and costing, and

e) identify research needs.

Two Technical Committees (North-Central Interior and Southern Interior) composed of tree improvement specialists from the participating organizations were established to make recommendations to the ITIC and to do the detailed planning.

Cooperatives in Canada vary tremendously in their structural complexities. The most informal with virtually no senior management input or any supporting committees is the NBTIC while the OTIC, at the other end of the spectrum, is an incorporated body of senior managers with two additional levels of supporting committees. The rest of the cooperatives fall in between. Only the OTIC has a permanent independent Director while the other cooperatives have a provincial government supported Technical Coordinator (Federal government in the case of NBTIC) to run the day-to-day operations. There is no one structural formula that guarantees success - each province has decided on a structure which best fits the requirements and the available resources at the time and all appear to be getting the job done.

ROLE AND FUNCTION OF THE COOPERATIVES IN THE PROVINCIAL TREE IMPROVEMENT PROGRAMS

The Nova Scotia Tree Improvement Working Group was formed during the early stages of tree improvement development in the Province and as such has instigated virtually the entire provincial program. The one small clonal orchard established by N.S. Department of Lands and Forests prior to 1977 has been incorporated into the NSTIWG program.

In Nova Scotia, the final responsibility to determine strategies for improvement, selection criteria, test and orchard design rests with the provincially supported Program Coordinator, however, the Technical Advisory Committee, composed of forest geneticists from CFS-M and UNB, provides advice on efficient designs and strategies. Implementation of these strategies rest with the NSTIWG members.

The New Brunswick Tree Improvement Council was also formed during the early stages of applied tree improvement in the Province. A few plus trees had been selected and a series of stand tests had been established by the N.B. Department of Natural Resources and Energy. The entire provincial program, both industry and government, is now under the direction of NBTIC. However, one company with a large amount of freehold land and a large planting program has developed its own tree improvement program in addition to cooperating in the NBTIC program. The responsibility of determining strategies, designs, and selection criteria for the provincial program and implementation rests with NBTIC. Recommended strategies have been published by Fowler (1986) which NBTIC will follow. Any company with programs over and above NBTIC's, determines its own strategies and criteria but relies on NBTIC breeding materials to supplement their programs.

The Ontario Ministry of Natural Resources has been pursuing a very active, but at the same time, very patchy tree improvement program across northern Ontario, for several years. The Ontario Tree Improvement Council, arriving late on the scene, is able to complement the ongoing OMNR program by concentrating efforts on those species or those breeding zones not covered by the OMNR. The OTIC and OMNR programs are carried on separately with no confluence. The responsibility to determine strategies and designs for the OTIC programs rests with the OMNR. Strategies and designs have been published by OMNR (1987). Some degree of flexibility in selection criteria and orchard design is permitted by OTIC members.

The North Shore Tree Improvement Cooperative, being in its formative months, has yet to clearly define its role with respect to the ongoing OMNR programs. It is envisioned, however, that existing OMNR programs will be amalgamated with new cooperative programs so that NSTIC will direct the total improvement effort for the Northeastern Region of Ontario. Strategies and designs will follow OMNR (1987).

In Alberta, tree improvement work had only just begun prior to the formation of the Alberta Cooperative Tree Improvement Program which is now responsible for the entire Province. Of the nine breeding zones delineated, five have AFS/industry cooperative programs while four, because of the absence of industrial Forest Management Agreements, have AFS programs only. The responsibility to determine strategies for improvement, test and orchard design rests with the AFS. Implementation is a joint AFS - industry decision where cooperative programs exist.

For several years prior to 1979, the British Columbia Ministry of Forests and industry had been actively involved in tree improvement, particularly in the coastal region where 25 Douglas-fir seed orchards (total area 65 ha) had been established. With the formation of the ITIC and the CTIC, all past installations and future activities came under the guidance of the cooperatives - except those established by industry in the coastal region to service freehold land. The responsibility to determine strategies for improvement and design rests with the British Columbia Ministry of Forests Research Branch. However, a Breeding Strategies Subcommittee of the Technical Planning Committee composed of geneticists and scientists from government, industry and university was formed to advise on long-term breeding options for all species in the program. The

TIC's in British Columbia make recommendations only to the Chief Forester of the Province as to which projects should be implemented. For financial reasons, it is the Chief Forester - not the TIC or its members - who decides if these projects can proceed.

In summary, there are substantial differences in the scope and role that these cooperatives play in the overall provincial programs. In Nova Scotia and New Brunswick, the co-ops and their members direct and implement the entire provincial programs, except that of industry on freehold. In British Columbia, the opposite appears to be true; the Ministry of Forests has the final say on strategy and implementation, with council members acting only as advisors and contractors. In Ontario, because current member companies operate on a limited land base, the OTIC programs form only a part of the provincial program.

FUNDING

Tree improvement programs are expensive. Cooperative structures, strategies, designs, and program plans are necessary but unless government and industry members are firmly committed to tree improvement or industry members are reimbursed by government in one form or another, little will be accomplished on the ground.

Across the country, there are differences in the approach to funding programs. In Nova Scotia and New Brunswick, industry and government members are firmly committed to tree improvement and industry shows this by implementing cooperative program plans on both Crown and freehold land without direct government funding or reimbursement. Both industry and government select trees and establish and maintain tests and orchards. During the years of restraint (1981 to 1983), programs did proceed despite the prevailing economic recession. The New Brunswick provincial government's program has been funded, to a large extent, by Federal/Provincial Development Agreements while Nova Scotia's provincial government has funded its program.

In Ontario, industry and government members, working almost totally on Crown land, committed themselves to tree improvement without the prospect of external funding. Shortly after the establishment of OTIC, however, funds became available through the Canada-Ontario Forest Resources Development Agreement. Proposals were submitted and now approximately one-half of the cooperative program is funded through the Agreement. Industry and the provincial government are directly responsible for the remainder. Each of the 10 agencies is assessed a standard membership fee to cover office, travel, and expenses of the Director.

In Alberta, where tree improvement is conducted almost solely on Crown land, industry members of joint projects fund their activities (selection, site preparation, orchard establishment, and operation) directly, while the AFS funds activities such as seed extraction, stock production, and data analysis.

In British Columbia, which is 96% Crown land, industry participation in the cooperative programs is funded indirectly by the Ministry of Forests through credit to stumpage under section 88 (1) of the Forest Act. Under the act, the work and expense must be approved in advance by the Chief Forester of B.C. Ministry of Forests and be performed to the satisfaction of the Chief Forester before the expense can be applied as a credit against stumpage payable in respect to timber harvested in a prescribed area.

DISTRIBUTION OF SEED

The Nova Scotia Tree Improvement Working Group has a unique seed distribution policy agreed to by all members. Although orchards are established primarily to meet the requirements of individual agencies, because of the vagary of nature and through no fault of the managing agency, individual orchards could be nonproductive or destroyed. Any member affected in this way will be assured of a continuing supply of seed from other members' orchards.

In New Brunswick, the provincial government establishes and manages seed orchards to produce seed for crown land reforestation. Companies with large freehold areas have established their own orchards to service their reforestation requirements. Companies with insufficient areas of freehold to justify establishment of seed orchards can obtain seed from the provincial government in proportion to their financial contribution to the program.

In Ontario, the work plans of the Regional Working Committees of OTIC clearly state the obligation of each member to the projects. The guiding principle is that distribution of improved material will reflect the contribution of OTIC members to any of the selection, testing, or orchard development phases of the programs.

In Alberta, industry is responsible for orchard establishment and operation in those breeding zones with joint industry/AFS programs and AFS has access to some of the seed. The AFS also manages orchards to produce seed for non-FMA Crown land.

In British Columbia, seed from orchards owned and managed by the B.C. Ministry of Forests is allocated to companies and the Ministry. Older orchards, established by companies prior to the formation of the Councils, which produce more than a 3-year seed supply for a company will have excess production distributed to other companies by the Regional Manager of the Ministry. Early production from new company orchards will be strictly for company use with the Chief Forester of the Ministry determining when there is an excess and allocating this to other companies.

APPROACH TO RESEARCH

The term "supportive research" may best describe this component necessary for continuation, justification, and development of tree breeding programs. Research is a major part of three cooperative programs while for others, which consider it important, it has yet to be initiated as a formal program. Data from progeny and family tests alone provide important information useful for guiding program development.

The Nova Scotia Tree Improvement Working Group has no formal research program but relies on CFS-M and UNB to provide basic background research. In the past, NSTIWG and NBTIC have cooperated in funding several projects.

All research support for the New Brunswick Tree Improvement Council is provided by CFS-M and UNB. The CFS-M has provided background research and breeding strategies. A Tree Improvement Chair was established at UNB in 1981 to advance teaching and research in tree genetics and to support the operations of NBTIC. Close collaboration exists between the Chair, CFS-M, and NBTIC. NBTIC is developing a more formalized approach to research. The one company conducting a separate tree improvement program is also conducting applied research.

Research is addressed in OTIC's terms of reference but a program has not been initiated to date.

A large portion of Alberta Forest Service's input into ACTIP is through their genetics and tree improvement research program. This was further strengthened in 1986 with the establishment of a Natural Sciences and Engineering Research Council/University of Alberta/Industrial Support program funded by AFS, Alberta Forest Products Association, and NSERC. The objective is to develop a scientific and applied research program to aid and complement ACTIP.

The Research Branch of B.C. Ministry of Forests provides most of the support to both CTIC and ITIC with the Silviculture Branch and CFS also contributing. A Tree Improvement Chair was established at the University of British Columbia in 1986 financed by grants from industry and the Ministry of Forests. The Technical Planning Committee of each Council identifies and recommends specific research projects to the various agencies including the Chair. The ITIC best exemplifies how research benefited a cooperative, particularly at the time of formation. Results from 10-year-old white spruce progeny and provenance test data allowed ITIC to proceed directly to 1.5 generation clonal seed orchards using genetically proven clones.

THE FUTURE

The seven cooperative tree improvement programs in Canada have demonstrated that government/industry cooperation is the most cost-efficient and effective means of justifying and operating large tree breeding programs. As these cooperatives continue to develop and mature, members will acquire more knowledge, competence, and awareness of needs. This will allow members to play an increasingly larger and important role in policy and strategy development and permit them to develop more specific tree improvement programs. Test measurement data generated from these programs contain a wealth of information. There is a need for additional personnel (quantitative geneticists) to evaluate these data to provide information for breeding strategy development and modification as rapidly as possible. Research will become an increasingly important and necessary part of all programs.

SUMMARY

The seven cooperative tree improvement programs in Canada vary in their structure, the role played in their respective province, funding, distribution of seed and approach to research. Despite these differences, each cooperative appears to function and operate effectively. These cooperatives should continue to develop and grow as experience, information, and results are acquired.

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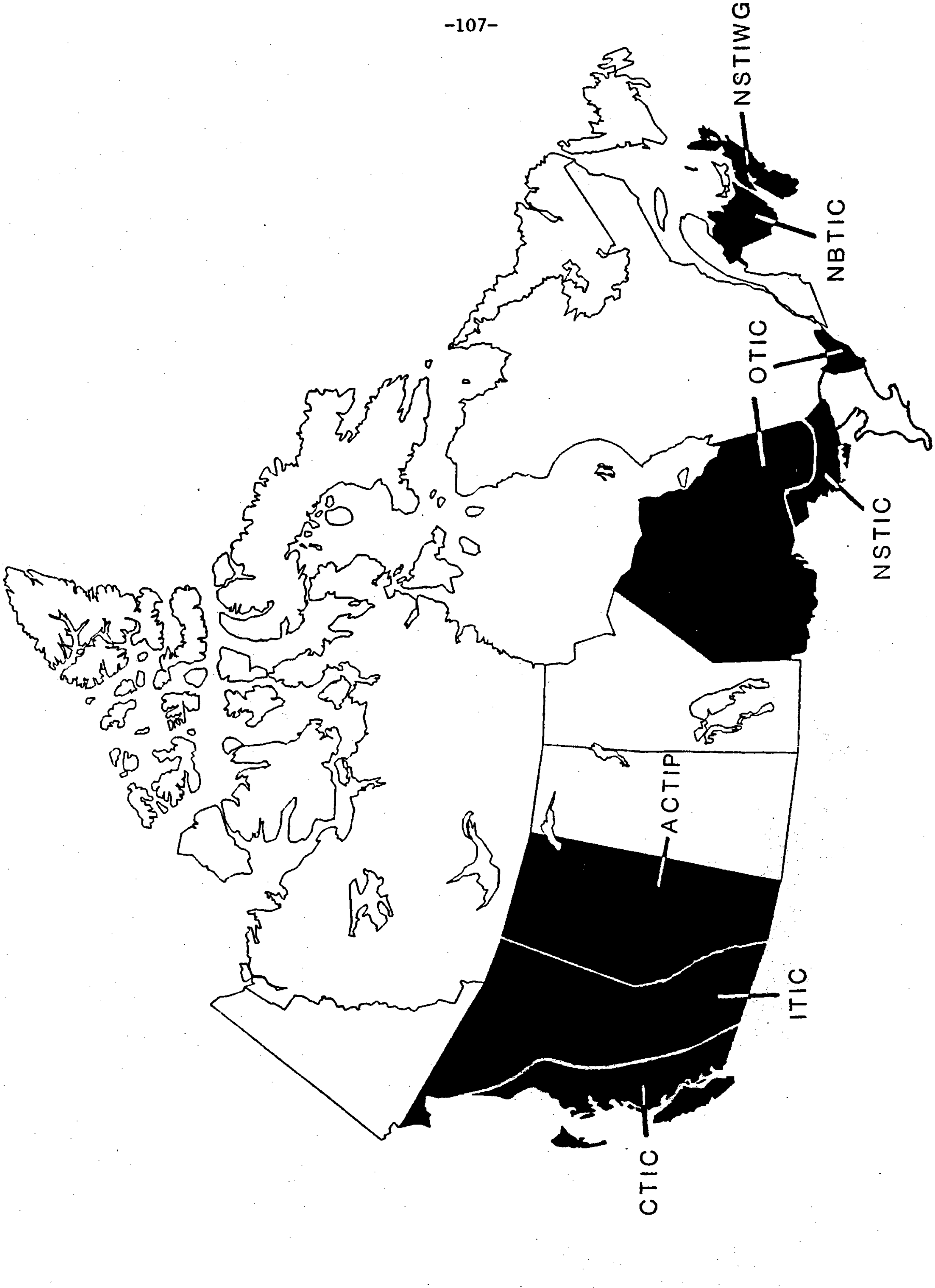

Fig. 1. Cooperative tree improvement programs in Canada.

PLUS-TREE SELECTION: CONTROLLING ITS COST

E.K. Morgenstern[1] and T.J. Mullin[2]

[1] Department of Forest Resources, University of New Brunswick, Fredericton, N.B., Canada E3B 6C2.

[2] Nova Scotia Department of Lands and Forests, Debert, N.S. BOM 1GO.

ABSTRACT

Research results and concepts are discussed that could make plus-tree selection more efficient, particularly in natural stands. In species that tend to develop a family structure with some inbreeding (e.g. white spruce), the comparison-tree technique should not be used. Selection based on growth efficiency (crown measurements) has not been substantiated so far and may not be promising. The ideotype concept requires further study before it can be applied.

Results of the New Brunswick tree improvement program indicate only limited genetic gains in tree height from plus-tree selection so far. For black spruce, it is expected that in the first generation, plus-tree selection will require 15% of total investment but will contribute only 9% of the genetic gain, the remainder coming from family selection. This justifies the current emphasis on family selection.

RÉSUMÉ

On fait état des résultats des recherches et des principes qui pourraient rendre plus efficace la sélection des arbres plus, particulièrement dans les peuplements naturels. On ne doit pas utiliser les techniques de comparaison des arbres pour les essences qui ont tendance à développer une structure familiale caractérisée par une certaine consanguinité (par ex. épinette blanche). La sélection fondée sur l'efficacité de la croissance (mesures de la cime) n'a pas été étayée jusqu'à présent et peut ne pas être prometteuse. Avant de mettre en application la notion d'idéotype, il faut effectuer d'autres études.

Les résultats du programme d'amélioration des arbres du Nouveau-Brunswick indiquent que les gains génétiques réalisés à l'aide de la sélection des arbres plus pour la hauteur des arbres sont limités jusqu'à présent. Dans le cas de l'épinette noire, on prévoit qu'au cours de la première génération, la sélection des arbres plus nécessitera 15 % de l'investissement total, mais ne produira que 9 % environ des gains génétiques, le reste provenant de la sélection de familles. Cela justifie l'accent mis actuellement sur la sélection de familles.

INTRODUCTION

Every tree improvement program with native species begins with the identification of individual trees which are above average in quality for some particular traits, and are used to establish a base population for future breeding. This initial step is often difficult: experience is limited, genetic theory may not be well understood, and guidelines from other species and regions may be of little value. In contrast to other stages of plant breeding, agricultural breeders do not have much to offer and the tree breeder stands alone.

In Canada, selection programs began first in Ontario and British Columbia (Carmichael 1960, Heaman 1967) and are now in progress in all provinces. The plus-tree selection phase has even been completed for some species, for example, jack pine (Pinus banksiana Lamb.) and black spruce (Picea mariana (Mill.) B.S.P.) in New Brunswick, and red spruce (Picea rubens Sarg.) and white spruce (Picea glauca (Moench) Voss) in Nova Scotia.

This paper will look at some of the problems associated with this activity particularly from the point of view of economic efficiency.

IMPROVING THE SELECTION PROCESS

Plus-tree selection is the very first stage of a breeding program with native species. In cooperative programs, training of field crews and careful organization of activities is necessary for efficiency and to impose some degree of standardization upon crews with varying backgrounds and experience. In both New Brunswick and Nova Scotia experienced technicians make the final choice.

A major problem is that selection must be based on the phenotype since no other information is available. Although the correlation between phenotype and genotype is stronger for morphological traits of stem and crown than for physiological traits related to growth, difficulties to overcome are still substantial, particularly in natural stands. There may be complications resulting from genetic population structure, uneven-aged stands and competition, and unequal growth due to microsite (Zobel and Talbert 1984). All these problems make objective comparisons almost impossible. Research is underway to gain a better understanding of natural forests or to use new selection concepts.

Population Structure

The most commonly used selection technique is that based on comparison or check trees (Morgenstern et al. 1975), which was devised in Sweden 45 years ago (Lindquist 1951). It has a weakness, as Ledig (1974) pointed out, in that comparisons among neighboring trees will be very ineffective if these trees are genetically related. In some species, groups of related trees may be easy to recognize - clones of aspen, for example, which may be very uniform in phenology and stem form. In other species studies of the population structure based on isoenzymes or controlled crosses will make it possible to identify species for which the technique cannot be used. For example, for white spruce, Park et al. (1984) have shown that the average relationship in natural stands in central New Brunswick approximates that of half-sibs. Cheliak et al. (1985) in Ontario confirmed that white spruce trees about 30 m from a central point were related and they recommended strict avoidance of the comparison-tree technique for this species.

In contrast, in black spruce, Boyle and Morgenstern (1987) found very little evidence of inbreeding and family structure in upland stands in central New Brunswick.

Instructions for plus-tree selection often give a minimum distance between selected trees to avoid the inclusion of relatives in the same seed orchard. Based on their findings, Cheliak et al. (1985) suggested a minimum distance of 63 m between selected trees while Boyle and Morgenstern (1987) indicated the possibility of taking trees that are growing more closely together.

Growth efficiency

Brown and Goddard (1961) expressed the idea that plus-tree selection should not identify the largest tree, but rather the tree that has utilized growing space most efficiently. Crown measurements are made and related to basal area and volume to identify plus trees.

Stanton and Canavera (1983) selected white birch (*Betula papyrifera* Marsh.) phenotypes on the basis of three techniques: (1) mean annual volume increment after adjustment by comparison trees, (2) without this adjustment, and (3) on the basis of differences between actual and predicted basal area increments regressed on crown surface area. Open-pollinated progeny tests from these trees were evaluated at age 17 weeks from seed. Results indicated significant differences between techniques but none of them produced progenies significantly taller than the controls. Another study by Van Damme and Parker (1987) applied to black spruce used (1) regressions of basal-area increment on crown-surface area, (2) stem volume on crown radius, and (3) height on age. Open-pollinated progenies from the selected trees at 2 years of age were significantly different but there were no differences among selection methods.

In both cases, it seems too optimistic to expect conclusive results at such young ages.

In a study of various competition indices applied to improve plus-tree selection, Thomas (1980) also found that this approach is not helpful.

There are other reasons why selection based on crown characteristics may not be effective. Although correlations of growth and leaf characters in young trees and in experiments are often strong, there are reasons why these relationships may not exist in mature stands or may not be easy to assess (Assmann 1970). Crown measurement may not accurately reflect leaf surface area; there may be differences in leaf arrangement and proportion of sun and shade leaves, and differences in phenology. Also, selection based on crown measurements cannot eliminate or account for differences in microsite. These reasons indicate that further work is this area may not be promising.

Crop ideotypes

The concept of the crop ideotypes was introduced by Donald (1968). Prior to this, plant breeding was based primarily on defect elimination or selection for yield. In contrast, Donald expected that a crop ideotype will be a weak competitor and will make minimum demand per unit of dry matter produced.

In forestry this concept has been given attention primarily in Estonia and Finland. Examples of individual Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst) have been given with narrow crowns, good stem form and small taper, which, when grown together in a stand, produce high volumes per unit area (Etverk 1972, Kärki 1983). In an experiment with Sitka spruce (*Picea sitchensis* (Bong) Carr.) in Scotland, Cannell (1982) demonstrated that progenies growing at a superior rate when widely spaced, failed to maintain this position when grown in closed stands. He concluded that there may be isolation ideotypes and crop ideotypes which perform well in competition, and that these types need to be distinguished.

A related idea is to measure productivity not simply by height or volume but by harvest index, particularly when total biomass is measured. This trait showed high heritability in experiments in Finland. As with the ideotype concept, the problem is that trees modify their crown structure when subjected to competition. More research is needed to see how the ideotype concept can best be applied (Prescher 1984).

COSTS AND GAINS

The difficulties with selecting plus trees, which are so evident particularly in natural stands (where nearly all our selections are made), should not lead to excessive costs. Plus-tree selection is of course a necessary first step in a selection program but the time and effort spent on it must be controlled. Cost must somehow be related to the genetic gain achieved - the cost-benefit ratio must be positive.

In the more mature selection programs of the southern United States, costs and gains are well documented, particularly for loblolly pine (*Pinus taeda* L.) (Talbert et al. 1985). For this species, Porterfield et al. (1975) have indicated that field selection costs of $200 per tree could be doubled and still be economically justified. It is significant that in this species economic improvement results from many traits including stem straightness, crown quality, wood density, fusiform rust resistance, and volume production. Several of these traits are fairly strongly inherited and can be reasonably well recognized in the field. In some of our species, e.g. black spruce, where growth is by far the most important selection criterion, the situation is entirely different.

