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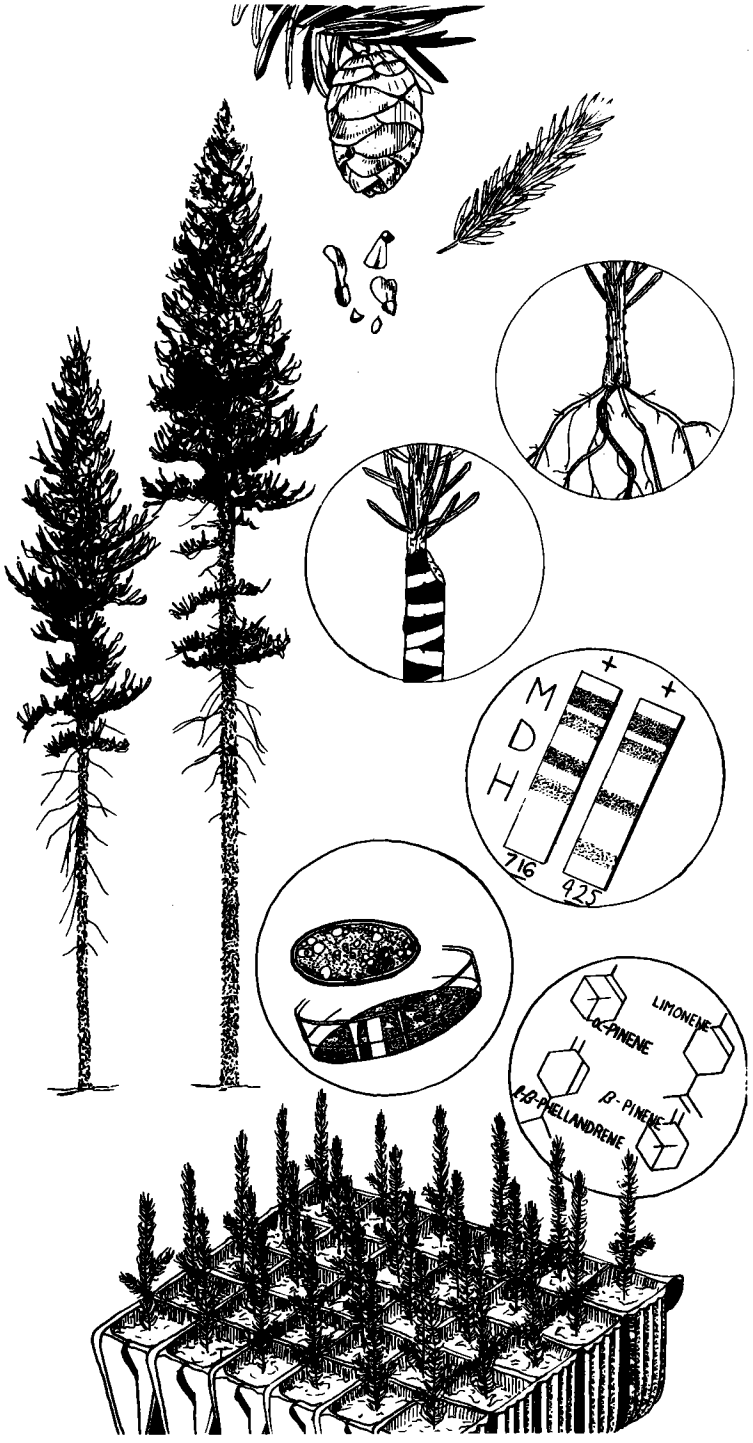
PROCEEDINGS
SEVENTEENTH MEETING
CANADIAN TREE IMPROVEMENT ASSOCIATION:
PART 2

COMPTES RENDUS
LA DIX-SEPTIÈME CONFÉRENCE DE L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES:
PARTIE 2



GANDER NEWFOUNDLAND
AUGUST 27-30, 1979
30 AOÛT 1979

SYMPOSIUM:
TREE IMPROVEMENT IN THE BOREAL FOREST:
TODAY AND TOMORROW

L'AMÉLIORATION DES ARBRES DE LA FORÊT BORÉALE:
AUJOURD' HUI ET DEMAIN

PROCEEDINGS OF THE SEVENTEENTH MEETING OF

THE CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 2

SYMPOSIUM ON

TREE IMPROVEMENT IN THE BOREAL FOREST: TODAY AND TOMORROW

GANDER, NEWFOUNDLAND

AUGUST 27-30, 1979

EDITOR: M.A.K. KHALIL

Part 1. Minutes and Members' Reports.

Distributed to Association members and to others on request to the Editor, C.T.I.A./A.C.A.A., Chalk River, Ontario, Canada, K0J 1J0.

Part 2. Symposium: Tree Improvement in the Boreal Forest: Today and Tomorrow

Distributed worldwide to persons and organizations actively engaged or interested in forest genetics and tree improvement.

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Ottawa, 1980

COMPTES RENDUS DE LA DIX-SEPTIÈME CONFÉRENCE DE

L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

PARTIE 2

SYMPOSIUM SUR

L'AMÉLIORATION DES ARBRES DE LA FORÊT BOREALE: AUJOURD' HUI ET DEMAIN

GANDER, NEWFOUNDLAND

27 au 30 août 1979

RÉDACTEUR: M.A.K. KHALIL

1re partie. Procès-verbaux et rapports des membres.

Distribués aux membres de l'Association et aux autres sur demande au rédacteur, C.T.I.A./A.C.A.A., Chalk River, Ontario, Canada, K0J 1J0

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Contributors of Papers to the Symposium with Chairman -

Left to Right: Dr. Richard B. Hall, Dr. M. Thompson Conkle, Dr. Francis C. Yeh, Dr. Robert C. Kellison, Miss Rose M. Rauter, Dr. Peter P. Feret, Dr. M.A.K. Khalil, Dr. D.J. Durzan, Dr. Hans Nienstaedt, Dr. Hyun Kang, Dr. E.K. Morgenstern, Dr. Louis Zsuffa.

PROCEEDINGS OF THE SEVENTEENTH MEETING OF

THE CANADIAN TREE IMPROVEMENT ASSOCIATION

With the compliments of the Association

Enquiries may be addressed to the authors or to Dr. N.K. Dhir, Executive Secretary, C.T.I.A./A.C.A.A.

The Eighteenth Meeting of the Association will be held in Victoria, British Columbia, August 18-21, 1981. The agenda for the meeting includes: Day 1, "Provincial Roundup" - a synopsis of progress among all active agencies in each province and field excursion to two seed orchards; Day 2, Panel Discussion and excursion to Cowichan Lake Experiment Station; Day 3, C.T.I.A. Symposium on Seed Orchards. Canadian and foreign visitors are welcome. Further information will be distributed in fall, 1980, to all members and to others on request. Enquiries regarding the Eighteenth Meeting should be addressed to Dr. D.F.W. Pollard (Vice-Chairman, Symposium), Pacific Forest Research Centre, 506 West Burnside Road, Victoria, B.C. or to Mr. M. Crown (Vice-Chairman, Local Arrangements), B.C. Forest Service, Box 816, Duncan, B.C.

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COMPTES RENDUS DE LA DIX-SEPTIÈME CONFERENCE DE L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

Gracieuseté de l'Association

Les demandes de renseignements peuvent être adressées aux auteurs ou à Dr. N.K. Dhir, secrétaire exécutif, A.C.A.A./C.T.I.A.

La dix-huitième conference de 1'Association aura lieu à Victoria, C.-B. du 18 au 21 août 1981. L'ordre du jour de la réunion comprend: Jour 1, "Rassamblements provinciaus" - un synopsis des progrès au sein de tous les organismes actifs dans chaque province et une excursion sur le terrain de deux vergers à graines; Jour 2, Discussion le groupe et excursion à la Station d'experimentation de Cowichan Lake; Jour 3, Symposium de 1'A.C.A.A. sur les vergers à graines. Les visiteurs canadiens etautres sont les bienvenus. De plus amples renseignments seront communiqués à 1'automne 1980, à tous nos membres et aux autres sur demande. Les demandes concernant la dix-huitième réunion devront être adressées à Dr. D.F.W. Pollard (vice-président du symposium) Centre de recherches forestières du Pacifique, 506 West Burnside Road, Victoria, C.-B. ou à M. M. Crown (vice-président arrangements locaux), B.C. Forest Service, Box 816, Duncan, C.-B.

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Association canadienne pour l'amélioration des arbres
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OPENING ADDRESS

Malcolm F. Squires

Chairman
Canadian Tree Improvement Association

Your Worship, Canadian Tree Improvement Association Members, Ladies and Gentlemen,

I have the honour as Chairman to welcome you to the seventeenth meeting of the Canadian Tree Improvement Association. To those of you to whom the Canadian Tree Improvement Association may not be a household topic of familiarity perhaps a short resume of what we are may be of some help. We are an association of scientists who work in tree breeding, genetics and allied fields. These are the people whom we call our "active members". Then there are people with a general interest in forest genetics who like to be kept informed on what is going on generally speaking in forestry. They are what we call "corresponding members". We have a third group who are generally managers of scientists or corresponding members. By virtue of that they too have an interest in tree improvement. These we call our "sponsoring members".