It is interesting to look at some of the figures on phenotypic selection released by the New Brunswick Tree Improvement Council (NBTIC). Table 1 shows percent height superiority of plus trees of black spruce, white spruce, jack pine and tamarack (Larix laricinia (Du Roi) K. Koch). There are no factual estimates of heritability of height growth in natural stands, but Namkoong (1970) states that they might reasonably be expected to lie at about 0.05 in the highly variable environments of natural forests. It is easy to see that with such low heritability the genetic gain will be low, since realistic selection differentials will not exceed 2 to 3 standard deviations (Wright 1976).

Costs in the NBTIC program have had a considerable range (Table 2). Selection of white spruce, red spruce, and tamarack is more intensive since clonal orchards are established, and costs are higher than for selection of black spruce and jack pine, which will lead to seedling orchards. This is justifiable since the former group of species are improved not only for growth but also for crown and stem form while growth is the primary trait to be considered in the latter group.

In a more detailed examination of the economics of black spruce breeding, Cornelius and Morgenstern (1986) found that total investment in a typical seedling orchard of 221 families and 5.2 ha with associated family tests ranged from $97,909 to $112,361 (in 1983 dollars) depending upon interest rate. Here the plus-tree selection cost ranged from $75 to $84 per tree, i.e. was much lower than indicated in Table 2 because open-pollinated families from each plus tree were established in more than one orchard. In spite of this advantage, plus-tree selection cost amounted to 14.8% of total investment (Table 3). The conclusion was that more intensive plus-tree selection and higher costs were not justifiable. It was better to keep the number of selected trees high since the benefits of family selection were much greater. Table 3 indicates that 91% of the genetic gain in volume came from family selection. The predicted total genetic gain of 20.4 m^3/ha constitutes a gain of 6.9% over an unimproved plantation (Cornelius and Morgenstern 1986).

To sum up, then, it is clear that the techniques for plus-tree selection and the degree of effort expended can vary with species and the breeding method applied. There are no general rules, but it is essential that supervisors know what can be achieved and realize that the point of diminishing return is very quickly reached in some species.

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Table 1. Results of phenotypic selection for height in New Brunswick, based on difference between selected trees and comparison trees[1].

Category calculated	Black spruce	White spruce	Jack pine	Tamarack
Phenotypic superiority (%)	10.3	9.9	8.1	5.9
Numerical basis (no. of trees)	707	251	758	159

[1] From a Table released by the New Brunswick Tree Improvement Council in 1985.

Table 2. Time and cost figures per tree from plus-tree selection in New Brunswick and Quebec (Morgenstern 1983)[1].

Species	Range		Mean	
	Time (Days)	Cost ($)	Time (Days)	Cost ($)
White and red spruce, tamarack	2.0-9.6	160-1118	5.3	558
Black spruce and jack pine	1.3-4.1	118-409	2.7	255

[1] Figures include scion or cone collection in most cases.

Table 3. Costs and genetic gains of a black spruce selection program (Cornelius and Morgenstern 1986).

Stage	Percent of total cost[1]	Genetic Gain	
		Volume (m^3/ha)	Percent of total (%)
Tree selection	14.8	1.8	8.8
First family selection (age 7)	-	1.2	5.8
Second family selection (age 16)	-	17.4	85.4
Total		20.4[2]	100.0

[1] Cost of family selection not calculated because of difficulties of separation.

[2] 20.4 m^3/ha is genetic gain at age of maximum mean annual increment (46 years).

SOME FACTORS INFLUENCING CHOICE OF MATING DESIGN FOR PRACTICAL TREE IMPROVEMENT

J.C. Heaman

B.C. Ministry of Forests and Lands
Research Branch
Victoria, B.C.
V8W 3E7

ABSTRACT

The role of mating designs is discussed as a means of manipulating relationships for study and application in tree improvement. A selective review of literature shows options available. From these options, objectives and common designs are reviewed and some critical factors listed. Applications to a practical, developing program are illustrated using the coastal Douglas-fir project in British Columbia. Historical decisions are followed to the present disconnected diallel design and some strengths and weaknesses noted. Simple alternatives are presented and advantages discussed. The need to review options and tie these to specific objectives, priorities and resources for individual programs is stressed.

RÉSUMÉ

On traite du rôle des dispositifs de croisement comme moyen de manipuler les relations pour l'étude et l'application de l'amélioration des arbres. Une étude sélective de la documentation montre les options disponibles, à partir desquelles on étudie les objectifs et les projets courants et énumère certains facteurs cruciaux. On illustre les applications d'un programme pratique en voie d'élaboration à l'aide du projet sur le sapin taxifolié de la Colombie-Britannique. On examine les décisions prises dans le passé jusqu'au croisement diallèle discontinu, et on relève certains points forts et faibles. On présente des options simples ainsi que leurs avantages. On souligne la nécessité d'étudier les options et de les relier à des priorités, des ressources et des objectifs précis pour chacun des programmes.

INTRODUCTION

The science of genetics is based on relationships and the manner in which traits are inherited. The fact that related individuals share some of the same genetic material which controls their form and performance and makes them more alike than those that are not related, provides the basis for all tree improvement work. These relationships must be manipulated for study and use and it is through a variety of mating designs that manipulation must be achieved.

The essential steps of a tree improvement program include study of the variation available and the isolation and evaluation of the genetic component of that variation. Through a variety of techniques, the variation is then channelled within a restricted population towards an accumulation of favourable traits which have economic or other advantages to the forest industry. It sounds simple but to requote Bob Campbell, it only becomes difficult when we try to make it efficient (Anon. 1977). Unfortunately, making it efficient has to be a primary goal in such long-term and expensive programs.

In the simplest procedure, cycles of selection to reduce the available population in a paritcular direction, followed by breeding amongst those selections to expand the population once more, thus enabling the selection process to be effective, represents the simplest procedure. In addition, tree improvement workers have to consider such questions as how to maintain adequate genetic variability as the program advances and how to avoid increasing levels of inbreeding which could in most species undo the gains the whole process has been set up to obtain. Ensuring adaptability represents another problem.

There are many modifications and variations of approach but this cycle of large and small populations will form the basis of most tree improvement efforts. These approaches represent different breeding strategies which must be tailored to each species program with its own conditions, priorities and environments. Information and new material must be generated and mating designs provide the mechanisms by which the strategies are carried out.

Mating designs must therefore serve three main functions:

1. Provide material for selection.

2. Provide material to test and evaluate genetic worth of the individual selections.

3. Provide material that can be used to shed light on how to choose an efficient breeding strategy through estimation of genetic parameters.

While there are many options for these designs, each meeting a particular need, practical breeding programs in forest trees are dependent upon combinations of full-sib and half-sib progenies. Some animal and crop breeding programs may draw more heavily on back-crosses, and progeny/-parent and inbreeding relationships but while these have a place in research, these are not usually used in practical programs. The value of full-sib and half-sib combinations depends on the fact that, when certain assumptions are met, the covariance of half-sibs can be used to estimate one quarter of the additive genetic variance and the covariance of full-sibs can estimate one half of the additive and one quarter of the dominance genetic variance of the populations. Heritabilities and genetic correlations can be calculated and the relative importance of additive and non-additive variances will provide the basis for making decisions on the best breeding approach.

REVIEW

As the selection of a suitable breeding strategy and accompanying mating design represents one of the key decisions in any breeding program, the problem has been addressed in considerable detail by several of the leading forest geneticists and there is little point in trying to repeat their detailed arguments. This paper is not intended to be a comprehensive review of all that has been written but some of the major papers will be mentioned. There will be some discussion of the application of the more common designs, and the mating designs being used, or planned for use, in the British Columbia breeding programs will be illustrated in more detail and some strengths and weaknesses discussed.

Strategies and mating designs are judged partly on their theoretical efficiencies, usually measured in terms of relative gain estimates and often with an attempt to bring in costs (Pepper and Namkoong 1978), and partly on the practical considerations such as biological limitations, program support, expertise available, time frame and overall program priority (Fowler 1986). Combinations of these two approaches are present in the literature.

Early concern for the topic was illustrated by the examination of diallel modifications by Hinkelman and Stern (1960) and the review of heritabilities and gain concepts for evaluating breeding approaches (Namkoong et al. 1966). The decision-making process for a specific program is illustrated for Pinus patula Schiede & Deppe in Africa (Burley et al. 1966). There it was recommended that three mating designs would best meet the needs of the total program, illustrating the need to clarify objectives before committing the program to a single or several approaches to meet the three needs of testing, selection and estimation.

In 1968 Dr. Libby was particularly involved in the development of single pair mating designs and their efficiencies in producing material for selection (Libby 1969). As it was pointed out later (Van Buijtenen 1972), this scheme was originally derived from animal work by Osborne (1957) using the hierarchical design, but its simplicity and relative efficiency which brings the numbers of crosses per parental entry to a minimum has many advantages for a particular set of circumstances (Libby 1972). Van Buijtenen (1972) reviewed efficiencies of mating designs for second generation selection and brought in the relationship with cost as a further means of reaching decisions. Shelbourne (1969) produced a comprehensive review of tree breeding methods as a whole, expanding on the earlier work of Namkoong et al. (1966) to examine a wider range of gain estimates. These estimates were based on Pinus radiata D. Don data from the New Zealand program but they form a useful guide to the approaches and decision-making. As a further development, the New Zealand program was reviewed and more specific plans were discussed in Burdon and Shelbourne (1971). Single pair mating and simple and nested polycross schemes were discussed again and once more the need to tailor the designs to specific objectives was underlined. There is no correct answer, but options for consideration are put forward. In North America, designs for estimation objectives were reviewed in detail (Namkoong and Roberds 1974) and efficiency parameters were illustrated graphically. Hinkelmann (1974) introduced a two level design in which both within and between population variation could be studied. This produced a complex design which is well suited to a research program but would be difficult to apply on a practical scale directly. It represents the more complex end of the design spectrum. In British Columbia, Gyula Kiss is using a similar design to study variation within and between his separate populations from within B.C. and from eastern North America (Kiss 1986).

During the mid-1970s, a number of major programs had become well established, usually along classical lines of wild stand phenotypic selection followed by clonal or seedling orchards with some testing plans and the need to develop second-generation populations was moving from the planning to the establishment phase. The alternatives were again reviewed and updated at a IUFRO meeting on advanced generation breeding at Bordeaux (Van Buijtenen 1976). Genetic gain formulae were provided for selection and for testing schemes to rogue the first-generation orchards. Polycross designs appeared most efficient for testing, while for breeding populations disconnected or partial diallel designs were favoured. Again, for the estimation objective, some form of partial diallel scheme appeared best. By this time trained geneticists were available in most programs and the level of expertise meant that in the major cooperative programs such as that associated with North Carolina State University and in the Western Gulf the more complex programs were practicable (Talbert 1979). Problems of avoiding inbreeding in the long run for the materials used to produce seed for reforestation were addressed. While inbreeding and selfing in an orchard can be a serious problem, certain planned levels may well be acceptable in the breeding population. This adds a topic for further research. By the end of the Bordeaux meeting there was a general tendency to favor complimentary mating designs, each contributing to a specific part of a total program. This was in contrast to the idea of choosing a single, perhaps complex and cumbersome design to attempt to answer the three basic needs in a single program. For example this change dates the coastal Douglas-fir program in British Columbia where in 1973 and "overall" project had been developed. The decisions in this program will be examined later to illustrate these points.

The FAO World Consultation at Canberra in 1977 produced another round of reviews including those from New Zealand (Burdon et al. 1978) and Sweden (Lindgren 1978). Options were displayed and the methods adopted for ongoing programs illustrated. Analytical techniques were developing at the same time and a quantitative genetics text provided details and in-depth review (Namkoong 1979). The disconnected factorial, a variation of the factorial design was also described at this time. Comparisons were made between the small disconnected factorials and the diallel designs and for some purposes the factorials appeared easier to use and analyze and yet met the same objectives (Anon. 1977). These small non-overlapping independent factorial (SNIF) designs are being used in British Columbia (Carlson 1985) and in Europe (Skrøppa 1984). In the faster growing species, programs are advancing rapidly and information on which to base decisions is being produced, taking away some of the assumptions and replacing them with facts. The pine programs in Australia provide an example and a recent review of alternative breeding strategies (Cotterill 1986) combined the theoretical aspects of gain arising from the different selection schemes with costs and time scale considerations. Cotterill (1986) made a strong case for using simple mating designs which were within the capabilities of even the less intensive programs and showed that if applied to a full scale program, the theoretical gain could be almost as high as that from more complex designs. He also stressed that the more complex programs left more room for errors and reduced efficiencies could well show up in practice. He also looked at two time frames, one as gain per generation of breeding, the other as gain per decade. He used Australian Pinus radiata data but the principles can be applied elsewhere. He did show that if an adequate number of disconnected diallels could be provided within an appropriate time frame, it still required complex index selection methods to take full advantage of the design. He also showed that on the per-decade basis and under his conditions, the advantage of the diallel program was lost. Where the rotations are short, juvenile/ mature correlations may be higher than in the slower growing temperate conifers and some of the findings may be modified. For example, the cost and effort of establishing a major disconnected diallel program for northern conifers may well still pay off if the index selection procedures can be worked out. One point made by Cotterill is that whether good or bad, the scale of a program must reach certain parental numbers before it can contribute to a practical objective. Even if the approach is not the best, the program should be large enough to allow conclusions to be drawn. There are examples where much effort has been wasted to provide information on too restricted a base. The process has been continued with a new review (Van Buijtenen and Tuskan 1986), including the consideration of planting designs and clonal tests.

However, integrated planning is now being followed and regional program plans are appearing (Fowler 1986, Harrison 1986) where these problems are addressed with prescriptions for practical programs. Fowler has taken the prescriptions through to breeding for third generation material using the selections made in the second and tackles the problems of increased chance of inbreeding by introducing breeding groups. This publication underlines the need for other programs to consolidate their plans.

The papers referred to in this review do not lend themselves to brief summary but they outline some of the important options and how they may be used. These options provide the tools which each of the breeders must adapt and apply to their own set of circumstances.

CHOICE OF DESIGN

With the large volume of literature to guide them, the breeders should be in a good position to draw up their plans. However, in practice considerable simplification is likely and some practical constraints or objectives may overrule many of the finer points.

The objectives are clear:

1. Testing
2. Selection
3. Estimation
4. A combination of these

A simplified listing of designs and their functions is as follows:

Design Group	Main Function
Wind pollination	Testing
Polymix	Testing
Single pair mating	Selection
Hierarchical	Multiple
Factorial	Testing, Estimation
SNIF (Small non-overlapping independent factorials)	Selection
Diallel	Multiple
Disconnected modified diallels	Selection, Estimation
Partial diallels	Multiple

When looking at the suitability of designs, it is perhaps better to look first at the critical restraints or bottlenecks. These will vary in importance but will all need to be considered.

1) Biological constraints of the organism
2) Costs: a) to carry out the mating design
   b) to carry out the resulting tests or selection
3) Availability of materials and expertise
4) Program priorities and level of support
5) Time frames
6) Scale
7) History of program to date
8) Weakness of a design
9) Strength of a design

To illustrate the way in which decisions have been made in an active program, I will use the coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) project in British Columbia (Heaman, 1978). The process has been far from smooth and as conditions and knowledge change, the decisions made can be questioned, but as an illustration the project can serve the purpose well. The support for tree improvement has increased greatly over the last thirty years and in the newer lodgepole pine (*Pinus contorta* Dougl.) and interior Douglas-fir programs a more direct approach to breeding strategies has been possible. The interior spruce (*Picea glauca* (Moench) Voss) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) programs illustrate some of the alternatives.

The coastal Douglas-fir program was started in 1957 with intensive phenotypic selection in wild stands for seed orchards. These clones were also grafted into clone banks (or breeding orchards) and wind pollinated seed was not available from the parent trees. The clone banks were expected to provide cones and pollen for progeny testing as they matured.

Apart from the appearance of widespread graft incompatibility, the clone bank located at Cowichan Lake did not produce reproductive buds readily and with limited funding, testing was delayed in favor of further field selection and research projects. Before testing could be started, clonal seed orchards lost favor for several reasons: a) incompatibility problems, b) slow cone production on the grafts, c) more rapid production of seedlings and d) increased maintenance of grafts rather than seedlings when resources were critical. A good wild stand crop in 1966 made widespread open pollinated collections possible and open pollinated seedling orchards were established instead. Low genetic gains, especially due to the low heritability of growth traits in wild stand selections meant that a selection objective at once took precedence over testing. Replacement of the first orchards rather than culling them would produce more gain. Open pollinated seed was collected from the selected plus trees for the orchards and weak nursery selection was practised. Seed was also collected for family tests from most of the trees but priorities did not permit its use. The clone banks were maturing and it was anticipated that full-sib crosses would soon become possible. These are the sort of background decisions which had to be made and which had to carry more weight in the old programs than efficiencies of design *per se*.

Major program costs had been switched from field selection to testing in the mid 1960s but with no production on the clones, the priorities were turned to breeding method research and Dr. Alan Orr-Ewing developed an interracial crossing project (Orr-Ewing 1966). With the wide range of Douglas-fir there was a possibility that wide interracial crossing might lead to heterosis and wide adaptability of the resulting material. This was a research program, but an imbalanced factorial mating design was used. Crossing was carried out on available, local female parents using pollen received from breeders across the species range. Formal mating designs, however desirable, were not possible under these circumstances and this has, in hindsight, led to reduced application of the data generated. A balance between doing something and standing back and waiting for the most complete approach always has to be maintained. This project does illustrate the need to spell out clear objectives and use a formal mating design to meet them if investments are to provide a useful return. This becomes even more important on the scale of an applied breeding program.

As the parent clones started to produce, a simple inexpensive design to meet the reselection objectives was needed and the single pair mating design was attractive. The features of the design were:

1. Simple, with a parent/cross ratio of 2:1.

2. For field testing the critical cost factor is the number of seedlings planted, which in turn is governed by the number of entries. Single pair mating requires minimum numbers.

3. Single pair mating is the simplest design where control of both parent identities is maintained. This permits knowledge and control of later inbreeding levels.

4. It is an ideal design where only small numbers of buds are available for controlled crossing, suiting developing clone banks and orchards. More classical hierarchical designs can take advantage of this situation but involve more crosses (Campbell 1972).

5. In theoretical terms, Libby (1969) has shown the design to be efficient for selection.

6. While this is a design with a single objective, this can be an advantage depending on the overall priorities etc.

These are the same features that favour the design in ongoing practical programs but the selection process will lead to greater gains if only those parents which have been shown to have high general combining abilities are brought into the next stage of the program. Testing using open pollinated seed either in the field or in farm-field tests, can reduce the wild stand selections and restrict investment to the better clones. Without the testing phase, and using phenotypically selected but random entries in the single pair mating design, will mean that some desirable parents will be masked by poor partners and potentially valuable materials may be rejected and lost.

Before the single pair mating design was in place, a further change occurred in the coastal Douglas-fir program and support from the B.C. Productivity Committee made some testing objectives possible. In 1971, a North Carolina II, balanced factorial design was added to provide for testing and estimation objectives, while the single pair mating design was continued on the same zonal populations for reselection A good crop in the clone banks and orchards that year meant the new double pronged approach could be started. The North Carolina design provides an example of how the objectives could be well met on a theoretical basis but the resulting program is extremely cumbersome, costly and, in effect, impractical. Reasonable numbers of progenies could give useful estimates of genetic parameters, but testing a useful population at even four crosses per parent soon becomes unrealistic. This was also exaggerated in the varied environments of coastal British Columbia where genotype by environment interactions could be anticipated and needed examination.

The selection objective was still of first importance and so the emphasis on testing was misplaced. Outside help was sought, and after consultation with Dr. Gene Namkoong the factorial design was relegated to a research project and the comprehensive aspects of the single disconnected modified diallel design appeared attractive and within our capability. By setting the priority on lower and middle elevation areas the current long-term breeding project was put in place. The disconnected modified diallel design meets the selection and estimation objectives efficiently although some doubts have been expressed (Van Buijtenen and Namkoong 1983), and with some compromises provides information for ranking parents on their general combining ability figures. The number of parents per diallel group was taken as six as a compromise figure, since it permits a little more flexibility than five which have been used in New Zealand and did not expand the project beyond our capability. Five crosses per parent gives a reasonable estimate of general combining ability. The complete diallel with n^2 crosses from n parents is not realistic, but the modified half diallel without inbreds and reciprocals which uses n(n-1)/2, was still practicable. The risks involved in the assumption that there are no reciprocal effects have not yet been fully explored but it has been possible to show that for this species no significant component for reciprocal effects was recognizable in a sample test at age seven.

The coastal Douglas-fir program has now reached the stage where early data are available and plans for the next generation of breeding and selection are being put together. A factual basis for efficiency and gain estimation will make it possible to evaluate the design. Cost-benefit assessments are not easy to produce but some ranking should soon be possible. We are already able to see some of the problems with the design, amongst which scale, cost and time must be considered.

In terms of scale, the decision was made to include about 350 trees in the breeding population and with the numbers needed for the field design, which incorporated study of performance across a variety of sites, it became necessary to divide the program into annual projects. Ten six-tree diallels amounted to the maximum planting program that could be handled in one year. Planting different annual groupings on different sites restricts the overall interpretation of performance but for practical purposes each annual series can be treated as a separate project. Planting numbers rather than difficulty in making crosses provided the major bottleneck for the project. By including some crosses from the previous year's project in each series of plantations a bridge was provided for gross comparison.

As the modified diallel units contain 15 crosses it was realistic to stipulate that all cells of each unit should be filled. Failure of a cross for example meant that a maximum of fourteen crosses would be held in storage until the cell could be completed. This was usually done the following year with stored pollen that was readily available. Bias by random sampling into six-tree groupings can also be seen with some significant set effects appearing (Yeh and Heaman 1987). Scale reflects more strongly the inclusion of genotype by environment interaction studies in the program and lack of sensitivity reflects the inherent variability of the test environments on coastal British Columbia and cannot be attributed to the choice of mating design. The assumption of random entry is not strictly valid since bud availability and phenology must influence the choice of parent trees included in any six-tree set, but it can be said that sampling is unbiased. Just as the project has been used here as an example, it can provide a basis for improving the selection of mating designs for other projects.

With our present knowledge, the lodgepole pine breeding strategy designed for the interior of British Columbia by M. Carlson represents a practical alternative. Population information was already available before the breeding strategy was put in place and with open pollinated seed readily available from a serotinous species, immediate testing of selected phenotypes within and between zones could be quickly established. Farm-field testing does not influence mating design directly but can influence speed of advance and later design questions. With general combining ability rankings available, the emphasis can be placed more heavily and more effectively on the selection objectives, and for that purpose the small non-overlapping independent factorials with a ratio of 1:1 for parental and progeny entries would be favoured over the disconnected diallels at 1:2.5. Time factors, bud induction techniques to overcome lagging male production and many other factors can still influence the final decision.

For species where open pollinated seed is not available but the genotypes have been moved into clonal orchards, polymix testing using an adequate number of males could give a quick ranking of genetic quality. By blocking the pollen parents, the efficiency can be increased for testing and these designs can also make reselection in the progenies a reasonably safe and simple procedure. Differential pollen viability or fertilization potential will always bring a risk but the design will be sufficiently robust for some programs. In British Columbia, blocked polymix designs are being used in parts of the interior spruce and western hemlock programs. They provide a good means of testing the existing orchards for roguing but the reselection aspects can also be valuable.

These examples emphasize once more that there is no simple answer when choosing a mating design for a practical program. The important considerations are to ensure that the specific objectives are met and that not only are the designs and strategies possible under the present resources, but also that the needed expenditures inherent in the design and approach can be met later. This applies particularly to test establishment, maintenance, assessment and evaluation. Clearly, if the same gains can be achieved by simple designs it is far safer to use these, but before deciding, the other options must be reviewed. Objectives must be clearly defined, priorities set and population numbers planned to balance resources with the scale of the project before a useful program on a practical scale can be put in place. All that remains then is to have faith and drive the program ahead.

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APPENDIX

(Figures)

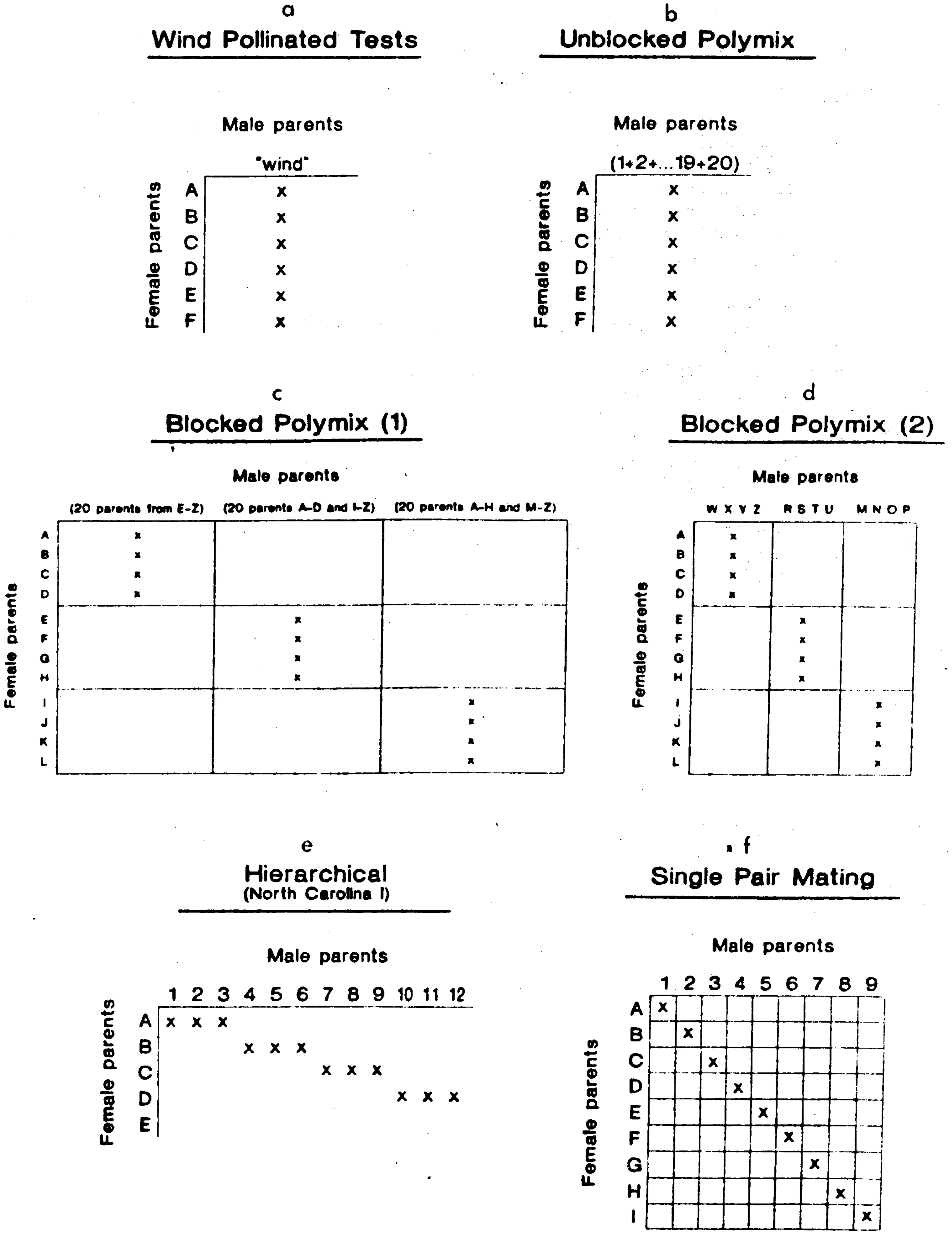
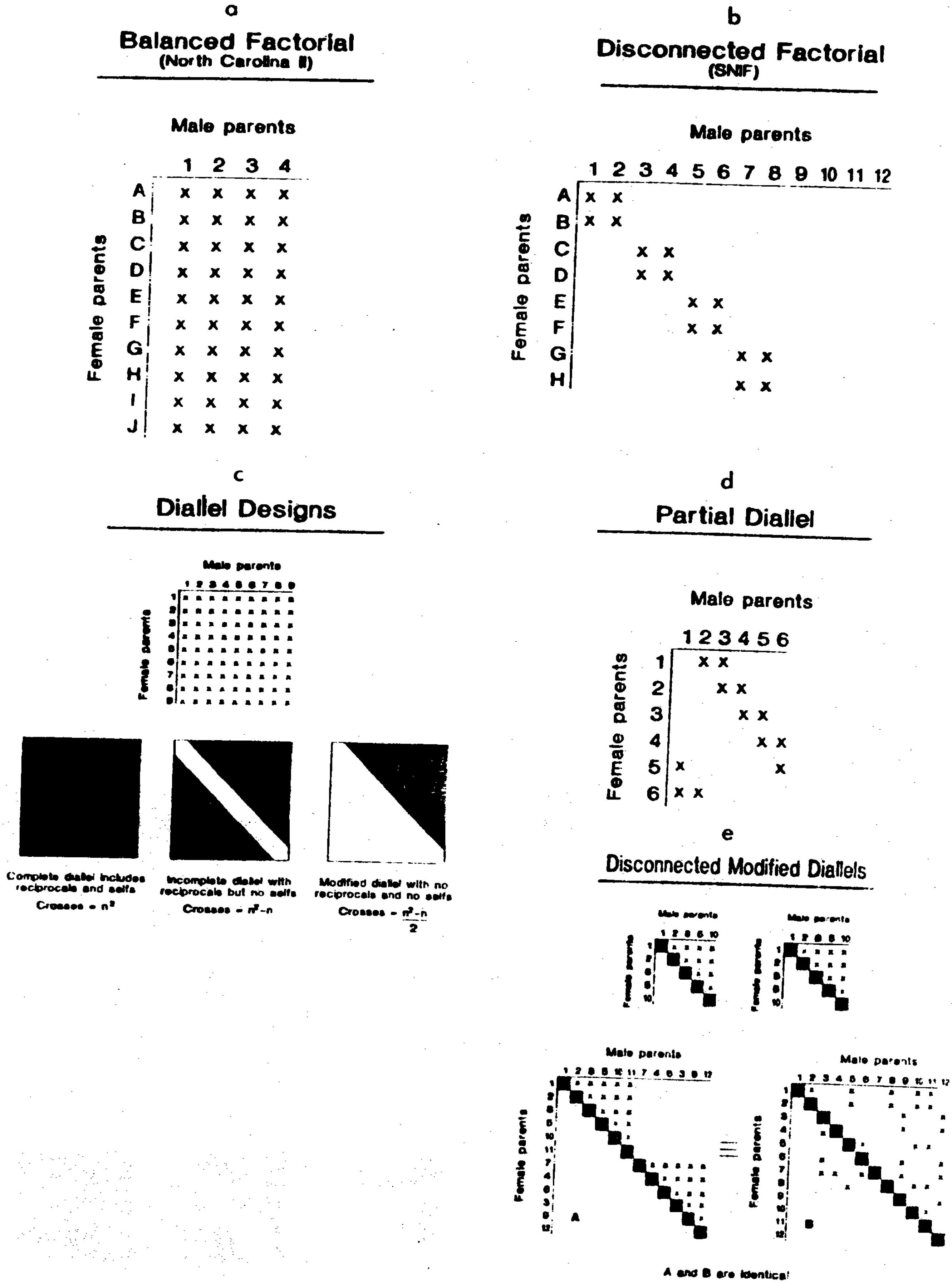

a
Wind Pollinated Tests
Male parents
"wind"
Female parents
A B C D E F
x x x x x x
b
Unblocked Polymix
Male parents
(1+2+...19+20)
Female parents
A B C D E F
x x x x x x
c
Blocked Polymix (1)
Male parents
(20 parents from E-Z)
(20 parents A-D and I-Z)
(20 parents A-H and M-Z)
Female parents
A B C D E F G H I J K L
d
Blocked Polymix (2)
Male parents
W X Y Z
R S T U
M N O P
Female parents
A B C D E F G H I J K L
e
Hierarchical
(North Carolina I)
Male parents
1 2 3 4 5 6 7 8 9 10 11 12
Female parents
A B C D E
f
Single Pair Mating
Male parents
1 2 3 4 5 6 7 8 9
Female parents
A B C D E F G H I

Figure I



a

Balanced Factorial
(North Carolina II)

Male parents

Female parents	1	2	3	4
A	x	x	x	x
B	x	x	x	x
C	x	x	x	x
D	x	x	x	x
E	x	x	x	x
F	x	x	x	x
G	x	x	x	x
H	x	x	x	x
I	x	x	x	x
J	x	x	x	x

b

Disconnected Factorial
(SNIF)

Male parents

Female parents	1	2	3	4	5	6	7	8	9	10	11	12
A	x	x										
B	x	x										
C			x	x								
D			x	x								
E					x	x						
F					x	x						
G							x	x				
H							x	x				

c

Diallel Designs

Complete diallel includes reciprocals and selfs
Crosses = n^2

Incomplete diallel with reciprocals but no selfs
Crosses = n^2-n

Modified diallel with no reciprocals and no selfs
Crosses = $\frac{n^2-n}{2}$

d

Partial Diallel

Male parents

Female parents	1	2	3	4	5	6
1		x	x			
2			x	x		
3				x	x	
4					x	x
5	x					x
6	x	x				

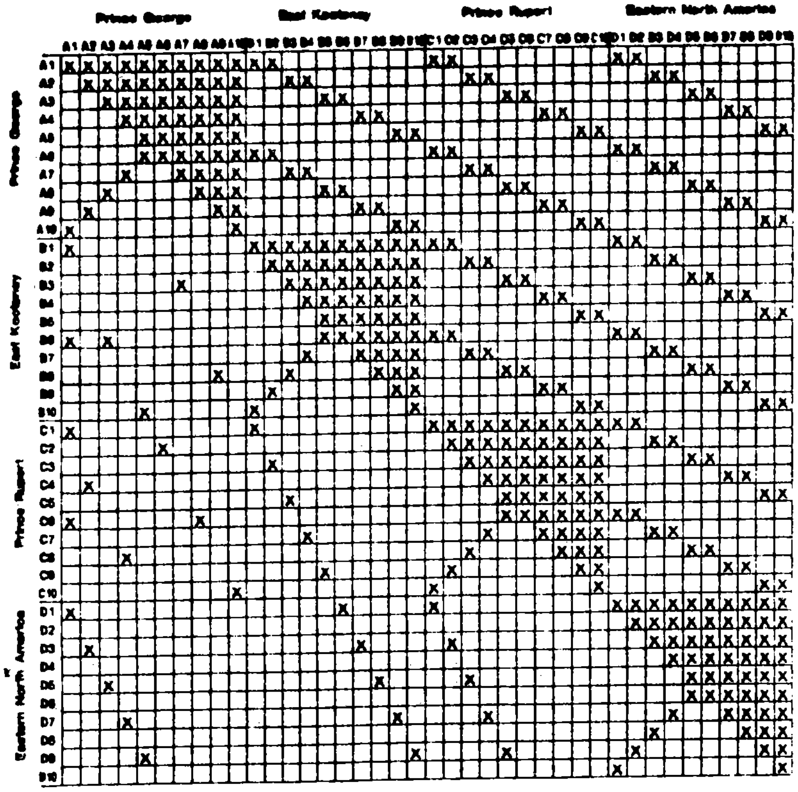
e

Disconnected Modified Diallels

A and B are identical

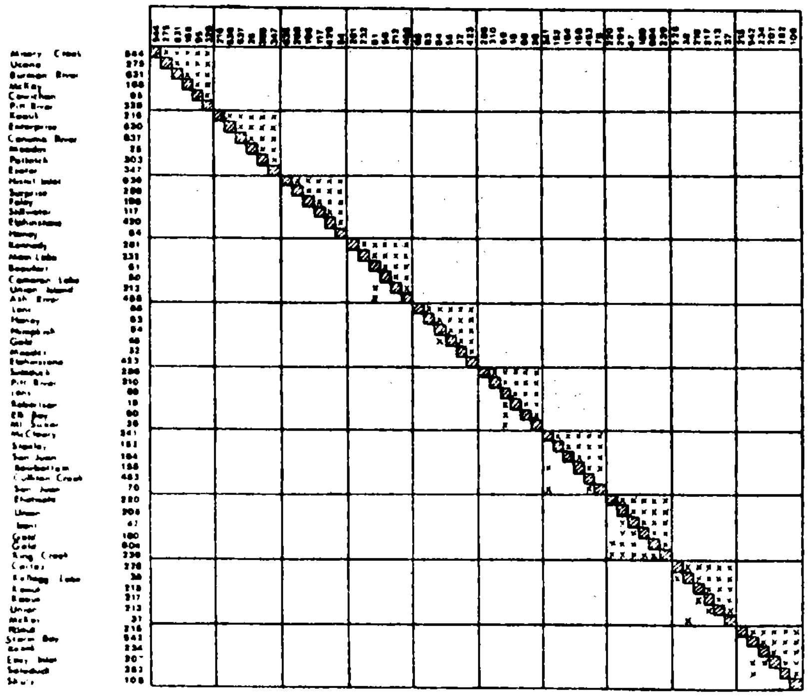
Figure II

a



Mating Designs
Two level design for research study of between and within population crossing
(Interior Spruce Program G. Kiss)
EP670.71

b



Mating design for diallel project.

c

Imbalanced Factorial
(Experimental Crossing)

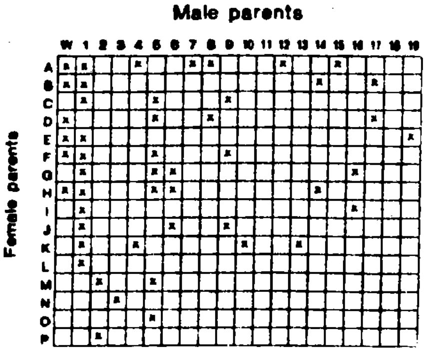


Figure III

a

Project Objectives

1. Testing: *Information to reduce population*
2. Selection: *Materials for new population with next level of gain*
3. Estimation: *Influence breeding strategy decisions*
4. Combinations (1-3)

b

Designs

Wind pollination *Polymix (full, nested)*

Hierarchical *Single pair mating*

Factorials *(balanced, imbalanced, SNIFS)*

Diallels *(full, partial, disconnected)*

Special designs *Two level*

c

Bottle Necks

NUMBERS

COST HISTORY

MATERIALS BIOLOGY EXPERTISE

TIME FRAME PRIORITIES

STRENGTHS → EFFICIENCY ← WEAKNESSES

Figure IV

FIELD TESTING IN OPERATIONAL BREEDING PROGRAMS

Yill Sung Park

Canadian Forestry Service - Maritimes
P.O. Box 4000, MacKay Drive
Fredericton, N.B.
E3B 5P7

ABSTRACT

Plot designs, analyses, and applications of genetic testing in operational tree improvement program are reviewed to provide guidelines for advanced generation testing and selection. The New Brunswick Tree Improvement Council's field testing procedure is used as an example. Single-tree plot or non-contigious plot designs should be given more consideration as they are statistically efficient. The implications of genotype x environment interactions in operational breeding program are discussed in terms of stability of genotypes and possible delineation of breeding zones. The variance components estimated from field tests can be used to predict genetic gains and to formulate a combined index selection strategy. Considerations for advanced generation testing and selection are discussed including those for clonal forestry.

RÉSUMÉ

On étudie la conception des parcelles, les analyses et les applications des tests génétiques dans des programmes opérationnels d'amélioration des arbres pour fournir des lignes directrices pour procéder à des tests et à une sélection perfectionnée. La méthode d'essai sur le terrain du Conseil d'amélioration génétique des arbres du Nouveau-Brunswick sert d'exemple. On devrait davantage songer à concevoir des parcelles ayant un seul arbre ou des parcelles non contiguës puisqu'elles sont efficaces du point de vue statistique. On expose les répercussions des interactions du milieu sur le génotype dans les programmes opérationnels d'amélioration en ce qui concerne la stabilité des génotypes et la délimitation possible des zones de reproduction. On peut utiliser les éléments de la variance, calculés d'après les tests sur le terrain, pour prévoir les gains génétiques et formuler une stratégie de sélection d'index combinés. On expose les points à considérer pour la sélection et les tests perfectionnés notamment en ce qui concerne la foresterie par clones.

INTRODUCTION

Field testing of genetic materials is an essential part of any tree improvement program, yet, there are many differing opinions on this subject. Genetic testing involves two equally important stages: 1) design and establishment of the test and 2) analyses and application of test results.

Field testing generally includes one or more of the following objectives:

1) Evaluation of families or parents for roguing of seed orchards. Ranking of the family means based on test results is required for this purpose.

2) Estimation of genetic variance components in the breeding population. Knowledge of the relative magnitude of variances due to additive, non-additive, and environmental causes is essential to develop an effective breeding strategy and to predict theoretical genetic gains.

3) Development of a base breeding population for the next generation. In multigeneration breeding programs, selected individuals in field tests can be used as parents in the next generation breeding population. For this purpose, a sufficient number of trees per family will be required to increase effectiveness of within-family selection.

In operational tree improvement programs, field testing is an expensive part of the program and may involve several objectives and constraints. Thus, in choosing test designs, breeders are concerned about statistical efficiency at minimum cost. It is difficult, if not impossible, to find a single test design to satisfy all the requirements of a breeding program. Therefore, designing a good field test is, in some respects, finding a compromise that will result in an efficient use of program resources.

The purposes of this paper are to review some elements of test design which should be considered to achieve this efficiency and to interpret test results in an operational program. As an example, I will discuss the New Brunswick Tree Improvement Council's (NBTIC) test designs already in place and how they can be used in the operation.

TEST DESIGNS

General considerations

Field testing involves the evaluation of breeding materials over the range of environmental conditions which the breeding program is intended to serve. Breeding materials are the collection of genotypes (genetic entries), such as clones, families, provenances, or populations. The fundamental principle of test design is to minimize biases when comparing breeding materials. It is also important to obtain a desired degree of statistical confidence (or accuracy) in such comparisons of the entries. The entries, environments and experimental design can introduce biases and influence accuracy.

The variation between and within the entries influence statistical efficiency. For example, if genetic differences are large between entries such as in species or provenance testing, the chance of finding significant differences is great with relatively few individuals. On the other hand, if the entries are intensively selected clones, genetic variation among them is likely to be small, and a somewhat larger number of individuals or an elaborate test design may be required. If the variation within an entry is small such as in ramets of a clone, few individuals per clone are required. The larger the number of entries under test, the more likely that significant differences are found.

Environmental variation among and within test sites influences the performance of the entries being tested. Among-site variation provides a breeder with useful information on the magnitude of environmental differences within breeding or planting zones. Furthermore, the characteristics of test sites influence genotype x environment (GE) interactions. The number of test sites required is mainly dependent on the magnitude of GE interactions. In the absence of strong GE interactions, only a few sites are necessary. A large number of test sites means high cost.

In field testing of forest trees, within-site variation exists due to microsite differences. The confounding of within-site variation and genetic potential of the entries could lead to a serious bias. Therefore, it is important to choose a design that samples microsite variation thoroughly, by adopting a proper combination of size and configuration of plots.

Statistical considerations

Perhaps, the most frequently asked questions on field testing are "What is a sufficient number of individuals to evaluate each family?", "How many test sites and replicates do we need?", or "What plot size and configuration shall we use?"

Cotterill and James (1984) studied these questions and provided some general solutions. The first solution was based on the minimum differences between the means of families which should be statistically significant, and they concluded that about 10 individuals per family is the lower limit while 20 individuals per family is the upper limit. A shortcoming of this solution is that "accuracy" is measured in terms of the probability that there is a significant difference among families. However, the objective of progeny testing is to determine the best families regardless of statistical significance. The second solution was focused on the expected probability that the best families are identified. Here the authors concluded that 10 to 20 individuals per family will give the breeder a good chance of making a correct decision.

The size and shape of plots also affects statistical efficiency. The influence of plot size in forest trees has been studied by Wright and Freeland (1960), who concluded that single-tree plots are most efficient for detecting significant differences among treatments. Lambeth et al. (1983) also examined the statistical efficiency of row and non-contiguous family plots in loblolly pine (Pinus taeda L.) tests and concluded that non-contiguous plots were more efficient than row plots. Despite the advantages of non-contiguous or single-tree plots, these plot designs have been seldom used in genetic testing.

To choose plot designs, it is useful to compare advantages and disadvantages of various plot sizes and shapes.

A single-tree plot consists of one tree per entry per block and is statistically most efficient (Wright and Freeland 1960). Such statistical efficiency is generally due to a reduction in block size such that microsite variation contributes little to within-plot variation. Because the number of individuals required per entry is smallest for a given area or block, this design makes it possible to test a large number of entries. The major criticisms or disadvantages of this design have been (1) the relative difficulty in establishing and keeping records of each tree over time, (2) the problem of missing plots due to mortality or damage, (3) thinning of test plantation will result in elimination of entries from a block and irregular spacing, (4) in some cases, within-family selection may not be effective as the number of individuals per entry is small, and (5) volume estimates are not possible. However, with the aid of modern computers and field data collectors, the first two problems become less important.

Non-contiguous plots are multitree plots consisting of several individuals which are separated from one another within a block, randomly or systematically. This design is more efficient than contiguous designs (Lambeth et al. 1983) because microsite variation in the test area is thoroughly sampled as in single-tree plots. An advantage of this design over single-tree plots is that the presence of several trees within a block usually avoids missing plots. As in single-tree plots, however, the thinning of a test plantation is a problem.

Small contiguous plots, such as 4- or 5-tree-row plots, are most commonly used. Many breeders consider this a good compromise between statistical efficiency and operational convenience. The contiguous plots usually introduce environmental covariances and, for this reason, have lower efficiencies than a non-contiguous design (Libby 1987). The smaller the plots, the more statistically efficient they are. An advantage of small contiguous plots is that they can provide desired spacing after thinning without eliminating entries from the block. Some traits, *e.g.*, survival, are evaluated as percentage data on the basis of plots. Also, plot means can be calculated and used in the analyses as a cost saving measure.

Large contiguous (block) plots consist of many individuals of an entry physically adjacent to one another. This design may be useful to select within families (van Buijtenen and Lowe 1979) or to quantify productivity per unit area (Libby 1987). If intergenotypic competition is a problem, it is necessary to use large plots with border rows. Large contiguous plots generally are not suitable for the evaluation of families, especially when the number of families is large.

Single-tree plots should be given more consideration in designing future tests. However, choosing a plot design is also influenced by growth rate and cultural practice of different species. For example, with current 2 x 2 m spacing, a black spruce (*Picea mariana* (Mill) B.S.P.) test may not require thinning for about 15 years and thus may be compatible with a single-tree plot design. But tamarack (*Larix laricina* (Du Roi) K. Koch) and jack pine (Pinus banksiana Lamb) tests will probably require thinning at about 10 years; therefore, small contiguous plots may be more suitable. Assuming 20 individuals per family per test site, the single-tree plot, 2-tree plot, and 4-tree plot will require 20, 10, and 5 replicates (blocks) per site, respectively. The size of block depends on uniformity within blocks; however, it should probably not exceed one-half acre (0.2 ha).

ANALYSES AND APPLICATIONS

NBTIC breeding strategy and testing program

Since the formation of the New Brunswick Tree Improvement Council (NBTIC) in 1976, operational tree improvement programs have been undertaken with black spruce, white spruce (Picea glauca (Moench) Voss), jack pine, and tamarack. Field testing in these programs guides seed orchard roguing and the acquisition of genetic information for long-term breeding strategies. Test plantations will also be used as a source of second generation breeding materials.

Recently, Fowler (1986) outlined multigeneration breeding strategies for the species in the NBTIC program. The effective implementation of these strategies is heavily dependent on the testing program. The black spruce breeding strategy will be discussed as an example of a field testing procedure.

The first generation improvement strategy for black spruce is based on a "seedling seed orchard" scheme using open-pollinated seeds from 1,200 plus trees selected from natural populations. The initial seedling orchards consist of single-tree plots planted a 1 x 2 m spacing. The "family tests," established at the same time as the orchard, are designed to provide information for roguing of the orchards. Because the selection of the required 1,200 plus trees was carried out over a period of 10 years, establishment of orchards and family tests were also completed over a 10-year period which ended in the spring of 1987. In any given year, at least one or two seed orchards were planted. The corresponding family tests were located at five or six representative sites in the same year. The typical design for family tests in a series (year) involved the testing of about 100 families at each test site, in 10 randomized blocks of 4-tree-row plots. To date, 12 of these test series have been established on a total area of over 100 ha.

The materials for the next generation improvement will be derived from these family tests by selecting one from each of the 400 best families. Single-pair mating is used to produce the next generation breeding population and polycross progenies are produced for testing each selection. The breeding population is sublined, and a partial diallel mating and polycross test cycle are repeated.

Statistical analysis and genetic interpretation

The analysis of variance is a standard technique for examining data from these tests. The linear model used in NBTIC family and polycross tests is

$$[1] \quad Y_{ijkl} = \mu + E_i + B_{j(i)} + G_k + GE_{ik} + GB_{ijk} + \varepsilon_{ijkl}$$

where

Y_{ijkl} = performance of l^{th} individual of k^{th} genotype (entry) growing in j^{th} block of i^{th} test site;
μ = overall mean;
E_i = effect of i^{th} test site, $i = 1, 2, .., e$;
$B_{j(i)}$ = effect of j^{th} block (replicate) within i^{th} site, $j = 1, 2, .., r$;
G_k = effect of k^{th} genotype, $k = 1, 2, .., g$;
GE_{ik} = interaction effect of k^{th} genotype and i^{th} test site;
GB_{ijk} = interaction effect of k^{th} genotype and ij^{th} block; and
ε_{ijkl} = random error associated with individuals within plot.

The form of analysis of variance, assuming all the terms in the model being random effects, is shown in Table 1. Other model assumptions, however, may be appropriate depending on the experimental conditions, such as considering environments and blocks as fixed effects. Estimation of variance components is carried out by equating expected mean squares to calculated mean squares and solving for the components.

The estimated variance components can be interpreted in terms of genetic and environmental variances and will help to choose effective breeding strategies. Heritability (h^2), i.e., the ratio of additive genetic variance to phenotypic variance, derived from these components can be used to predict theoretical genetic gains from appropriate selection systems. The predicted genetic gain (ΔG) is calculated as:

$$\Delta G = \underline{i}\, \sigma_u h^2$$

where $\underline{i}$ is standard selection intensity and σ_u is phenotypic standard deviation.

The relative magnitude of the following variance components is important as they are the parts of phenotypic variation expressed by the tested trees (Table 1).

The variance component due to genotypes (σ^2_G) represents the genetic covariance based on relatedness within entries. It is interprested as one-quarter of additive genetic variance (σ^2_A) if the entries are half-sib or polycross families and as approximately one-third of σ^2_A if the entries are open-pollinated families (Squillace 1974). If the entries are full-sibs, this variance component represents one-half of

σ^2_A and one-quarter of dominance variance (σ^2_D). If the entries are clones, this component represents total genetic variation.

The variance component due to the interaction of genotypes and environment (σ^2_{GE}) is the most important component used to determine planting areas (zones) of the genetic entries. The GE interactions can occur at the provenance, family and clone levels due to differential response to various environmental components such as climate, edaphic conditions, and cultural practices. It is also a major factor in determining the number of test sites. If this component is insignificant, testing can be done on one typical site.

The variance component due to interactions of genotypes and blocks (σ^2_{GB}) is caused by differential response of genotypes in the blocks within test sites, which is commonly called a plot error variance (σ^2_p).

The variance component due to trees within plots (σ^2_w) contains two variances (Cockerham 1963). One is tree-to-tree environmental variance (σ^2_{ew}), and the other is the remaining genetic variance among the members of the genetic entry (σ^2_{gw}), *i.e.*,

$$\sigma^2_w = \sigma^2_{ew} + \sigma^2_{gw} \; .$$

If we denote σ^2_{TG} as total genetic variation in the tested population, the following relationship holds:

$$\begin{aligned} \sigma^2_{gw} &= \sigma^2_{TG} - \sigma^2_G \\ &= \sigma^2_A + \sigma^2_D - \sigma^2_G \end{aligned}$$

since total genetic variance consists of additive (σ^2_A) and dominance (σ^2_D) variances.

The total phenotypic variance (σ^2_{TP}) is the sum of the above variances:

$$\begin{aligned} \sigma^2_{TP} &= \sigma^2_w + \sigma^2_{GB} + \sigma^2_{GE} + \sigma^2_G \\ &= \sigma^2_{ew} + \sigma^2_{gw} + \sigma^2_p + \sigma^2_{GE} + \sigma^2_G \\ &= \sigma^2_{ew} + \sigma^2_p + \sigma^2_{GE} + \sigma^2_{TG} \; . \end{aligned}$$

The variance for entry means (σ^2_{GM}) if the tests include g families in n tree plots established at e sites, with r blocks within each site is

$$\sigma^2_{GM} = \frac{\sigma^2_w}{nre} + \frac{\sigma^2_{GB}}{re} + \frac{\sigma^2_{GE}}{e} + \sigma^2_G \; .$$

The variance within entries (σ^2_{TW}) is

$$\begin{aligned}\sigma^2_{TW} &= \sigma^2_{TP} - \sigma^2_{G} \\ &= \sigma^2_{w} + \sigma^2_{GR} + \sigma^2_{GE} \\ &= \sigma^2_{ew} + \sigma^2_{gw} + \sigma^2_{p} + \sigma^2_{GE} .\end{aligned}$$

Using these variances, different types of heritabilities can be constructed depending on the selection method (Namkoong 1979). For example, the calculation of heritability to use for half-sib family mean selection is $\frac{1}{4}\ \sigma^2_A\ /\ \sigma^2_{GM}$, and that for individual selection within half-sib families is $\frac{3}{4}\ \sigma^2_A\ /\ \sigma^2_{TW}$. For other types of heritability, see Namkoong (1979, pp 74-88). Once heritabilities of particular selection systems are constructed, a breeder can compare relative gains of different selection systems for given selection intensities.

In forest tree breeding, the variance components due to test sites (σ^2_E) and blocks within sites (σ^2_B) are traditionally excluded in estimation of heritabilities based on individual data. The exclusion of these components from the denominator (total phenotypic variance) is based on the assumption that corrections will be made for the site and block effects before using phenotypic measurements in selection. Cotterill (1987) discussed the consequence of using heritability estimates with the environmental components omitted in practical applications and provided possible correction methods. He demonstrated that the failure to make such corrections can have a substantial influence on the magnitude of both heritability and genetic gain estimates. He found that the gain estimates based on heritabilities when the environmental components are omitted may significantly overestimate the response which can be expected from selection. A method of correction is to convert raw measurements to deviations from site and block means. For example, the correction for block means on one site is

$$d_{jk} = Y_{jk} - Y_{.k}$$

where d_{jk}, Y_{jk}, and $Y_{.k}$ are deviation from block mean, measurement on j^{th} tree in the k^{th} block, and k^{th} block mean, respectively. The corrected absolute values rather than the deviation can be obtained by adding the overall mean ($Y_{..}$) of the test to the deviation, i.e., $d_{jk} + Y_{..}$. If no corrections for block or site effects are made prior to selection, these effects should be included as part of the total phenotypic variance. For family mean heritability, these components can be excluded from the denominator as they are accounted for in the averaging of family performance across sites and blocks (Cotterill 1987).

Namkoong et al. (1966) formulated expected genetic gains from various tree breeding methods. In the seedling seed orchard method, they recognized three stages of gain: phenotypic selection in the wild, selection of best open-pollinated families, and selection of best trees within best families in a half-sib progeny test itself; symbolically,

$$\Delta_G = \tfrac{1}{2}\,\underline{i}_1 \frac{\tfrac{1}{2}\,\sigma^2_A}{\sigma_1} + \underline{i}_2 \frac{\tfrac{1}{4}\,\sigma^2_{A''}}{\sigma_2} + \underline{i}_3 \frac{\tfrac{3}{4}\,\sigma^2_{A''}}{\sigma_3}$$

where $\underline{i}$'s are selection intensities, σ_1, σ_2, and σ_3 are standard deviations of total phenotypic variance $(\surd\sigma^2 3dTP)$, half-sib family means $(\surd\sigma^2_{GM})$, and within half-sibs $(\surd\sigma^2_{TW})$, respectively. The term $\sigma^2_{A''}$ is additive genetic variance among open-pollinated progenies of selected trees and, because genetic recombination occurs through mating, the genetic variance is assumed to be regenerated, i.e., $\sigma^2_{A''} = \sigma^2_A$. The term $\tfrac{3}{4}\sigma^2_{A''}$ represents additive variance within half-sib families.

Some of NBTIC family tests series have been analyzed for 5-year performance (Table 2). The results indicated that there were large differences among test sites and blocks. The variance components due to families (σ^2_G) were about 2-4% of total variance from all sources. The largest component of variance was due to within-plot errors (σ^2_W). The estimated heritability based on individuals (h^2) ranged from 0.09 to 0.14 while those based on family means (h^2_f) ranged from 0.70 to 0.75, indicating family selection can be effective. The magnitude of genotype x environment interactions (σ^2_{GE}) is relatively large amounting, to about one-half the variance due to genotypes. Therefore, further analysis is required to reduce its impact.

In the NBTIC program, the first roguing recommendation is likely to be based on 10-year data from the family tests and will remove about 50% of the poorest families in the seed orchard. However, at the final stage, when 15- and 20-year data are available, roguing will have removed about 80% of all families. Selection of better trees in the best families will be carried out on the basis of individual phenotypes in the orchard.

Genotype x environment interactions

The magnitude of genotype x environment interactions is important for the deployment of genetic entries and for determining number of test sites. The GE interactions are caused by differential response of genotypes in a range of environments, and often result in changes in rank of genotypes at different environments. If the variance due to GE interaction reaches more than one-half that due to genotypes, it is likely to have a serious impact on selection and breeding (Shelbourne 1972). Once significant GE interactions are found, there are two general actions that a breeder can take to reduce their impact: (1) stratify genotypes suitable for deployment in the entire region and (2) group the environments so that interactions within groups are minimized.

The first strategy, stratification of genotypes, involves detailed examination of genotypes relative to other genotypes in the test over a range of environments. Many statistical techniques are available (Hill 1975); however, stability analysis based on regression is used most frequently (Finlay and Wilkinson 1963; Eberhart and Russell 1966). A linear regression of genotypic performances is calculated for each environment on an environmental index, such as the means at each test location. The stability of entries is determined by two parameters: one, the regression coefficient that measures the linear response of entries over a range of environments and the other, the deviation mean square from the regression. For a given set of environmental indices, the regression coefficient defines the predictable portion of the entries' response, while the deviation mean square measures the unpredictable response. Both measures of stability should be considered together when evaluating the adaptability of entries.

The second strategy, grouping of environments can be achieved by several methods. The most widely used technique has been cluster analysis (Lin 1982; Ramey and Rosielle 1983). The cluster analysis is generally done on a matrix of similarity indices which is often based on GE interaction mean squares (Lin 1982). At each fusion cycle of this technique, the locations with the smallest index are clustered, and the process is stopped when the smallest similarity in the cycle exceeds the critical F-value. The resulting group will have no significant GE interactions.

A breeder can group test sites by empirically examining interaction mean squares for various combinations of test locations so that interaction is not important. A previously defined seed or breeding zone, if available, would provide some insight on initial groupings. This procedure can be laborious and produce several possible solutions.

Park and Fowler (1987b) delineated black spruce planting zones, i.e., the area which a breeding program is to service, as well as breeding zones, i.e., the area from which the base breeding population is to be derived. Based on the data from a range-wide provenance test established at 10 locations in the Maritime Provinces, they proposed revision of the 11 existing seed zones of the Maritime Provinces (Fowler and MacGillivray 1967) to three planting zones after considering provincial jurisdictions. However, the breeding zones for black spruce can be much larger than planting zones (Fig. 1).

Combined index selection

The theory of the selection index was first developed by Smith (1936), and later by Hazel and Lush (1942). For simultaneous improvement of several traits, the method provides an index score for each family or tree so that the selection is equivalent to the single stage truncation type. The selection index (I) is defined as:

$$\begin{aligned} I &= b_1x_1 + b_2x_2 + \ldots + b_mx_m \\ &= \Sigma\, b_ix_i \\ &= \underline{x}'\underline{b} \end{aligned}$$

where $\underline{x}$ is a known vector of phenotypic values for the traits and $\underline{b}$ is an unknown vector of index coefficients to be calculated. The solution for $\underline{b}$ is given by the expression,

$$\underline{b} = \underline{P}^{-1}\,\underline{G}\,\underline{a}\ ,$$

where $\underline{P}$ and $\underline{G}$ are phenotypic and genotypic variance-covariance matrices, respectively, and $\underline{a}$ is a vector of known economic weights.

Despite many optimal properties of index selection, the difficulty of estimating economic weights for the component traits has been a reason for its limited use (Namkoong 1976). The problem of estimating economic weights can be compromised by use of a 'restricted selection index' approach such as setting gains for some traits by a fixed amount while maximizing other traits (for review see Lin 1978, Cotterill and Jackson 1981, 1985). A multitrait index for roguing of J.D. Irving Ltd's 1979 jack pine seedling seed orchard has been developed (Adams, pers. comm.). This study indicated that selection based on height alone will result in a substantial loss in quality characters, and the restricted index procedure was effective in holding quality characters constant.

The selection index approach is also efficient even for single trait improvement by utilizing genetic information from relatives, such as treating the performance of parents and sibs as different traits (Cotterill 1986). The genetic and phenotypic variance-covariance matrices required for constructing such 'combined sib-family plus individual' index is available from progeny tests as outlined by Burdon (1982).

CONSIDERATIONS FOR ADVANCED GENERATION TESTING AND SELECTION

Test sites

As previously mentioned, small plots are statistically efficient, and we should give more attention to the single-tree plot design. One of the shortcomings of the single-tree plot design is imbalance created by mortality. Although unbalanced data can be analyzed and parameters can be estimated, the imbalance is likely to influence precision of the estimates. Generally, the larger the plot size, the less sensitive is the test to imbalance.

Site selection, site preparation, and care of field tests are crucially important in obtaining valid and fair estimates. In the past, many of our tests have been established on typical reforestation sites, and several of these tests have had confounding damage and unacceptable levels of mortality. Protection and care of tests from confounding damage and losses will become increasingly important as small-plot designs are adopted. Perhaps, homogeneity within the test should be given emphasis. Genetic testing should be done on "testing nursery" or prime sites so that the trees can express optimal potential. One may argue that survival in operational field conditions is important; however, many of our provenance tests indicate that provenance x site interactions are not important, i.e., provenances which survived better on better sites also survived better on poor sites.

Adams, G. J.D. Irving Ltd. Sussex, N.B.

It is generally agreed that genetic testing should be carried out at about one-half the rotation age. However, the information on juvenile-mature correlation is the major factor determining duration of the genetic testing and will be available from family and provenance tests in the near future. If the correlation is sufficiently high such that effective evaluations can be made in 5 to 10 years, trees can be evaluated in a "testing nursery" at a narrow spacing. The use of a narrow spacing in the test reduces the size of the test, which in turn reduces the environmental variation and, thus, increases precision.

Polycross test

The conventional tree breeding strategy based on sexual reproduction relies on the additive or general combining ability (GCA) variance. In screening for high GCA parents in a breeding population, one can use an inexpensive open-pollinated progeny test; however, these tests may be confounded in that the number of effective males and relatedness among pollen parents may differ for different progenies. The polycross is an alternative where a standard pollen mix, e.g., equal volumes of pollen, is used on each mother tree to be tested. Although it may cost more, the polycross should correct the deficiencies of the open-pollinated test, and thus promote fairness of the test. Fowler (1987) suggested a pollen mix from 20 trees for most northern conifers to remove bias due to differential pollen competition as speculated by Cheliak et al. (1987).

More elaborate mating designs, e.g., diallel, factorial and hierarchical designs, are also used in tree breeding. These designs not only require more effort, but for purpose of ranking parents they are not as fair as the polycross (Libby 1987), since different males are involved. The intent of these mating designs is to obtain additional information on non-additive variance and specific combining ability of crosses as well as to produce progenies for the next generation of selection and breeding.

While the polycross test is used to select high GCA parents, the single-pair mating or disconnected diallel mating among the selected parents should provide full-sib families from which the next generation breeding population can be derived. Selection within these full-sib families presents an opportunity for additional gain. 'Selection plantations' composed of large contiguous family blocks may be useful for this purpose. In the NBTIC program, selection plantations are to be established on two good, uniform sites using 49 (7 x 7) or 100 (10 x 10) tree plots to increase efficiency of within-family selection (Fowler 1987). Libby (1987) cautions that small contiguous plots, i.e., 25 or fewer trees per plot, may not be suitable for within-family selection. If edge or intergenotypic competition effects are important, two border rows in the contiguous plots cannot be used (Bridgewater and McKeand 1983). For example, the 7 x 7 plot design will provide only 25 candidate trees for within-family selection.

Clonal forestry

In recent years, clonal forestry has received increasing attention (Zsuffa et al. 1985). Vegetative propagation and clonal testing provide a means of obtaining genetic information and an opportunity to capture a greater portion of the genetic variation in a tree improvement program. Large scale mass vegetative propagation of juvenile materials in black spruce is already underway (Armson et al. 1980, Mullin pers. comm.) and is also applicable to tamarack (Park and Fowler 1987a). The 'tree improvement and mass vegetative propagation' strategy in these species may replace wind-pollinated orchard systems.

One approach, the general combing ability (GCA) type, involves selection of a few high GCA parents, controlled crosses to produce the desired number of families, and then mass propagation of these families for planting. The principle of this approach is essentially similar to a seed orchard system but it relies on controlled crosses and vegetative propagation system while a seed orchard system is based on panmictic wind-pollination and seed propagation. Therefore, with this approach, initial screening of high GCA parents can be done by conventional polycross or open-pollinated progeny tests as described previously, but a breeding hall or greenhouse can be used instead of a seed orchard. Genetic gain with this approach should be higher than the conventional seed orchard system, because the possibilities of selfing, non-random pollen dispersion, and pollen contamination in the orchard are eliminated.

The other approach involves clonal selection based on the combined family-clonal test. The plant material for the combined family-clonal test is obtained by vegetative propagation (rooted cuttings) of several trees within each family under consideration. For example, suppose that s rooted cuttings were made from c trees each of the g families, and the field test is established at e sites with r replicates within each site. Then the linear model for this analysis is

$$[2] \quad Y_{ijklm} = \mu + E_i + B_{j(i)} + G_k + C_{l(k)} + GE_{ik} + CE_{ikl} + GB_{ijk} + CB_{ijkl} + \varepsilon_{ijklm}$$

where

Y_{ijklm} = performance of mth ramet of lth clone within kth family growing in jth block of ith site;

$C_{l(k)}$ = effect of lth clone within kth family;

CE_{ikl} = interaction effect of klth clone with ith test site;

CB_{ijkl} = interaction effect of klth clone with ijth block;

ε_{ijklm} = random error associated with ramets within plot

and all other terms are defined as in the previous model (Model [1]). The form of analysis of variance with expected mean squares is shown in Table 3. If single tree plots are used, the random error term will be dropped, and the clone x block interaction term will become the error term.

Mullin, T.J. Tree Breeding centre, Nova Scotia Dep. of Lands and Forests. Debert, N.S.

Interpretation of variance components is similar to Model [1]. The family component of variance (σ^2_G) is interpreted as one-half, one-third, and one-quarter of the additive variance if the families tested are full-sibs, open-pollinated, and half-sibs (polycross), respectively. The variance component due to clones (σ^2_C) consists of total genetic variance minus σ^2_G. For example, if the open-pollinated families are used for cloning, the following relationship holds:

$$\sigma^2_C = {}^2/_3\ \sigma^2_A + \sigma^2_D \ .$$

Different kinds of heritability can be constructed. The narrow-sense heritability (h^2) for predicting mass selection gain is calculated as

$$h^2 = \sigma^2_A \ / \ \sigma^2_{ph} = 3\ \sigma^2_G \ / \ \sigma^2_{ph} \ ,$$

where σ^2_{ph} is total phenotypic variance, *i.e.*,

$$\sigma^2_{ph} = \sigma^2_G + \sigma^2_C + \sigma^2_{GE} + \sigma^2_{CE} + \sigma^2_{GB} + \sigma^2_{CB} + \sigma^2_{\varepsilon} \ .$$

The broad-sense heritability of individual ramets (H^2) is calculated as

$$H^2 = (\sigma^2_A + \sigma^2_D) \ / \ \sigma^2_{ph} = (\sigma^2_G + \sigma^2_C) \ / \ \sigma^2_{ph} \ .$$

Since clonal selection is likely to be based on clone means, the appropriate broad-sense heritability of clone means (H^2_{CM}) is calculated as

$$H^2_{CM} = (\sigma^2_G + \sigma^2_C) \ / \ \sigma^2_{CM} \ ,$$

where σ^2_{CM} is the phenotypic variance of clone mean, *i.e.*,

$$\sigma^2_{CM} = \sigma^2_G + \sigma^2_C + \sigma^2_{GE}/e + \sigma^2_{CE}/e + \sigma^2_{GB}/re + \sigma^2_{CB}/re + \sigma^2_{\varepsilon}/nre \ .$$

If the clonal approach is used, the maximum genetic gain is theoretically achieved when only the one very best clone in the test is selected for deployment (Libby 1987), although this is not an acceptable solution because of biological or policy considerations, such as avoidance of monoculture. Once an acceptable number of clones in a clonal plantation is determined, clonal selection efficiency becomes important to obtain maximum gain. Russell and Libby (1986) explored clonal selection and test efficiencies as a function of the number of clones in the test, ramets per clone, and clones selected. They concluded that, except at very low heritabilities and high selection intensities, the optimum number of ramets per clone per test site is usually six or less. This result indicates that a large number of candidate clones can be tested by using few ramets per clone.

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Table 1. Form of analysis of variance for family and polycross tests in NBTIC program

Sources of Variation	d.f.	Mean squares	Expected mean squares
Environments (E)	e-1	MS(E)	$\sigma^2_W + n\sigma^2_{GB} + rn\sigma^2_{GE} + gn\sigma^2_B + rgn\sigma^2_E$
Blocks in E (B)	e(r-1)	MS(B)	$\sigma^2_W + n\sigma^2_{GB} + gn\sigma^2_B$
Genotypes (G)	g-1	MS(G)	$\sigma^2_W + n\sigma^2_{GB} + rn\sigma^2_{GE} + ern\sigma^2_G$
G x E	(e-1)(g-1)	MS(GE)	$\sigma^2_W + n\sigma^2_{GB} + rn\sigma^2_{GE}$
G x B	e(r-1)(g-1)	MS(GB)	$\sigma^2_W + n\sigma^2_{GB}$
Within-plot error	erg(n-1)	MS(W)	σ^2_W

e, r, g, and n are the number of test sites, blocks within test site, families, and trees within plot, respectively; σ^2_W, σ^2_{GB}, σ^2_{GE}, σ^2_G, σ^2_B, and σ^2_E are the variance components due to within plot error, genotype x block interactions (plot error), genotype x environment interactions, genotypes, blocks within test site, and test sites, respectively. The coefficients of expected mean squares will vary if data are unbalanced.

Table 2. Variances (%) and heritabilities of 5-year heights of black spruce estimated from NBTIC family tests

Variance components		1978 test at 3 sites	1979 test at 5 sites	1980 test at 4 sites
Site (E)	σ^2_E	15.1	20.9	41.6
Block (B)	σ^2_B	3.7	4.8	1.9
Family (G)	σ^2_G	3.7	2.1	2.2
G x E	σ^2_{GE}	1.6	1.9	1.2
G x B	σ^2_{GB}	7.2	10.0	6.3
Within plot	σ^2_w	68.7	60.3	46.8
Total		100.0	100.0	100.0
Individual heritability (h^2)[1]:		0.14	0.09	0.12
Family mean heritability (h^2_f)[2]:		0.73	0.70	0.75

[1] $h^2 = 3\,\sigma^2_G / (\sigma^2_G + \sigma^2_{GE} + \sigma^2_{GB} + \sigma^2_w)$

[2] $h^2_f = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE}/e + \sigma^2_{GB}/re + \sigma^2_w/nre)$,

where e, r, and n are number of test sites, blocks, and trees per plot, respectively.

Table 3. Form of analysis of variance for combined family-clonal test

Sources of variation	d.f.	Mean square	Expected mean square
Environments (E)	e-1	MS(E)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nc\sigma^2_{GB} + nr\sigma^2_{CE} + n\,cr\sigma^2_{GE} + ncg\sigma^2_{B} + ncgr\sigma^2_{E}$
Blocks in E (B)	e(r-1)	MS(R)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nc\sigma^2_{GB} + ncg\sigma^2_{B}$
Families (G)	g-1	MS(G)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nc\sigma^2_{GB} + nr\sigma^2_{CE} + n\,cr\sigma^2_{GE} + nre\sigma^2_{C} + ncre\sigma^2_{G}$
Clones in G (C)	g(c-1)	MS(C)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nr\sigma^2_{CE} + nre\sigma^2_{C}$
G x E	(e-1)(g-1)	MS(GE)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nc\sigma^2_{GB} + nr\sigma^2_{CE} + n\,cr\sigma^2_{GE}$
C x E	(e-1)r(g-1)	MS(CE)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nr\sigma^2_{CE}$
G x B	e(r-1)(g-1)	MS(GB)	$\sigma^2_\varepsilon + n\sigma^2_{CB} + nc\sigma^2_{GB}$
C x B	e(r-1)r(g-1)	MS(CB)	$\sigma^2_\varepsilon + n\sigma^2_{CB}$
Error	ergc(n-1)	MS(e)	σ^2_ε

c, σ^2_ε, σ^2_{CB}, and σ^2_{CE} are the number of clones within family, variance components due to within plots, clone x block interactions, and clone x test site interactions, respectively; all other terms are defined in Table 1.

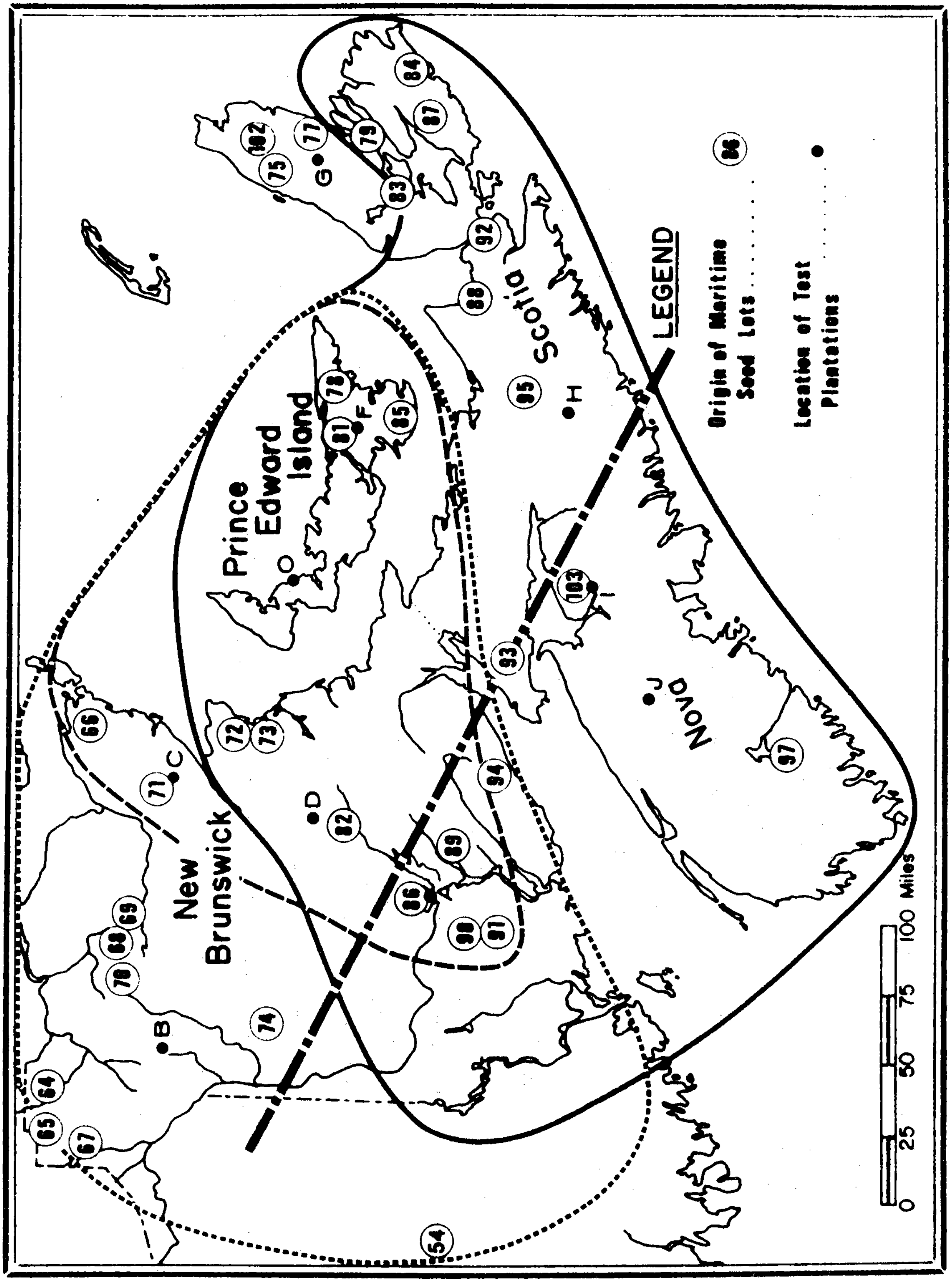

Figure 1. Maritime provenances with test locations and breeding zones. The line, ▬ ▪ ▬, indicates two breeding zones based on the cluster analysis. The lines ———, -------, and ··········, indicate three overlapping breeding zones recommended.

SEED PRODUCTION STRATEGIES: CURRENT vs. FUTURE

by

Hugh O. Schooley[1] and T.J. Mullin[2]

[1] Canadian Forestry Service, Petawawa National Forestry Institute, Chalk River, Ont., K0J 1J0.

[2] Nova Scotia Dept. of Lands and Forests, Debert Tree Breeding Centre, Debert, NS, B0M 1G0.

ABSTRACT

Canadian statistics on conifer tree seed procured and utilized since 1978 are reviewed in relation to increased seed requirements for reforestation. Seed utilized increased from about 4.2 billion in 1978 to about 7.3 billion in 1984 and has continued to increase. Sufficient seed has been collected but most have originated from unimproved natural stands. Maximum annual production from seed collection and seed production areas was less than 12% of total seed production. The establishment of seed orchards has progressed at an accelerating rate in recent years and should exceed a total of 2000 ha by 1988. As these orchards become productive the supply of genetically improved seed will increase but, for many years, this supply is unlikely to exceed total seed requirements. Seed collection and seed production areas are the preferred seed sources to satisfy the deficiency between available orchard seed and total seed required.

RÉSUMÉ

On étudie les statistiques canadiennes sur les graines de conifères obtenues et utilisées depuis 1978 en relation avec les besoins croissants de graines pour le reboisement. Le nombre de graines utilisées est passé de 4200 millions en 1978 à environ 7300 millions en 1984 et n'a cessé d'augmenter. On a récolté suffisamment de graines, mais la plupart proviennent de peuplements naturels non améliorés. La production annuelle maximale réalisée à partir de zones de production et de récolte de graines était inférieure à 12 % de la production totale de graines. L'établissement de vergers à graines a progressé à un rythme accéléré ces dernières années et devrait dépasser 2000 ha avant 1988. À mesure que ces vergers deviendront productifs, les réserves de graines génétiquement améliorées continueront d'augmenter, mais ces réserves ne dépasseront probablement pas tous les besoins en graines avant de nombreuses années. Les zones de production et de récolte de graines sont les sources préférées pour combler la différence entre les graines de vergers disponibles et le total de graines nécessaires.

INTRODUCTION

Forestry authorities throughout Canada are demonstrating a high interest in reforestation. Since 1982, financial assistance provided through federal/provincial agreements under the authority of the Forest Sector Strategy for Canada Program has contributed significantly to this increased activity. It is generally recognized that the greatest potential for future economic gain from the forests that are being planted lies in the use of the highest quality, suitable seed sources available (Faulkner 1982). In this report we briefly outline the quantity of seed being utilized in Canada, and the amounts obtained from various sources. We then discuss how successful Canada has been at increasing the quality of seed being utilized and what course seed production strategies might follow in the near future.

Seed Utilization

National statistics for all conifer seed utilized in 1978, 1980, 1982, and 1984 and the projected target for 1987 are shown in Table 1. There are large differences in the numbers of seed utilized in different parts of the country and from one year to the next within a given province. A trend towards increasing seed utilization is clear in most provinces. For example, in Alberta, New Brunswick, Ontario, and Quebec the amount used in 1984 already exceeded the quantity that Morgenstern (1979) forecast would be utilized in 1987. It would not be unreasonable to expect total seed utilization in 1988 to be double the amount in 1978.

Seed utilization statistics for the eight most important conifer species in 1980, 1982, and 1984 (Table 2) show that demand for jack pine and white and black spruce seed greatly exceeded that of other species. Also, the utilization of white spruce seed increased more than any other species during the period 1980 to 1984.

Smyth and Brownwright (1986) reported from a Canadian survey that nursery and greenhouse production centres shipped about 450 million trees in 1984. There were 119 production centres at that time; 43 reared seedlings as bare-root stock and 101 reared containerized seedlings. During the period 1979-1986, shipments of bare-root seedlings remained fairly constant at about 210 million. In contrast, the production of containerized stock increased substantially during the same period, from 100 million to about 570 million. If this trend is maintained, more than twice as many containerized seedlings as bare-root seedlings will be produced in 1987. This shift in production methods has also been well described for the Maritimes by Hallett and Lanteigre (1986, pers. comm.); container stock accounted for only 17% of seedling shipments in 1975, but increased to 62% in 1979, 77% in 1985, and 82% in 1986.

Table 1. Number of conifer seed utilized in Canada in 1978, '80, '82, '84 and expected in 1987 by province.

	Millions of Seed				
Province	1978[1]	1980[2]	1982[3]	1984[4]	Forecast 1987[1]
BC & Yukon	325	320	347	384	550
Alta.	1607	1137	895	2804	2406
Sask.	203	136	192	65	1349
Man.	91	16	26	54	97
Ont.	1255	3230	2416	2753	1500
Que.	170	623	918	1172	420
N.B.	92	123	350	--[5]	288
N.S.	23	77	80	--	107
P.E.I.	1	0.3	9	--	3
Nfld.	381	41	56	49	611
Totals	4148	5705	5291	7281	7331

[1] Morgenstern 1979

[2] Janas, Haddon 1984

[3] Janas 1985

[4] Wang, unpublished data

[5] Data not available

Table 2. Relative consumption of eight most important forestry species in Canada in 1980, '82, and '84, based on total viable seed used in reforestation

	% of total seed utilized		
Species	1980[1]	1982[2]	1984[3]
Jack pine	59.2	43.0	26.1
White spruce	21.8	23.6	44.9
Black spruce	5.6	17.8	18.4
Lodgepole pine	3.8	4.2	6.9
Interior spruce	2.8	1.6	1.2
Douglas-fir	1.1	1.0	1.2
White pine	1.0	1.5	1.1
Engelmann spruce	--	1.0	1.0

[1] Janas, Haddon 1984

[2] Janas 1985

[3] Wang, unpublished data, does not include information from New Brunswick, Nova Scotia, or Prince Edward Island

Forest stands are also being regenerated using direct seeding techniques on both prepared and unprepared sites (Cayford 1974). Seed is hand broadcast or sown in prepared spots and may be distributed aerially from fixed-wing aircraft or helicopters. The quantities of seed used in this way are large, especially for species such as jack pine (Yeatman 1985). More than 4 billion seed were used for direct seeding in 1984 (Table 3) and this type of utilization appears to be on the increase. British Columbia, Alberta, Manitoba, and Ontario have direct-seeded large areas in the past (Morgenstern 1979) and are continuing to do so.

The amount of seed utilized in seedling production and direct seeding varies greatly among Canada's eight major tree species. In 1984, for example, more than 70% of the jack pine, lodgepole pine, and white spruce seed was used in direct seeding (Table 3). Bare-root seedling production was the major use for white pine and black spruce seed while the majority of Douglas-fir and British Columbia spruce seed was used for containerized stock production. Year-to-year changes in utilization are determined in part by increasing planting stock production targets and in part by the shift from bare-root to containerized seedling production. However, the total amount utilized is influenced primarily by the quantity used in direct seeding.

The method of artificial regeneration determines the quantity of seed required per unit area. Wang (1985) and Skeates (1985) have indicated that the high cost and low availability of improved seed demand that the efficiency of seed utilization be improved. Seed requirements using various regeneration methods differ greatly:

Method	Seed per seedling
container seeding	1-5
bare-root seeding	2-10
seed spot	5-15
hand broadcasting	5-30
aerial seeding	50-150

Clearly, containerized seedling production results in the most efficient use of seed. However, seed utilization efficiency is not the only factor requiring consideration. For example, it may be less expensive to reforest extensive burned areas by direct seeding suitable species than to plant seedlings.

Seed Sources

At present, agencies responsible for obtaining seed recognize the following major sources of seed (listed in order of decreasing seed quality):

1. seed orchards (SO)
2. seed production areas (SPA)
3. seed collection areas (SCA)
4. controlled general collections
5. uncontrolled general collections

Table 3. Methods of seed utilization and seed inventory in Canada[1] by species in 1984 (Wang, unpublished data).

Method	Douglas-fir	Jack Pine	Lodgepole Pine	White Pine	White Spruce	Black Spruce	Interior Spruce	Engelmann Spruce	Total (All species)
	% of total seed utilized by species								
Direct seeding	26	73	81	6	78	17	--	--	62.3
Bare-root seedlings	23	9	6	83	21	50	11	24	23.5
Containerized seedlings	51	18	13	11	1	33	89	76	14.2
Seed utilized (millions)	84.8	1,536.0	500.6	82.9	3,211.6	1,337.7	85.3	57.2	6,896.0
Seed inventory (millions)	929.2	8,377.8	3,528.3	1,103.90	23,528.7	5,008.1	2,535.5	1,771.1	46,781.7

[1] Data from New Brunswick, Nova Scotia, and Prince Edward Island are not included.

Uncontrolled general collections presently represent a limited source of seed because the quality of trees that cones are collected from is frequently poor. Controlled general collections are a major seed procurement source, but the level of 'control' implemented varies across the country. All provinces have identified seed zones based either on ecological or forest site classification systems or on a system of administrative districts (Konishi 1979, Skeates 1979). In some provinces, the level of control executed is a simple designation of the seed zone in which collections are made. In other provinces, controls are more restrictive and may identify specific stands for collection. In British Columbia, for example, cones are only collected from stands inspected by the B.C. Forest Service or forest industry personnel who characterize each stand's quality as a seed source (Konishi 1979).

Seed collection areas (SCA) are uniform tree stands of average or above-average quality at a harvestable age. These stands are reserved from cutting until a good seed year occurs, at which time harvesting is coordinated with cone collection. The seed obtained is frequently better-than-average quality but is not genetically improved.

Seed production areas (SPA) are usually young stands of above average quality which have been upgraded by thinning and the removal of undesirable trees. They may also be treated (ex. fertilized) for early and abundant seed production (Lamontagne 1979). Natural stands or plantations can be selected as SPAs. They should be accessible, on reasonably level terrain, and young enough to respond to thinning with increased crown development. The merits and potential problems of SPAs in plantations established with planting stock grown from seed of superior parents (i.e. plus trees, super seedlings, seed production areas, or seed orchards) have been discussed for decades. Decisions to develop plantations into SPAs must be made on an individual basis, with consideration given to such factors as the size of the genetic base represented in the plantation, its origin, and the intended use of the seed. Once established, SPAs provide convenient, accessible locations for cone collection during moderate or better crop years. Seed is obtained in larger quantities per tree than would be possible in untreated stands.

A Canadian survey conducted in 1978 indicated that in some provinces only a few SCAs and SPAs had been established (Lamontagne 1979) and seed was obtained mainly from uncontrolled collections. At that time most provinces indicated a desire to complete their SCA and SPA development programs within 15 to 20 years and there is ample evidence to indicate considerable progress. In Ontario, for example, the total area of SCAs for black spruce and white pine increased greatly between 1978 and 1986 (Table 4). More significantly the size of SPAs was increased greatly for white and jack pine and white and black spruce. A drop in the size of SCAs for jack pine and white spruce was compensated for by the substantial increase in SPAs.

Table 4. Changes in the size (ha) of SCA and SPAs in Ontario.

	SCA		SPA	
Species	1978[1]	1986[2]	1978	1986
white pine	40	348	11	595
red pine	0	0	72	0
jack pine	19,950	12,059	12	1,193
white spruce	320	119	115	202
black spruce	120	557	54	180

[1] 1978 data from Lamontagne (1979).
[2] 1986 data (Rauter, pers. comm.).

It is our impression that progress with SCA programs has proceeded successfully in most of Canada. For example, much of Ontario's jack pine seed is now collected from SCAs. Most of the province developed in a way similar to that reported for the Gogama District by Oldford et al. (1979). Here, there was a progressive switch from nearly 100% of seed being obtained from general collections prior to 1975 to 72% in 1978, and 80% in 1980. By 1983 nearly 100% of seed was obtained from SCAs.

Progress in the establishment and development of SPAs has been equally successful. In New Brunswick, for example, J.D. Irving Ltd. was the pioneer in establishing SCAs and SPAs, and has maintained its lead for more than a decade. Unfortunately, an initial enthusiasm for establishing and utilizing SPAs may wane with the passage of time in localized areas. This situation can be illustrated with another example from Ontario. Starr (1979) described the productivity of two white spruce seed production areas of 1.2 ha and 6.5 ha in Clute township, Cochrane district established in 1964 and 1970, respectively. Cone crop forecasts for the Cochrane district indicated a heavy cone crop in 1964, 1970, 1974, and 1982 and a medium crop in 1976 but no SPA cones were collected in 1964 or 1976 (Table 5). In some suitable years, collections were made in one but not both SPAs. Clearly failure to collect cones when they were available represents a missed opportunity for obtaining quality seed. Also, in 1982, many more cones than those tabulated (Table 5) were collected from the SPAs. Unfortunately, however, their uniqueness was lost because they were bulked with other collections made at the same time.

The history of seed orchard (SO) development in Canada, though slow to start, has been impressive. Pollard (1982) reported there were 364 ha of orchards in Canada in 1981. At the 20th CTIA meeting Morgenstern (1986) reported that the total area of orchards had expanded to 618 ha and that planned orchard development should bring the total area to more than 2000 ha by 1988. Given the current high interest in SO establishment across the country (Coles and Simpson 1987), we expect that this goal will be met.

Table 5. Cochrane district cone crop forecast, and cone collection[1] for Clute Township white spruce seed production areas.

Year	Crop forecast	SPA	Cones collected (hl)
1962 and 63	light		-
1964	heavy	No. 1 (established)	none
1965	light		-
1966 to 69	failures		-
1970	heavy	No. 1	none
		No. 2 (established)	18.27[2]
1971	light	No. 1	1.05
		No. 2	0.80
1972 and 73	failures		-
1974	heavy	No. 1	none
		No. 2	8.86
1975	light	No. 2	-
1976	medium	No. 1	none
		No. 2	none
1977	failure		-
1978	light		-
1979 to 81	failures		-
1980	failure		-
1981	failure		-
1982	heavy	No. 1	2.40
		No. 2	1.50
1983	failure		-
1984	light		-
1985 and 86	failures		

[1] Update of data from Starr (1979) collected by Skeates (unpublished) and Swaile (Angus Seed Plant records)

[2] Collection from felled trees during establishment of SPA not the retained SPA trees.

The progress in SO development has not been uniform in all provinces. British Columbia has been the leader. It began selecting superior trees and the development of SOs more than 25 years ago. Since then BCs continued effort has been fruitful. Their first orchard was established in 1963, but today British Columbia has 52 orchards with a total area of 164 ha (Hanson 1985). British Columbia, Ontario, Quebec, and the Maritimes Provinces all expect to utilize orchard seed for most of their planting stock production by the year 2000 (Ontario's plans are outlined in Silviculture, Vol. 1(3) p. 34; Maritimes, by Simpson 1985; Quebec, by Lamontagne 1987). Ontario has been doing tree breeding work as long as British Columbia but their SO development program, until recently, had progressed more slowly. In the other provinces, SO developments have been more recent. Quebec, for example, had no SOs until 1978 but their establishment record and plans are spectacular (Lamontagne 1987):

1980 with 80 ha established
1984 with 129 ha established
1985 with 300 ha established
1986 with 531 ha established
1988 with 840 ha planned
1990 with 247 ha planned

On the other hand, Alberta has only recently initiated a SO establishment program.

Seed Production

Surveys have determined that about 7.6, 14.3, and 12.3 billions of viable conifer seed were produced from cones collected, respectively, in 1980, 1982, and 1984 (Table 6). The proportions of total seed collected from general collections, SCAs plus SPAs, and SOs remained about the same throughout this period and did not show any progressive shifting from general collections to sources of better quality seed. Similarly, seed obtained from the various sources for each of Canada's eight major tree species also fail to show a consistent year to year trend towards increased collections from better sources (Table 7). These national statistics are discouraging but, on closer examination, the higher production of seed from SCAs or SPAs and SOs in 1982, and the fact that cones were collected from these sources for seven of the eight species listed, indicates progress towards making better quality seed available. Also, it is a fact that most of the orchards established in Canada are presently too young to produce seed but some will become productive within a few years. Across the country there are striking examples of specific efforts that indicate the soon-to-be significant availability of SO seed. British Columbia, for example, is already producing most of its requirements for coastal Douglas-fir seed in SOs (Hanson 1985). In the Maritimes, 80% of the jack pine seed sown in 1987 by the J.D. Irving Co. was produced in their own seedling SOs (Greg Adams, pers. comm.).

Table 6. Sources of seed production as a percentage of total seed produced in Canada (Janas and Haddon 1984, Janas 1985, Wang, unpublished data, Morgenstern 1979).

	1980	1982	1984[2]	1987 (forecast)[1]
Total seed produced (billions)	7.623	14.340	12.271	7.3 (required)
General collections (%)	90.5	88.6	94.7	42
SCA plus SPA (%)	9.3	11.3	5.2	55
SO (%)	0.2	0.1	<0.1	3

[1] Data from New Brunswick, Nova Scotia, and Prince Edward Island not available

Future Supply of Seed

Although the production of orchard seed will increase each year it could be as many as 20 years before production is adequate to meet requirements for improved seed throughout all of Canada. This long a period is necessary because many parts of Canada's productive forests are not yet represented in seed orchards and the increasing demand for seed is expected to continue. Orchard managers will endeavour to initiate production as soon as possible, and implement crop enhancement procedures such as fertilization, GA 4/7 hormonal treatments, and irrigation to maximize seed production. Although this will speed up availability of improved seed, many problems that influence production are yet to be solved. These facts bring up the question: What seed sources will we utilize until sufficient quantities of orchard seed become available?

Forestry authorities have a choice of strategies they can follow until improved seed from SOs becomes available. Perhaps the easiest, but probably the most unsatisfactory, course of action would be to cut back on our reforestation efforts and simply wait until SO seed is available. Forestry authorities could also decrease seed collection efforts, and utilize only the seed available from SOs and from existing inventories of seed in storage. If the seed in storage were utilized at the 1984 rate, and current inventories equalled those available at the beginning of 1985, there would be enough seed available for four to 30 years depending on tree species (Table 1). The only possible merit of these two courses of action is that funds normally allocated to seed collection programs might be reallocated to intensify efforts in SO development. Another alternative is to continue procuring seed as we are doing at present. However, this alternative is unsatisfactory because we should be using seed of the highest possible quality. We must intensify our efforts to obtain more seed from SCAs and SPAs and less seed from general collections. These sources are currently not providing anywhere near the 55% of seed requirements that was forecast for 1987 (Morgenstern 1979) (Table 6).

The productivity of existing SCAs and SPAs can be greatly improved by increasing seed collection efforts during good crop years. Administrative arrangements and flexible funding required to permit increased cone collection efforts when crops are available are essential. Existing cone crops can be protected from cone and seed insect pests. Insect pests and the damage they cause are well illustrated by Hedlin et al. (1980) and control procedures are being developed for those pests causing significant damage (Fogal and Lopushanski 1984, 1985, Fogal et al. 1981, Amirault 1986). Seed orchard management procedures that stimulate crown development and enhance cone production, such as treatments utilizing fertilizers and irrigation, might also be employed in SPAs.

Increasing the proportion of seed obtained from SCAs and SPAs further requires that additional areas be identified and developed for various species in some parts of the country. In these cases an expanded investment in SCAs and SPAs during the next 10 years would be money well spent. It is wrong for anyone to think that the need for seed from SCAs and SPAs will end abruptly when present orchards become fully productive.

Table 7. Productivity of major species by source (Janas and Haddon 1984, Janas 1985, Wang, unpublished data).

Species	1980			1982			1984[1]		
	Total seed (x 10^6)	% from SCA + SPA	% from SO	Total seed (x 10^6)	% from SCA + SPA	% from SO	Total seed (x 10^6)	% from SCA + SPA	% from SO
Douglas-fir	102.7	-	2.8	207.3	22	9.6	19.7	-	0.1
Jack pine	2,049.6	17	-	3,181.8	37	-	2,019.5	31	-
Lodgepole pine	460.3	-	-	863.5	1	<0.1	471.1	-	-
White pine	47.7	-	1.3	134.1	-	-	131.5	1	5.1
Black spruce	1,221.3	9	0.2	2,291.0	3	-	572.8	<1	<0.1
White spruce	2,203.9	12	0.3	5,608.2	2	<0.1	8,884.1	<1	<0.1
Interior spruce	96.3	-	-	324.3	44	-	26.6	-	-
Engelmann spruce	-	-	-	369.3	24	-	7.4	-	-
Totals	5,358.0	13	0.2	13,092.7	14	0.2	12,132.7	5	<0.1

[1] Data from New Brunswick, Nova Scotia, and Prince Edward Island not available

These SOs will be able to supply a large portion of the seed required for planting stock production, it is unlikely that SO production will be sufficient to fill the large requirements of direct seeding. If direct seeding remains a viable reforestation procedure, the seed requirements should come from SCAs and SPAs.

Effort is also required to maximize returns from the limited quantity of improved orchard seed currently available. Most reforestation agencies will likely direct this seed towards containerized seedling production, where utilization efficiency is much higher than it is with bare-root stock production or direct seeding. To make even more efficient use of small quantities of improved seed, agencies may also consider the use of clonal vegetative propagation techniques. In Nova Scotia, for example, 3.0 million black spruce rooted cuttings are produced annually for operational planting on the Cape Breton Highlands. The rooted cutting program was originally based on 180 000 donor plants raised from a small seed collection made on the Highlands, where collecting cones had been hampered by several years of heavy budworm feeding. This rooted cutting program now also includes parent material produced by controlled breeding among unrelated individual trees (Mullin and Frame 1986).

Assuming that the best quality seed from advanced generation orchards will always be in limited supply, there will be strong incentive to pursue other new methods of clonal planting stock production. Developing research in biotechnology is of particular interest (Thorpe and Hasnain 1987). Advances in cell and tissue culture now offer the realistic hope that labour intensive and expensive production of clonal planting stock as rooted cuttings will one day be replaced by highly efficient cell culture procedures. Conventional nursery production and seed orchard mangement will remain with us for some time to come. Over time, however, we expect continuous modifications as technological advances change the economics of forest renewal.

CONCLUSIONS

SPAs and SCAs can provide large quantities of average or better quality, source-identified seed. If adequate programs of SPA establishment and maintenance and SCA identification and utilization are encouraged, there should be no shortages of quality seed for Canadian reforestation programs. Once SO production becomes adequate to meet the requirements for planting stock production, seed from SCAs and SPAs can continue to provide the large volumes of seed required for direct seeding. As even more seed from SOs becomes available, it should displace seed from SCAs, so that the highest quality seed available is utilized in our reforestation programs.

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SELECTION AND MATING STRATEGIES IN SECOND GENERATION BREEDING POPULATIONS OF CONIFER TREE IMPROVEMENT PROGRAMS

J.I. Klein

Northern Forestry Centre
Canadian Forestry Service
Edmonton, Alberta, CANADA T6H 3S5

ABSTRACT

Strategies for selection and mating in second generation breeding populations are compared for eight tree improvement programs in Canada, southeastern U.S.A., Australia, and New Zealand. Second generation breeding populations in these programs consist of progenies produced by open pollination, factorial mating, or partial diallel mating. Selection of parents for the next generation is always based on family means and performance of individual progeny trees, but with various ways of allocating selections among and within families. All programs plan to use controlled pollination for mating of second generation selections. Mating designs for producing third generation breeding populations include polycross, single-pair mating, and various forms of partial diallel. Sublining, and use of polycross progenies for genetic assessment of pedigreed breeding population progenies, are proposed in combination with various selection and mating procedures. Advantages of the various strategies used or proposed in these programs are discussed, but there is no advocacy of any particular strategy over others.

RÉSUMÉ

On compare les stratégies de sélection et de croisement des populations améliorées de la deuxième génération appliquées dans huit programmes d'amélioration des arbres au Canada, au sud-est des États-Unis, en Australie et en Nouvelle-Zélande. Les populations améliorées de la deuxième génération dans ces programmes comprennent des descendants produits par la pollinisation libre, le croisement factoriel ou le croisement diallèle partiel. La sélection des parents de la génération suivante est toujours fondée sur les moyennes des familles et le rendement de chacun des arbres descendants, mais en tenant compte des diverses manières d'attribuer les sélections entre et chez les familles. On prévoit d'utiliser, dans tous les programmes, la pollinisation contrôlée pour le croisement des sélections de la deuxième génération. Les dispositifs de croisement pour la production des populations améliorées de la troisième génération comprennent les tests de polycross, le croisement de couples uniques et diverses formes de croisement diallèle partiel. On propose d'utiliser le sous-repiquage et les descendances polycross pour l'évaluation génétique des descendants de populations améliorées et pédigrées de concert avec les diverses méthodes de sélection et de croisement. On expose les avantages des diverses stratégies utilisées ou proposées dans ces programmes, mais on ne favorise aucune stratégie en particulier par rapport aux autres.

INTRODUCTION

Canadian tree breeding programs are progressing beyond the initial phases of identifying phenotypically superior wild parent trees and assembling genetic material from those trees in tree breeding plantations. Reports on measurement of plantations for genetic assessment of breeding populations of important species at ages up to 10 years have appeared in these Proceedings. We will soon begin to see reports of selection and mating activities in these plantations directed toward establishment of third generation breeding populations. Informed planning is vital in developing and refining plans for the procedures to be used in selecting the most genetically superior trees from the appropriate number of families and in mating those selected trees to produce the following generation (Burdon et al. 1971, Cotterill 1986). It is the purpose of this paper to describe options and offer some opinions so as to stimulate discussion to promote the quality of planning for selection and mating in established conifer tree breeding programs in Canada.

The subject area on which I was invited to speak was second generation selection and breeding. There is some variation in the counting of generations in tree breeding programs, but the second generation is usually understood to consist of the progenies of the original parent trees selected to start a program, whether those progenies are open-pollinated or control-pollinated.

The approach chosen was to describe and comment on a sample of actual programs. Programs described in this paper have in common the existence of a report in my collection or in the library of the Northern Forestry Centre, in English. Reporting of these programs furthermore includes progress or explicit planning at least as far as selection in the second generation population.

DESCRIPTION OF BREEDING PROGRAMS

Eight programs will be used as the basis for discussion. They are identified in Table 1 as to general location, species, literature references used here, and number of parent trees in the first generation breeding population. To save space in the following tables, an identifying label is introduced in the last column for each program. For the North Carolina and New Zealand programs the references are from earlier than 1980, and there may be more recent reports describing further progress. The New Brunswick black spruce (*Picea mariana* (Mill.) B.S.P.) and Nova Scotia white spruce (*P. glauca* (Moench) Voss) programs were selected from a larger number described in the cited paper (Fowler 1986) and are representative of the others.

Composition, selection, and breeding of the second generation breeding populations are in Tables 2, 3, and 4, respectively.

Populations

The eight programs fall into two equal groups having open-pollinated (op) progenies and control-pollinated (cp) progenies for the second generation breeding population. Op progenies are used for New Zealand and Australia radiata pine (*Pinus radiata* D. Don), New Brunswick black spruce, and Manitoba jack pine (*Pinus banksiana* Lamb.). Cp progenies are used for the North Carolina State University - Industry and Western Gulf (based in Texas) loblolly pine (*Pinus taeda* L.), British Columbia coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and Nova Scotia white spruce programs. A factorial mating design was used for the North Carolina program, whereas cp progenies for the other programs were produced by partial diallel mating designs.

For the purposes of this paper we are interested in the second generation populations primarily as a source of parents for the next breeding population, but these progenies are also used as a source of information and materials for seed orchards.

Assessment and selection

Generally, the progenies of the second generation populations were planted in replicated designs at several locations, where they were measured or scored at intervals of several years for traits targeted for genetic improvement. Selection of parents for the next breeding cycle uses mean performance of the families and individual performance of progeny trees in various ways.

For radiata pine in South Australia and black spruce in New Brunswick, the 400 best open-pollinated families were or will be selected, to be followed by selection of the one best tree in each of these families. In New Zealand, 80 radiata pine progeny trees were selected in total from a population of 800 families. Number and distribution of selections were not specified for the Manitoba jack pine program, but that program differs from the others in planning to use parent clones as the selected genotype in some selected families. Selection of a parent clone (backward selection) rather than a progeny individual (forward selection) can be genetically advantageous for the best of the superior families (Lindgren 1986), but it does complicate counting of generations.

Another unusual option proposed for the Manitoba jack pine program is the use of a short-term polycross progeny test of candidate parents to complete second generation selection. This procedure would delay completion of selection by about five years (assuming a test duration of three years), but could enhance the efficiency of selecting for within-family genetic effects.

For the North Carolina loblolly pine program, each member of the cooperative is expected to select about 100 individuals in their plantations of factorial progenies. The distribution of selected individuals among and within half-sib and full-sib families was not described in the cited report. Pedigrees of all selected trees will be examined to exclude from breeding use excess individuals of over-represented parents. Additional parents for breeding of the next generation are to be selected, 100 per member, from unimproved plantations.

Mid-parent general combining ability (gca) is used as the criterion for family selection in the other three programs with control-pollinated breeding populations. Each parent's gca is calculated from the means of its full-sib partial diallel progenies for the Western Gulf loblolly pine and coastal B.C. Douglas-fir programs. The Nova Scotia white spruce program, still at an early stage of implementation, plans to estimate gca for each parent from polycross progenies which would be grown concurrently with the breeding population, but would not be part of the breeding population. In the Western Gulf program, about 5500 full-sib families would be ranked for gca and the top 15% of the families would be screened for superior individuals. Only one tree would be retained as a selection for any full-sib family and plantation, but this constraint would not prevent selection of half-sibs, or of full-sibs from different plantations. Twenty-five second-generation trees are to be selected in each of 115 breeding groups (sublines). Parent trees are being selected within full-sib families having the highest gca's in the B.C. Douglas-fir program, but proportions and constraints were not specified. The four best trees are to be selected within each of the 100 best families for Nova Scotia white spruce.

Mating of Second Generation Selections

There is no apparent correlation between type of progeny used for the second generation population and type of mating plan for producing the third generation populations. Partial diallel mating is planned for two cp programs (North Carolina loblolly pine and Nova Scotia white spruce) and one op program (New Zealand radiata pine), and is mentioned as an option in a second op program (Manitoba jack pine). Polycross mating to produce assessment progenies separate from the breeding populations would be grown concurrently with the breeding population families for the Western Gulf loblolly pine (cp), Nova Scotia white spruce (cp) and New Brunswick black spruce (op) programs. These procedures are analogous to the polycross option proposed for the Manitoba jack pine program, but here the genetic assessment of second-generation selections occurs after they are mated. Delay is avoided at the cost of breeding extra families. Polycross pollen would be obtained from trees unrelated to those in the breeding population. Where polycross mating would be used to assess parents or families, the mating design for producing breeding population families would be single-pair (black spruce and jack pine) or partial diallel (loblolly pine and white spruce).

Mating within sublines would be initiated at this stage for the Australia radiata pine and the two spruce programs, in addition to the Western Gulf program, which used sublines for the second generation breeding population. Because the use of sublining controls the effects of inbreeding, pedigree maintenance was judged unnecessary for the Australia radiata pine program, which consequently plans to use polycross mating to produce the third generation breeding population.

DISCUSSION

Selection

Second-generation breeding populations provide genetic quality assessment on their first-generation parent trees and on families and trees of the second generation, ortets or parents for second generation seed orchards or cutting orchards, and parents of the third generation breeding populations. The main concern of this paper is with the last output. With this frame of reference, the goal of selection in the second generation breeding population is to find the right balance between immediate genetic gain and the potential for continuation of genetic gain for many subsequent generations of recurrent selection and breeding.

Both immediate and long-term gain are enhanced by procedures which promote accurate expression of genetic quality of families and individual trees in the breeding population. Other selection parameters may favor one benefit at the expense of the other. Given a fixed number of families and trees in a breeding population, immediate gain can be increased by reducing the proportion of individuals selected, while the potential for gain in later generations is increased by increasing effective population size after selection. It is easy to agree that the selected portion of the breeding population should include representation from an adequate number of families. Despite the considerable body of opinion and theoretical calculation on this subject, a breeder informed by this literature will still rely partly on instinct in deciding on constraints to multiple selections within families. Some tree breeding strategists place more emphasis on the additional early gains from selecting individuals in proportion to family merit, than on enforcing diversity through equal representation of selected families (Lindgren 1986).

Of the programs described here which specified the distribution of selected trees among families, the Western Gulf loblolly pine program apparently plans to place most weight on individual performance within qualifying families. In the South Australia radiata pine, New Brunswick black spruce, and Nova Scotia white spruce programs, there is a fixed intensity of family selection, followed by selection of one or four individuals in each selected family. Gain can be increased beyond either of these approaches by combined family-plus-individual index selection (Cotterill 1986), but losses in respect to effective population size could result if there are no constraints on trees per family. Apart from selecting more trees from the most superior families, combined selection can increase gain by allowing large individual differences to outweigh small family differences near the truncation point, at no cost to effective population size.

Breeding

Sublining is the first step in grouping selected trees for mating in the Western Gulf loblolly pine program and both eastern Canada programs. The North Carolina and Australia programs mention sublining as a possibility. One reason offered for the use of sublining is to avoid inbreeding in the production population in a later generation when relatedness in the breeding population can not be avoided. For the Manitoba jack pine program, an inbreeding coefficient of 0.03125 per generation was calculated, beginning in the seventh generation. That level of inbreeding would occur if each generation's breeding population were the result of single-pair mating involving the two best trees of all 16 families of the previous generation. From the sixth generations onward, all trees would be 16-fold fourth cousins. The effect of inbreeding at the calculated rate is not known. There are other advantages of sublining, including convenient division of work among cooperators, avoidance of the need for pedigree maintenance, possible gain from heterosis when slightly inbred but unrelated genotypes mate in a production seed orchard, and the absence of any serious disadvantage. In regard to the last reason, the radiata pine program for part of New Zealand and the Manitoba jack pine program have population sizes that would be marginal for sublining.

All of the mating designs planned for second generation breeding in these programs offer unique advantages. Assuming appropriate selection procedures in the third generation, partial diallel mating offers maximum gain per generation over a wide range of heritability values, while single-pair mating has the advantage in gain per decade and polycross mating is most efficient in terms of effort (Cotterill 1986). As programs approach and reach the point at which assessment data support selection and breeding, the programs' breeding strategists need to inform themselves of the most realistic theoretical calculations in the literature and heritability results from their plantations, and consult their cooperators to determine feasibility of alternative mating designs. The ideal mating design for second-generation breeding depends as much on the circumstances of a particular program as it does on the attributes of alternative designs.

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Table 1. A sample of tree breeding programs reporting second generation procedures.

Organization or place	Species	Reference	Parent trees	Program code
North Carolina State University - Industry Cooperative	loblolly pine	Talbert 1979	30/member	NC lp
Western Gulf Forest Tree Improvement Program	loblolly pine	Lowe and van Buijtenen 1987	3200	WG lp
British Columbia coastal region	Douglas-fir	Heaman 1982, 1986	372	BC Df
Nova Scotia	white spruce	Fowler 1986	468	NS ws
Part of New Zealand	radiata pine	Burdon and Shelbourne 1971, Burdon et al. 1977	800	NZ rp
Part of South Australia	radiata pine	Cotterill 1984, 1986	700	AUS rp
New Brunswick	black spruce	Fowler 1986	1200	NB bs
Eastern Manitoba	jack pine	Klein 1982	209	MAN jp

Table 2. Composition of second generation breeding populations of sample programs.

Program	Parent trees	Composition of second generation breeding population
NC lp	30/member	Factorial progenies from 4-5 pollen parents mated to all other parents
WG lp	3200	Disconnected partial diallel progenies in 115 sublines
BC Df	372	6-parent blocked disconnected partial diallel progenies
NS ws	468	Partial diallel progenies
NZ rp	800	Open-pollinated progenies
AUS rp	700	Open-pollinated progenies
NB bs	1200	Open-pollinated progenies
MAN jp	209	Open-pollinated progenies plus parent clones

Table 3. Selection in second generation breeding populations of sample programs.

Program	Parent trees	Second generation selection
NC lp	30/member	100-200 progeny trees/region, 1 tree/family except best families; 500-1000 new selections/region from unimproved populations
WG lp	3200	Exclude families not in top 15% for mid-parent gca. Select 25 progeny trees/subline
BC Df	372	Best progeny trees in families with best mid-parent gca
NS ws	468	Best 4 trees in each of 100 families with best mid-parent gca from polycross test
NZ rp	800	80 progeny trees
AUS rp	700	Best progeny tree in each of 400 best families
NB bs	1200	Best progeny tree in each 400 best families
MAN jp	209	Parent clone or best progeny tree of best families, possibly polycross test of candidate selections

Table 4. Mating designs for breeding of second generation selections to produce third generation breeding populations of sample programs

Program	Parent trees	Second generation selection
NC lp	30/member	6-parent blocked disconnected diallel
WG lp	3200	Partial diallel for breeding population, unrelated polycross for gca test
BC Df	372	Not reported
NS ws	468	Partial diallel in 20 sublines, unrelated polycross for gca test
NZ rp	800	5-parent blocked disconnected partial diallel
AUS rp	700	Polycross in sublines
NB bs	1200	10 single pair matings in each of 20 sublines, unrelated polycross for gca test
MAN jp	209	Partial diallel or single pair mating if polycross test done

CONFÉRENCIERS VOLONTAIRES

VOLUNTARY PAPERS

A CLINAL MODEL OF TREE IMPROVEMENT

Roy R. Silen

U.S. Forest Service
Pacific N.W. Research Station
3200 Jefferson Way
Corvallis, OR 97331 U.S.A.

The words UNIFORM and CLINAL are used here to describe contrasting tree improvement models. As with agricultural crops, an assumption for both models is that improved seed should genetically match the effective growing season length of the planting site within 10 days to assure full biomass productivity. The present worldwide tree improvement model is termed UNIFORM because it delineates a zone of growing season or other climatic uniformity from which parent trees are chosen. Cuttings or seedling families from them are arranged randomly in seed orchards to produce a single seed mixture suitable for planting anywhere in the zone. Since growing seasons change by only about 10 days per degree of latitude in the gentle topography of eastern United States or western Europe, an area of over 2-million acres can often be found to meet this criterion in a one-degree by one-degree square of latitude and longitude. The summer rains and high humidities permit growth through all the summer months.

The same criterion is difficult to meet in the complex mountainous Pacific Northwest. Each 400-foot rise in elevation shortens growing seasons about 10 days (from Hopkins' Law). Summer drought regularly truncates growing seasons. Wilting point commonly occurs by late July, but can occur as early as mid-June in rain shadows of mountains. Snow packs on north exposures delay onset of spring growth. Frosts can occur any month in valleys that drain cold air from mountains. Uniform zones of the above criterion become ridiculously small in such environments forcing most western tree improvement programs to accept larger growing-season variation than 10 days in most commercial breeding zones.

The CLINAL model is proposed in order to meet the 10-day growing-season matchup of seed with planting site. The key to the model is the concept of producing many seed mixes in the same orchard. The simplest example would be for one aspect on a uniformly sloping mountainside. Parent trees could be placed in the orchard in essentially elevational bands and seed could be collected from the band that matched the planting-site elevation on the mountainside. For complex mountainous topography more typical of the West, an elegant solution is a maplike placement of clones or families in orchards to match their corresponding coordinate location in the forest. Such orchards have been established at the J.E. Schroeder orchard complex in the Williamette Valley owned by the State of Oregon. With abundant tested parents, essentially local seed of correct growing season length and desired gains could be provided for each planting site. But even with limited parent numbers in a multiple arrangement of an orchard, seed collection can start with such selected local parentage. Any additional seed needs would come from the band of adjacent parents in the orchard having equivalent growing season length.

Growing season maps are inadequate in the Pacific Northwest due to poor weather station coverage. Fortunately, the relationship between inherent growth rate and the effective growing season appears strong. Maps of inherent growth rate are being prepared for much of the Douglas-fir region from progeny data of commercial tree improvement programs that now test more than 25,000 parent trees. These maps appear to sample the long-term climatic variation better than is possible with present weather records. Where sample cold or drought gradients are documented, the matchup appears essentially templatelike on several maps.

The computations of genetic gains in growth rate are more complex for the CLINAL than the UNIFORM model. For each specific seed mix the parental population size is limited to the parents in the orchard that contribute to it.

A PILOT COOPERATIVE ACCELERATED TREE IMPROVEMENT PROGRAMME

T.J.B. Boyle[1], J.F. Coles[2], and J.V. Hood[3]

[1] Canadian Forestry Service, Petawawa Nat. For. Inst., Chalk River, Ont.
[2] Ontario Tree Improvement Council, Guelph, Ont.
[3] Ontario Ministry of Natural Resources, Queen's Park, Toronto, Ont.

Black spruce (Picea mariana [Mill.] B.S.P.) is the major commercial species in the Northern Region of Ontario's Ministry of Natural Resources, with a projected annual planting stock requirement of 50 million plants. To meet this requirement, operational tree improvement activities have recently been initiated involving programmes of plus-tree selection, testing, and seed orchard establishment, together with some vegetative propagation of seedlings from plus-tree selections.

In view of the very significant economic benefits from black spruce improvement in the region, a co-operative accelerated programme has been developed to take advantage of two favorable factors. These are the ease of rooting of juvenile cuttings and the existence of sexually mature research tests in the region. The accelerated programme is divided into three sections: exploitation of immediate gain, breeding for additional gain, and research and re-testing.

Reserved seed from parents of the best families in each of five breeding zones, as well as open- and control-pollinated seed of those proven superior parents that have survived are being vegetatively propagated to obtain immediate genetic gain. The magnitude of this gain, for open-pollinated seed, is estimated to be of the order of 3% as a result of using tested material plus 1% due to previous "super-seedling" selection. In addition, the exploitation of non-additive genetic variation through vegetative propagation will increase these figures by up to a half. Assortative mating in producing control-pollinated seed will produce further gain.

Breeding work currently involves assortative single-pair matings and polycrosses on surviving first-generation parents. Selection of first-generation progeny by breeding zone is underway and will lead to the establishment of a clonal breeding archive for further controlled breeding. Newly generated material will be tested in close-spaced and accelerated tests. By inclusion of previously-tested material, selection in these tests will be more efficient than would otherwise be possible and will effectively reduce generation times.

Similar opportunities may exist in many cases and should be carefully investigated in order to avoid duplication of effort and to provide lasting genetic benefits.

BREEDING BARRIERS AND INTERSPECIFIC HYBRIDIZATION IN WILLOWS

A. Mosseler

University of Toronto
Faculty of Forestry
Toronto, Ontario
M5S 1A1

Some of the post-mating reproductive barriers restricting artificial interspecific hybridization between *S. amygdaloides* Anderss., *S. bebbiana* Sarg., *S. discolor* Muhl., *S. eriocephala* Michx., *S. exigua* Nutt., *S. lucida* Muhl., *S. pellita* Anderss., and *S. petiolaris* Smith were investigated. Interspecific pollen-pistil incongruity represented a major internal barrier to cross fertilization. Post-zygotic inviability barriers included seed incompatibility, seedling inviability, inferior growth in most hybrid progeny, and reduced levels of fertility.

Although many hybrid combinations produced high proportions of inferior progeny, some interspecific hybrids performed very well and often demonstrated unique combinations of morphological traits potentially useful in breeding for improvements in biomass quality. Preliminary tests on the breeding parents revealed significant differences between species and between clones within species for wood specific gravity and moisture content. Strong differences in coppice form, rootability, and disease susceptibilities have also been observed among these species. Selected clones from both intra- and interspecific crosses are being tested at several locations in Ontario.

GENETIC VARIATION IN SUGAR MAPLE (ACER SACCHARUM MARSH.)

D.J. Perry and P. Knowles

School of Forestry
Lakehead University
Thunder Bay, Ontario
P7B 5E1

Genetic variation in 5 populations of sugar maple (*Acer saccharum* Marsh.) from northern Ontario was analyzed electrophoretically for 7 enzymes encoded by 11 structural loci. On average, populations were polymorphic at 38.2% of the loci with 1.95 alleles per locus. Analysis of F-statistics revealed an overall 0.2% deficiency of heterozygotes relative to Hardy-Weinberg expectations. Although only 3% of the genetic variability was among populations, there was significant allelic heterogeneity at all polymorphic loci. Genetic distance between populations ranged from 0.0205 to 0.0612, but no relationship with geographic distance was observed. The outcrossing rate appeared to be high ($\bar{t} = 1.006$) and there is some evidence of population sub-structuring. Indications are that there is considerable genetic variation and population differentiation within the species, however, more thorough investigation of the mating system and structure of populations is necessary for a better understanding of the genetics of sugar maple.

EARLY FLOWERING AND SEED PRODUCTION OF WHITE SPRUCE GRAFTS AT FOUR LOCATIONS IN ALBERTA AND BRITISH COLUMBIA

N.K. Dhir and J.M. Schilf

Alberta Forest Service
Edmonton, Alberta
T5K 2G6

Flowering and seed production of white spruce grafts of northern Alberta origin was studied at four locations in Alberta and British Columbia. Grafts were made in 1979 and the plantings were established in 1981 and 1982. The outplantings contained 3 or 4 ramets of 12-17 clones. Outplanting locations were: (1) a 'local' northern Alberta site at Grande Prairie, (2) a central Alberta site at Smoky Lake, (3) a southern Alberta site at Brooks, and (4) a south-western British Columbia site at Vernon.

Percentage of flowering ramets, combined over all locations, steadily increased from 23 percent in 1983 to 91 percent in 1987. Flowering at the warmer southern locations commenced at an earlier age and was more consistent. Male and female flowering commenced at same age and increased in a similar manner during the study period. Male flowering was more abundant at southern locations than at northern locations (38.6, 42.6, 16.8 and 6.1 strobili/ramet/year at Vernon, Brooks, Smoky Lake and Grande Prairie respectively). In comparison, location effects on average female strobilus production were relatively small, with better production realized at the northern locations (15.9, 16.5, 18.5 and 20.0 strobili/ramet/year at Vernon, Brooks, Smoky Lake and Grande Prairie respectively). Data indicated strong location x clone interactions and differences among clones were found to be statistically significant ($p \leq .05$) only for earliness of flowering.

Average number of sound seeds produced per cone fluctuated widely among locations (0-18.3 seeds/cone) for two study years (1985 and 1986). A detailed analysis was carried out on 1986 seed crop at Grande Prairie. It showed that clonal means for average number of seeds/cone varied from 1.8-11.6 (mean = 6.2) and clonal differences were statistically significant. It was estimated that 20 percent of the clones produced approximately 40 percent of the seed crop. Germination quality of seed was very good (mean germination = 87.3%, range of clonal means = 75-95%), but clonal differnces were not significant.

PRELIMINARY ASSESSMENT OF JAPANESE LARCH (*LARIX LEPTOLEPIS* [SIEB. AND ZUCC.] GORD.) ESTABLISHED FOR LAND RACE DEVELOPMENT IN EASTERN ONTARIO

F. Schnekenburger[1], and C.W. Yeatman[2]

[1] Ontario Ministry of Natural Resources, Box 605, Brockville, Ontario, K6V 5Y8
[2] Canadian Forestry Service, Petawawa National Forestry Institute, Chalk River, Ontario, K0J 1J0

In 1980, two Japanese larch land race trials were established in in eastern Ontario by the Ministry of Natural Resources in conjunction with Petawawa National Forestry Institute. The trials contain 79 seedlots collected from plantations, orchards and natural stands in Japan, Europe, and North America. Assessments were made in 1983 and 1986 for height and diameter growth. Mean seedlot heights in 1986 ranged from approximately 3.0 to 5.5 m; mean diameters ranged from approximately 3.0 to 8.0 cm. Variation among seedlots in terms of 1983 survival and 1986 growth was significant. Over half of the total variation was accounted for by individuals within seedlots. The trial will be progressively rogued and thinned over time and the best trees of the best families will be selected and used for seed collection.

GENETIC CHARACTERIZATION OF SOME WHITE PINE SPECIES AND THEIR INTERSPECIFIC HYBRIDS BY ISOZYMES

E. Chagala

University of Toronto, Faculty of Forestry
Toronto, Ontario
M5S 1A1

One of the ways of achieving genetic improvement in Eastern white pine (*Pinus strobus* L.), especially in breeding for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch ex. Rabenh), has been through interspecific hybridization. Of importance for interspecific hybridization with Eastern white pine were, in Ontario studies, *Pinus griffithii* McClelland, *Pinus monticola* Lamb. and *Pinus peuce* Griseb. The genetic structure and variation in these species is being studied by electrophoresis. A fuller knowledge of the evolutionary trend, inheritance patterns and a better utilization of the interspecific hybrids is hoped to be realized by these studies. In addition to allozyme frequencies and variation studies, gene markers for species, hybrids and clones will be described and related to traits such as blister rust resistance. Results to date show that species differ in their allelic frequencies, number of polymorphic loci and heterozygosity for some enzyme systems. The study is in progress.

REPLICATING BLACK SPRUCE PROGENY TESTS OVER TIME MAY NOT BE NECESSARY

J.D. Simpson

Canadian Forestry Service - Maritimes
Box 4000
Fredericton, N.B.
E3B TP7

The New Brunswick Tree Improvement Council is about to embark upon a second generation improvement program for black spruce utilizing trees selected in first generation family tests. Since these family tests were not replicated in each of several years the question arises whether there is a need to replicate future control-pollinated progeny tests over several years. Data from seven black spruce families, planted in fifteen family tests in three years by Council members were analyzed. Correlations for five-year height of different planting years were significant, ranging from .80 to .90, indicating family performance was consistent from year to year. Height and survival were generally, not significantly correlated with precipitation and growing degree days. Based on these results, there is no need to consider replicating black spruce progeny tests over several years.

WHITE PINE BREEDING AND FLOWER INDUCTION STUDIES IN EASTERN ONTARIO

F. Schnekenburger

Ontario Ministry of Natural Resources
Box 605
Brockville, Ontario
K6V 5Y8

White pine (*Pinus strobus* L.) is considered the highest priority species for regeneration and tree improvement effort in eastern Ontario. Large scale breeding efforts are currently underway to support a clonal production seed orchard program and clonal forestry efforts. The breeding program and testing program are outlined in detail. Breeding is conducted in a large breeding hall facility in Kemptville, Ontario. Flower induction and accelerated growth studies have been established to support the white pine breeding program in the breeding hall. Preliminary results of these studies will be profiled, as well as details on planned flower induction studies.

IN VITRO CULTURE OF EXPLANTS FROM MATURE BLACK SPRUCE*

R.H. Ho, and A.Y. Raj

Ontario Tree Improvement and Forest Biomass Institute
Ministry of Natural Resources
Maple, Ontario
L0J 1E0

* Supported by a research grant from the Canadian-Ontario Forest Research and Development Agreement

Fifteen black spruce (Picea mariana [Mill.] B.S.P.) trees, which were growing in the arboreta at Maple, Ontario in three age classes of 15, 25 and 30 years were sampled. Buds were collected from the lower crown of each tree one day before incubation. Bud scales were removed and the embryonic shoots were excised above or below the crown. The embryonic shoots with or without crown were soaked in a solution for an hour before they were sterilized with a bleach solution followed by rinsing with sterile distilled water. The embryonic shoots were then incubated on a medium supplemented with cytokinin and auxin. The media used were Murashige and Skoog (MS), Gresshoff and Doy (GD), Quoirin and Lepoivre (LP), Schenk and Hildebrandt (SH), Woody plant (WPM), and Loblolly pine (LM) medium. There were nine shoots per petri dish and at least six dishes were made for each medium per incubation. The culture was placed in a growth room with a 16-hour photoperiod (60 $\mu E/m^2/s$) and temperature at 25° during the day and 15°C at night.

The soaking solution contained one or two antioxidants including 1% ascorbic acid, 1% activated charcoal, a combination of ascorbic acid and activated charcoal, 1% butylated hydroxytoluene (BHT), 1% butylated hydroxyanisole (BHA), 10% ethanol, and autoclaved distilled water. After the soaking and sterilization, the embryonic shoots with or without crown were incubated on MS medium supplemented with 10 mg/L benzylaminopurine (BAP) and 0.2 mg/L naphthaleneacetic acid (NAA) for three months. It was found that embryonic shoots with crown grew best at 95% after soaking in 1% activated charcoal or autoclaved distilled water. None survived the BHA pretreatment.

Different media with supplements of various combinations of cytokinins and auxins were used for culture in order to define a suitable combination. Four media (MS, ½ MS, GD, ½ GD) were factored with four concentrations of BAP (0, 2, 5 and 10 mg/L) when NAA was at 0.01 mg/L, and the four media with five concentrations of NAA (0, 0.01, 0.1, 1 and 5 mg/L) when BAP was at 2 mg/L. Embryonic shoots with crown were cultured on each medium for three months. The results showed that 5 mg/L BAP was best when each of ½ MS, GD, and ½ GD contained 0.01 mg/L NAA, and 0.1 mg/L NAA was best when each of the three media contained 2 mg/L BAP. Little or no growth was found when media was supplemented with 2 mg/l BAP and 5 mg/L NAA.

Media were screened for the induction of adventitious shoots on the embryonic shoots with crown. Monthly incubation from August to February was carried out to define the best time for sampling for culture. Each of the media outlined above was supplemented with 5 mg/L BAP and 0.2 mg/L NAA. The embryonic shoots were cultured on each medium for one to eight weeks before they were transferred onto the medium without any supplement. It appeared that GD and LP medium were most suitable for black spruce explants and four weeks of treatment were optimal. The best induction frequency was 56% when embryonic shoots from a 15-year-old tree were incubated on GD medium in December followed by those from a 25-year-old tree on LP medium in December at 48%. Embryonic shoots which were collected in October from a 30-year-old tree produced adventitious shoots from 16% of the explants. Adventitious shoots were regenerated either from axillary area of leaf primordia on an embryonic shoot or through the redifferentiation of the leaf primoridia. The redifferentiation occurred occasionally and the shoots were not as vigorous as those from the axillary area. Many adventitious shoots had elongated to about 5 mm in height and could be excised for rooting to produce plantlets.

TOWARDS COMMERCIALIZATION OF DOUGLAS FIR MICROPROPAGATION

Gina H. Mohammed, and Kamlesh R. Patel

AgriForest Technologies Ltd.
2330 Enterprise Way
P.O. Box 178
Kelowna, B.C.
V1Y 7N5

Tissue culture micropropagation of forest trees is widely recognized as potentially having an important role to play in reforestation and tree improvement programmes. To evaluate the economic feasibility of large scale micropropagation of Douglas-fir, experiments were carried out for the selection of an ideal explant and the optimum requirements for each stage of _in vitro_ plantlet production, i.e. induction of the shoot buds, development and multiplication of these buds, rooting of the shoots and hardening of the plantlets.

Embryos and seedling parts obtained from 3 open-pollinated and 5 half-sib seed lots were used as explants. The explants were inoculated on various media containing N^6-benzyladenine (BA), however maximum bud induction was obtained on the medium for conifer morphogenesis (MCM). Embryonic explants were preferred over the seedling parts because of their consistent and synchronous response to culture as well as low contamination rate. Exposure of the explants for 17 days to the BA containing medium was optimum for bud induction. Up to 100% of the explants produced on average of 8.6 primordia per embryo. Development of the bud primordia was achieved by transferring the explants to hormone-free medium. Shoot elongation was enhanced by inclusion of 0.05-0.1% conifer derived activated charcoal in Gelrite or agar solidified basal medium. Depending upon the seed lot, up to 92.9% of the primordia elongated to 1.0-1.5 cm in height by the 28th week in culture. These shoots were then remultiplied by axillary bud breaking or rooting. For remultiplication the shoots were cultured for 4 weeks on the medium containing low levels of BA (0.5-1.0 μM) followed by one or two subcultures on basal medium. Shoots from the elite clones can be bulked up in large numbers through axillary branching to obtain the fullest potential from tree improvement programmes. Rooting was achieved in 84.6% shoots after 5-10 weeks on peat: perlite mixture containing reduced MCM salts and 2.7 μM naphthalene acetic acid. The plantlets were successfully transferred to the greenhouse and acclimatized. Investigation on the field performance of the tissue cultured Douglas-fir plantlets is underway in collaboration with scientists from the B.C. Ministry of Forests.

SEED ORCHARDS IN ROMANIA

V. Enescu

Forest Research and Management Institute
Sos.Stefanesti 128
Bucuresti II
Romania

Research on breeding by means of selection and crossing of plus trees and the establishment of seed orchards was started in early 1959. In 1961 the first seed orchard programme was established and put to practice. It stipulated the establishment of 200 ha of introduced hardwood and softwood seed orchards.

Since 1976 the tasks of seed orchard establishment have been stipulated in the National Program for the preservation and development of the forest for the period 1976-2010, approved by Law no. 2/1976.

Seedling seed orchards (Eastern white pine, black pine, Scots pine, Norway spruce, red oak) were established, but clonal seed orchards were more numerous.

The seedling seed orchards were thinned, selecting the most valuable families, and the most valuable individual trees within these families, calculating the main genetic parameters. Up to now mainly first generation seed orchards have been established and, to a smaller extent, 1.5 generation seed orchards (in Scots pine, black pine, Eastern white pine and red oak).

Up to the end of 1986, over 950 ha of seed orchards have been established, almost half of them consisting of indigenous hardwood species. Each seed orchard is made up of several clones, at least 40 for small orchards and about 100 clones in most cases. The clones sometimes came from plus trees selected in the same provenance region, but most of the seed orchards were established from clones obtained from plus trees selected in several provenance regions.

Hardwood and softwood seed orchards of adequate ages are producing commercial crops. In softwood trees, the highest crop was obtained in larch (33 kg/ha pure seed) and in hardwood trees, in common oak (_Quercus robur_ L.) oak (900 kg/ha) and black locust (40 kg/ha).

THE SEASONAL CHANGES IN THE PHYSICAL AND CHEMICAL COMPOSITION OF CONES OF BLACK SPRUCE, PICEA MARIANA (MILL.) B.S.P.

Y.H. Prévost

Service canadien des forêts
C.P. 3800
Ste-Foy, Québec
G1V 4C7

Seasonal growth of female buds, flowers and cones of black spruce, *Picea mariana* [Mill] B.S.P., was divided into four phases based on changes in length and diameter. Flowers were formed by the end of the first phase when the length and diameter of buds had increased to 50% and 44% respectively, of the maximum seasonal values of the mature cones. Water content and dry weight of flowers had reached 12% and 4% respectively, of their maximum. By the end of the second phase, length increased by another 40% and diameter by 46%, which correlated respectively with 62% and 33% increases in water and dry weight. During the early part of phase three, length and diameter of cones increased by an additional 3% and 5% respectively which related to increases of 26% in water content and 39% in dry weight. In the latter part of phase three, length and diameter increased by 7% and 5% respectively while dry weight increased by 24% and water decreased by 12%. Length and diameter of cones decreased slightly in the fourth phase during which time water and dry weight decreased respectively by 65% and 7% to the end of the season. Seasonal changes in water, dry weight, sugars and crude fats per cone could be explained by the synthesis and hydrolysis of indigestible fibres, and their hydrophobic and hydrophilic properties. Concentrations of N, P and K, appeared to be positively correlated with metabolic activities in the cone tissues.

SYLLEPSIS: POSSIBLE RAMIFICATIONS FOR SELECTION OF TAMARACK TREES

G.R. Powell

Department of Forest Resources
University of New Brunswick
Bag Service No. 44555
Fredericton, N.B.
E3B 6C2

Syllepsis, the production of leafy branches on a long shoot while that long shoot is itself elongating, is a common feature of young tamarack (Larix laricina [Du Roi] K. Koch) trees. It is expressed in both short shoots and long shoots. Up to 90% of the leaders of young trees in plantations carry long-shoot syllepsis, but quantities vary by tree and by year. Syllepsis modifies the crown structure. It increases the number of shoots directly and indirectly over time. Hence, the amount of foliage and the potential for cone production are also increased. Tree height and stem diameter growth are increased in proportion to the amounts of syllepsis. Thus, although branchiness is increased by syllepsis, other features associated with syllepsis appear desirable. Furthermore, the fact that some trees in given populations remain non-long-shoot sylleptic, suggests that syllepsis may be a heritable trait.

Numbers of sylleptic long shoots produced in three successive years on leaders of families of tamarack trees provided narrow-sense, individual-tree, and full-sib-family heritability estimates which ranged from 0.25 to 0.76 and 0.64 to 0.98, respectively. This indicates a genetic basis for selecting for syllepsis. Syllepsis does not often occur on older trees, but trees selected for their superior growth may have grown well because of syllepsis early in life. Progeny should not be scored without regard to syllepsis.

THE USE OF DEVELOPMENTAL STUDIES IN REFINING DORMANCY INDUCTION TREATMENTS FOR CONTAINERIZED SEEDLINGS: A DOUGLAS-FIR EXAMPLE

Joanne E. MacDonald, and John N. Owens

Department of Biology
University of Victoria
Victoria, B.C.
V8W 2Y2

On the basis of growth chamber experiments on dormancy induction treatments, a greenhouse experiment was conducted to determine: (1) the minimum time that seedlings must be in short days (SD) to maximize shoot preformation and cold-hardiness acquisition, and (2) the maximum time that seedlings can be in SD before photosynthesis is reduced to the extent that stem diameter (caliper) and root dry weight are affected. Coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) seedlings, from a mid-elevation seed source, were grown under operational conditions at the MacMillan Bloedel nursery. In early July, dormancy induction treatments began. They were: (1) SD with drought-stress, (2) SD without drought-stress, and (3) drought stress. In a dark-out house, an 8 hr SD was used. Seedlings either were or were not placed under a 2-week drought-stress cycle. Styroblocks were removed after 3, 4, 5, and 6 weeks in SD, and placed in another house. In a house without dark-out, an operational control was set up. Seedlings were given a 2-week drought-stress cycle. The experimental design was a randomized split-block.

Seedlings were sampled just prior to the start of treatments. After treatments began, seedlings were sampled weekly until mid-August, then bi-weekly until mid-October. Shoot tips of seedlings were dissected, fixed, and processed for examination under the light and scanning electron microscope. On seedlings sampled in mid-September and mid-October, caliper and shoot and root dry weights were determined. In October, additional seedlings were sampled for cold-hardiness assessment.

Just prior to treatments, seedlings in the two houses were in different developmental stages. Seedlings in the dark-out house were initiating leaves, whereas seedlings in the house without dark-out were initiating budscales. After 1 week of SD, seedlings had switched from leaf to budscale initiation. After 1 week of drought-stress, seedlings were still initiating budscales. Subsequently, on seedlings grown under SD: (1) the transition from budscale to leaf initiation was completed more quickly, (2) leaf initiation during shoot preformation was accelerated, (3) larger preformed shoots were formed, and (4) calipers were larger than on drought-stressed seedlings. Drought-stress in combination with SD delayed the developmental response and reduced caliper. SD enhanced cold-hardiness acquisition, drought-stressing hindered it. Drought-stress in combination with SD improved acquisition of the initial stage of cold- hardiness, but hindered acquisition of the second stage of cold-hardiness.

GENETIC CONTROL OF SOME WOOD PROPERTIES IN BLACK SPRUCE

T.J.B. Boyle[1], J.J. Balatinecz[2], and P.M. McCaw[2]

[1] Canadian Forestry Service, Petawawa Nat. For. Inst., Chalk River, Ont.
[2] Faculty of Forestry, University of Toronto, Toronto, Ont.

Wood properties were assessed on 15-year-old trees from a complete 7 x 7 diallel cross of black spruce (*Picea mariana* [Mill.] B.S.P.), planted in four locations at Petawawa Nat. For. Inst. A total of 152 basal diameter cores were taken from trees planted in replicated experiments at three locations and the average core specific gravity was measured, together with diameter growth rate and pilodyn penetration. At a fourth location, 185 trees were destructively sampled, disks taken from up to four heights in the stem and the specific gravity, tracheid length and diameter, wood to bark ratio, total disk diameter, diameter growth rate, and moisture content measured. Heights at ages 14 or 15 were also available for all trees.

Correlations of specific gravity with both height and diameter were equal to -0.35 and were highly significant. The correlation between specific gravity and pilodyn penetration was also highly significant and equal to -0.36. In contrast to height growth, variance components due to locations and replications within locations for specific gravity amounted to only a small proportion of the total variation (17%), indicating a high heritability for this trait. Also, specific gravity, together with tracheid diameter and moisture content, varied only slightly with height in the tree. However, tracheid length decreased markedly with height above 1.3 m.

The relative magnitude of variance components due to general combining ability (gca) and specific combining ability (sca) give an indication of the importance of additive, compared with non-additive, genetic variation. This, in turn, determines the suitability of a purely additive strategy for genetic improvement of the trait. Inheritance of specific gravity appeared to be predominantly additive, although the gca/sca ratio declined markedly with height in the tree. Similarly, gca accounted for virtually all the genetic variation observed for tracheid length and diameter and moisture content. In contrast, for total disk diameter, diameter growth rate and wood to bark ratio, sca (non-additive) was the dominant form of genetic variation. In addition to greatly suppressed growth rates, selfs had a significantly higher specific gravity than outcrosses of the same size.

CIRCUMFERENTIAL VARIABILITY IN THE RELATIVE DENSITY OF DISCS TAKEN AT BREAST HEIGHT OF LODGEPOLE PINE PLUS TREES

J.S. Gonzalez

Canada Forintek Corp.
6620 N.W. Marine Drive
Vancouver, B.C.
V6T 1X2

The circumferential variability of relative density in breast-height discs of lodgepole pine was examined. The objective was to assess the number of increment cores needed to get a good estimate of the mean relative density at breast height. The study was done on nine discs taken from plus trees from three different provenances as part of the selection made in a B.C. tree improvement program. The discs were carefully selected to obtain materials with the minimum amount of defects which are known to affect density. The discs were sawn into wedges. Each wedge was further divided into an inner half: the portion from the pith to half the radial distance to the bark, and an outer half: the portion from half the radial distance to the bark. The mean density and variance for each disc based on the inner and outer half portions were calculated. Using the disc variance as population variance, 95 percent confidence limits for the mean were calculated based on one, two, three, and four samples randomly selected from the disc. Also, the 95 percent confidence limits for the mean were calculated on the basis of two samples taken at 180 degrees to each other. Results showed that in most discs, the 95 percent confidence limits for two samples taken 180 degrees apart were lower or equal to the 95 percent confidence limits obtained for three or four samples randomly selected.

RELATIONSHIP BETWEEN BREAST HEIGHT AND WHOLE-STEM DENSITY IN 50-YEAR OLD COASTAL DOUGLAS-FIR

J.S. Gonzalez

Canada Forintek Corp.
6620 N.W. Marine Drive
Vancouver, B.C.
V6T 1X2

Density data collected by the Douglas-fir task force were analyzed to examine the feasibility of predicting whole-stem density of mature trees from breast-height density of the juvenile trees. Test trees consisted of sixty dominant trees from medium to good sites on Vancouver Island. Using the X-ray densitometry method, density was determined at breast height, at 20, 40, 60, and 80% of total height. Whole-stem density was estimated as the weighted mean of the densities at the different height levels sampled. Density at breast height of the juvenile trees at ages 5, 10, 15, and 20 years were regressed against whole-stem density of the same trees at ages 35, 40, 45, and 50 years. Comparisons of the coefficients of determination of the various regressions and the 95 percent prediction intervals about the regression lines were made. Trees were ranked according to breast-height density of juvenile trees and whole-stem density of mature trees at age 50. All the relationships were linear and statistically significant at the 5% level. However, the variation in the density of the mature trees accounted for by the density of the juvenile trees was much lower at ages 5 and 10 than at ages 15 and 20 years. Breast-height density at age 15 and 20 were approximately equal as good predictors of whole-stem density at age 50 and for ranking trees according to density for purposes of selection. it is concluded that progeny testing of coastal Douglas-fir for wood density evaluation could be done at age 15.

SOME BASIC PROPERTIES OF WOOD OF EUROPEAN LARCH FROM FAST-GROWTH PLANTATIONS IN EASTERN CANADA

C.T. Keith

Forintek Canada Corp.
800 Montreal Rd.
Ottawa, Ontario
K1G 3Z5

A number of basic wood quality characteristics were evaluated in ten trees of European larch; five from each of two rapidly growing plantations in eastern Ontario and western Quebec. The plantations were approximately 30 years old (Ontario) and 25 years old (Quebec). The samples were collected in the form of cross-sectional discs cut at breast height and at 20%, 40%, 60% and 80% of total tree height for each stem. Characteristics evaluated included growth rate and relative density, longitudinal shrinkage, contents of alcohol-benzene extractives and Klason lignin. An attempt was made to identify differences between juvenile and mature wood and to assess distribution within the stems.

Radial and longitudinal patterns of variation in wood characteristics are illustrated graphically. Extractive contents were found to be more closely related to the extent of heartwood and sapwood in the stems than to juvenile wood-mature wood differences. Relative density was generally lower at the centre of the stem than in the mature outer wood but the change was usually gradual with no recognizable boundary between juvenile and mature wood zones. The most useful characteristic for delimiting the juvenile wood zone appeared to be longitudinal shrinkage. On this basis, the transition is placed at about 15 rings out from the pith at breast height. Ranking of individual trees for high or low density wood was judged to be reliable at about 7 years of age at this height level.

THE ESTABLISHMENT OF END-PRODUCT BASED CRITERIA FOR THE EVALUATION OF WOOD DENSITY IN TREE IMPROVEMENT PROGRAMS

Jean Cook

In this paper a method for evaluating young trees for wood density in terms of the density found in currently produced end-products is outlined. The relationship between the breast-height density of young trees and their whole-stem density at a projected age of harvest is used to establish limits that can be applied in the selection of suitable trees at a young age which will ensure satisfactory product quality when the trees are harvested. The method is illustrated using data from 16-year-old coastal Douglas-fir trees and the distribution of relative density found in visually graded Douglas-fir structural lumber.

TABLE OF CONTENTS/TABLE DES MATIÈRES

Page

BUSINESS MEETING - MINUTES

MEMBERS' REPORTS

Newfoundland - Department of Forest Resources and Lands

Newfoundland - Canadian Forestry Service

New Brunswick - Department of Natural Resources and Energy

New Brunswick - Canadian Forestry Service

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New Brunswick - Université de Moncton

New Brunswick - University of New Brunswick

Québec - Ministère de l'Énergie et des Ressources

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Ontario - Ministry of Natural Resources

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Ontario - University of Toronto

Saskatchewan - Forestry Branch, Parks, Recreation and Culture

Saskatchewan - Agriculture Canada, Tree Nursery Division, PFRA

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GUELPH ONTARIO
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CROOK GREGORY
C I P INC
R R NO 2
CALUMET
QUEBEC
J0V 1B0

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SERV CANADIEN DES FORETS
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C P 3800
SAINTE FOY QUEBEC
G1V 4C7

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WINNIPEG
MANITOBA
R3N 1Z4

FARMER R E DR
SCHOOL OF FORESTRY
LAKEHEAD UNIVERSITY
THUNDER BAY
ONTARIO
P7B 5E1

FRAME HOWARD M
DEPT OF LANDS &FORESTS
TREE BREEDING CENTRE
P O BOX 190
DEBERT N S
B0M 1G0

GAMBLES ROBERT DR
RESEARCH ASSOCIATE
FACULTY OF FORESTRY
UNIVERSITY OF TORONO
203 COLLEGE ST
TORONTO ONT M5S 1A1

GLEN WILLIAM M
DEPT ENERGY AND FORESTRY
P O BOX 2000
CHARLOTTETOWN
P E I
C1A 7N8

GONZALES J
FORINTEK CANADA LTD
6620 NW MARINE DR
VANCOUVER
B C
V6T 1X2

GORDON A G DR
ONT TREE IMPROV AND
FOREST BIOMASS INST
P O BOX 490
SAULT ST MARIE ONT
P6A 5M7

GREENWOOD RICHARD
ONT MINISTRY NATURAL RES
60 WILSON AVENUE
TIMMINS ONT
P4N 2S7

HALL J P DR
NEWFOUNDLAND FOR RES CNTR
CANADIAN FORESTRY SERVICE
P O BOX 6028
ST JOHN'S
NEWFOUNDLAND A1L 5X8

HARRISON CHARLES M DR
DEPT FOR RES & LANDS
P O BOX 2006
CORNER BROOK
NEWFOUNDLAND
A2H 6J8

HEAMAN J C
MIN OF FORESTS & LANDS
1450 GOVERNMENT ST
VICTORIA B C
V8W 3E7

HEWSON CLARE A
MINISTRY OF FORESTS
3401 RESERVIOR RD
VERNON
BRITISH COLUMBIA
V1B 2C7

HO R H DR
ONT TREE IMPROV AND
FOREST BIOMASS INST
MIN NATURAL RESOURCES
MAPLE ONTARIO
L0J 1E0

HOOD J V
FOREST RESOURCES BRANCH
MIN NATURAL RESOURCES
PARLIAMENT BUILDINGS
TORONTO ONTARIO
M7A 1W3

HUNT RICHARD S
CANADIAN FORESTRY SERVICE
506 WEST BURNSIDE RD
VICTORIA
B C
V8Z 1M5

INNES M R
ASST MAN OF FORESTRY &
RESOURCE PLANNING
ABITIBI-PRICE INC
TORONTO-DOMINION CENTRE
TORONTO ONT M5K 1B3

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VERNON RESEARCH CENTRE
BC MIN OF FORESTS & LANDS
3401 RESERVOIR RD
VERNON B C
V1B 2C7

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FORINTEK CANADA CORP
800 MONTREAL ROAD
OTTAWA
ONTARIO
K1G 3Z5

KELLOGG R M DR
FORINTEK CANADA CORP
6620 N W MARINE DR
VANCOUVER
BRITISH COLUMBIA
V6T 1X2

KHALIL M A K DR
17 DIEFENBAKER ST
ST JOHN'S
NEWFOUNDLAND
A1A 2M2

KISS G K
VERNON RESEARCH STATION
BC MIN OF FORESTS & LANDS
3401 RESERVOIR RD
VERNON B C
V1B 2C7

KLEIN J I DR
NORTHERN FOREST RES CNTR
CANADIAN FORESTRY SERVICE
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EDMONTON ALTA
T6H 3S5

KNOWLES MARGARET H DR
SCHOOL OF FORESTRY
LAKEHEAD UNIVERSITY
THUNDER BAY ONT
P7B 5E1

KONISHI J
SILVICULTURE BRANCH
BC MIN OF FORESTS & LANDS
1450 GOVERNMENT ST
VICTORIA B C
V8W 3E7

KORELUS VLAD J R P F
C I P INC
TAHSIS PACIFIC REGION
8067 EAST SAANICH RD
R R # 1 SAANICHTON
BRITISH COLUMBIA VOS 1MO

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SAINTE-FOY PQ G1P 3W8

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FRASER INC
27 RUE RICE
EDMUNSTON
N B
E3V 1S9

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UNIV OF BRITISH COLUMBIA
VANCOUVER B C
V6T 1W5

LOO-DINKINS J DR
FACULTY OF FORESTRY
UNIV OF BRITISH COLUMBIA
VANCOUVER B C
V6T 1W5

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CHALK RIVER
ONTARIO
K0J 1J0

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FORT FRANCES
ONTARIO
P9A 3M3

MAZE JACK DR
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VANCOUVER B C
V6T 2B1

MEAGHER M D DR
PACIFIC FOR RES CNTR
CANADIAN FORESTRY SERVICE
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VICTORIA B C
V8Z 1M5

MOORE W
GREAT LAKES FOR PROD LTD
P O BOX 430
THUNDER BAY
ONTARIO
P7C 4N3

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UNIVERSITY NEW BRUNSWICK
FACULTY OF FORESTRY
BAG NO 44555
FREDERICTON NB
E3B 6C2

MULLIN T J
DEPT OF LANDS & FORESTS
TREE BREEDING CENTRE
P O BOX 190
DEBERT NOVA SCOTIA
B0M 1G0

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WINNIPEG
MANITOBA
R3C 1A6

NICKS BRYAN
E B EDDY FORESTRY LTD
STATION ROAD
ESPANOLA
ONTARIO
P0P 1C0

NIEMAN THOMAS C
CANADIAN FORESTY SERVICE
PETAWAWA NAT FOR INSTIT
CHALK RIVER
ONTARIO
K0J 1J0

PANDILA M DR
SASKATCHEWAN DEPT OF PARK
& RENEWABLE RESOURCES
FORESTRY DIVISION PO 3003
PRINCE ALBERT
SASKATCHEWAN S6V 6G1

PARK Y S DR
CFS- MARITIMES
P O BOX 4000
FREDERICTON
N B
E3B 5P7

PARKER WILLIAM H DR
SCHOOL OF FORESTRY
LAKEHEAD UNIVERSITY
THUNDER BAY ONTARIO
P7B 5E1

PARROT LOUIS DR
FACULTE DE FORESTERIE
ET DE GEODESIE
UNIVERSITE LAVAL
QUEBEC QUEBEC
G1K 7P4

PHARIS R P DR
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UNIVERSITY OF CALGARY
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CALGARY
ALBERTA T2N 1N4

PITEL JACK A
CANADIAN FORESTRY SERVICE
PETAWAWA NAT FOR INST
CHALK RIVER
ONTARIO
K0J 1J0

POLIQUIN J DR
FACULTE DE FORESTERIE
ET DE GEODESIE
UNIVERSITE LAVAL
QUEBEC QUEBEC
G1K 7P4

POWELL G R DR
UNIV OF NEW BRUNSWICK
DEPT OF FOREST RESOURCES
BAG SERVICE NO 44555
FREDERICTON N B
E3B 6C2

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SERVICE DE L'AMELIORATION
DES ARBES
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SAINTE-FOT PQ G1P 3W8

RAJORA O P DR
DEPT OF FOREST SCIENCE
UNIVERSITY OF ALBERTA
720 CHEM MIN ENG BLDG
EDMONTON AB
T6G 2G6

RAITANEN W E
MIN NATURAL RESOURCES
60 WILSON AVE
TIMMINS ONTARIO
P4N 2S7

RAUTER ROSE MARIE
FOREST RESOURCES BRANCH
ONTARIO MINISTRY
OF NATURAL RESOURCES
PARLIAMENT BUILDINGS
TORONTO ONTARIO M7A 1W3

ROBERTSON S
NEWFOUNDLAND FOR RES CNTR
CANADIAN FORESTRY SERVICE
P O BOX 6028
ST JOHN'S
NEWFOUNDLAND A1C 5X8

RODDY DIANE M
PRINCE ALBERT PULPWOOD LT
P O BOX 1720
PRINCE ALBERT SASK
S6V 5T3

ROGERS D
TIMMINS REGIONAL OFFICE
O M N R AND ENERGY
60 WILSON ST
TIMMINS ONTARIO
P4N 2S7

ROSS S D DR
BC MIN OF FORESTS & LANDS
RESEARCH LABORATORY
1320 GLYN ROAD
VICTORIA B C
V8Z 3A6

RUSSELL JOHN
BC MIN OF FORESTS & LANDS
COWICHAN LAKE RES STATION
P O BOX 335
MESACHIE LAKE B C
VOR 2NO

SAVIDGE R DR
DEPT OF FOREST RESOURCES
UNIV OF NEW BRUNSWICK
BAG NO 44555
FREDERICTON N B
E3B 6C2

SCHILF JANET M
ALBERTA FOREST SERVICE
ENERGY & NATURAL RESOUCE
P O BOX 634
SMOKEY LAKE ALBERTA
TOA 3CO

SCHOEN D
MCGILL UNIVERSITY
DEPT OF BIOLOGY
1205 AVE DR PENFIELD
MONTREAL PQ
H3A 1B1

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PETAWAWA NAT FOR INST
CHALK RIVER
ONTARIO
KOJ 1JO

SCHROEDER WILLIAM
PFRA TREE NURSERY
INDIAN HEAD
SASKATCHEWAN
SOG 2KO

SEABROOK R
GREAT LAKES FOR PROD LTD
P O BOX 430
THUNDER BAY
ONTARIO
P7C 4N3

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FORESTRY BRANCH
DEPT OF NATURAL RESOURCES
300-530 KENASTON BLVD
WINNIPEG MAN
L3N 1Z4

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MARITIMES FORESTRY CENTRE
CANADIAN FORESTRY SERVICE
P O BOX 4000
FREDERICTON N B
E3B 5P7

SKEATES D A
ONT TREE IMPROV AND
FOREST BIOMASS INST
MIN NATURAL RESOURCES
MAPLE ONTARIO
LOJ 1EO

SMITH R F
MARITIMES FORESTRY CENTRE
CANADIAN FORESTRY SERVICE
P O BOX 4000 FREDERICTON
N B
E3B 5P7

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MIN ENERGIE ET RESSOURCES
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DES ARBRES
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SAINTE-FOY PQ G1P 3W8

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UNIVERSITY OF B C
207-2357 MAIN MALL
VANCOUVER B C
V6T 1W5

THORPE TREVOR DR
DEPT OF BIOLOGY
UNIVERSITY OF CALGARY
CALGARY
ALBERTA
T2N 1N4

TOSH KATHLEEN
DEPT OF NATURAL RESOURCES
PROVINCIAL FOREST NURSERY
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NEW BRUNSWICK
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MIN ENERGIE ET RESSOURCES
SERVICE DE L'AMELIORATION
DES ARBRES
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SAINTE-FOY PQ G1P 3W8

VILLENEUVE M
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SERVICE DE L'AMELIORATION
DES ARBES
2700 RUE EINSTEIN
SAINTE-FOT PQ G1P 3W8

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CHALK RIVER ONT
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J D IRVING CO LTD
SUSSEX TREE NURSERY
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PROCTER & GAMBLE LTD
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POSTAL BAG 1020
GRANDE PRAIRIE
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NORTHN FOREST DEVEL GROUP
TIMMINS REGIONAL OFFICE
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P4N 2S7

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AGROGEN
BIOTECHNOLOGIES INC
AGROGEN BLDG
520 W 6TH AVE
VANCOUVER B C V5Z 4H5

WEBBER J E DR
RESEARCH LABORATORY
BC MIN OF FORESTS & LANDS
1320 GLYN ROAD
VICTORIA B C
V8Z 3A6

WHITE E DR
PACIFIC FOREST RES CENTRE
CANADIAN FORESTRY SERVICE
506 WEST BURNSIDE ROAD
VICTORIA B C
V8Z 1M5

WILD JOAN
ONTARIO MINISTRY NAT RES
ONTARIO GOVERNMENT BLDG
10 TH FLOOR 199 LARCH ST
SUDBURY ONTARIO
P3E 5P9

WOOD ANNE MRS
GENERAL DELIVERY
HARWOOD
ONTARIO
K0K 2H0

WOODS J H
COWICHAN LAKE RES STN
BC MIN OF FORESTS & LANDS
P O BOX 335
MESACHIE LAKE B C
V0R 2N0

YANCHUK A
REFOR & REC BRANCH
ALBERTA FOREST SERVICE
9915-108 ST
8TH FLOOR MAIL CAGE
EDMONTON ALTA T5K 2G9

YEATMAN C W DR
CANADIAN FORESTRY SERVICE
PETAWAWA NAT FOR INST
CHALK RIVER ONT
K0J 1J0

YEH F DR
DEPT OF FOREST SCIENCE
UNIVERSITY OF ALBERTA
720 CHEM MIN ENG BLDG
EDMONTON AB
T6G 2G6

YING CHENG DR
RESEARCH BRANCH
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1450 GOVERNMENT ST
VICTORIA
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ZSUFFA L DR
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