The objectives of the Canadian Tree Improvement Association are primarily:

(1) Promotion and use of scientifically and technically sound genetic practices in forestry. This is done by exchange of information in meetings such as the one we are having here today;

(2) Promotion of communication and liaison between scientists and workers in the field on routine basis;

(3) Encouraging participation of foresters and managers in government and industry to analyse problems and set priorities in the field of tree improvement.

We also hope for advice and assistance to encourage formulation of policies leading to better tree improvement practices. We feel these are worthy objectives, especially now when we, in Canada, are rapidly moving into a new era of intensive forest management. We say rapidly because over the past ten years the concern for the future viability of our Canadian forests and the economy based on them has expanded from the minds of only a few foresters, and not all foresters, to the minds of most Canadians and to the political scene, both at the provincial and the federal levels. Across Canada forest policies to ensure our future economic viability have been and are being developed. Legislation has been and is being passed and positive incentives are becoming realities and active forest management programs are being developed with ever increasing momentum. I think you can summarize that by saying that the future certainly is for intensive management.

As an example, during the past few months the forest products manufacturing company that employs me has entered into two long term forest management agreements, in which the Company will be doing the forest improvement work, with two provinces and is currently nearing the final stages with the third province.

At this time the Canadian Tree Improvement Association's role has become critical. On the one hand there is going to be a tremendous expansion of all aspects of forest improvement. Tree improvement will be seen as a keystone in the long term viability of forest management programs. This expansion will generate demands for faster answers which current forest tree improvement efforts and methods are unable to provide in the slow growing boreal forest. That means geneticists are going to turn more and more to modern sophisticated techniques or tools which will be necessary to contribute any significant impact over the next few years. On the other hand, because of the tremendous investments that will be made, the results of tree improvement research and development will be critically important so that conclusions and practices must be sound. Hence, the theme of our meeting:

TREE IMPROVEMENT IN THE BOREAL FOREST:
TODAY AND TOMORROW

KEYNOTE ADDRESS

The Honourable C. James Morgan

Minister for Forestry Resources and Lands,
Government of Newfoundland and Labrador

Mr. Chairman, Mr. Mayor of Gander and distinguished guests.

I have called you "distinguished guests" because you all are, in my view, very distinguished guests in our Province of Newfoundland and Labrador.

First of all, on behalf of Premier Peckford and his administration I welcome you here as very distinguished guests, indeed, to our Province, and I hope you have an enjoyable stay here.

Your work is going to have a very important impact on what we are doing in the Province of Newfoundland and Labrador in forestry, especially in the policy formulation. I am also convinced it will have a very significant impact on what the nation will be doing throughout Canada and also our neighbours to the south. I am pleased to note, looking at the delegates here, that there are quite a few from U.S.A. I do not know whether there are any from outside these two countries - our own and U.S.A. - but if there are some, of course we will be benefitting from their views as well.

I am convinced that it is very significant to us that it is the first time that you are holding a meeting of this nature in our Province. It is your seventeenth meeting but the first time here. I say it is significant because we are now in a very important stage for our program of management, regeneration and reforestation in this Province. We are sitting on a major resource which we consider a very important and vital resource for this Province.

The fact that our forest industry in the past number of years served our people well and the fact that we had two large mills operating here justifies our concern about intensive forest management. Last year the export value of the produce from these two mills was $220 million and in the coming year (1980) we will have a third mill on stream, producing a further 150 000 tons of newsprint and further beyond 1987, a second machine in the same mill at Stephenville would produce further 150 000 tons of newsprint. In addition to these we have over 1 400 licensed sawmills, some of which are a way of life in our Province because they are family owned operations. But they are still important to our overall general forest industry. And of course, we all know the value of non-industrial uses, if you want, of our forests. So, the forest is indeed a very important natural resource, especially at a time and age when we are experiencing shortage of resources. Therefore, as a government we are convinced that we have to reinvest in this vital resource. This is why, I, as a Minister and my Deputy, here, Mr. Kinsman and the officials from the Department of Forest Resources and Lands who are here, consider

your role so important to assist us and advise our government, and in fact, governments across the nation to make sure that we are using the right policies. You are the key advisors, you are the experts, scientists and specialists and we count on you for views and opinions as to what we should be doing and if we are doing wrong what we should do to correct the problems we have.

I mentioned that we have a substantial resource and I give an example. Looking at commercially productive forest area with 5 or more cunits of wood per acre in our Province today, we have 18 million acres of commercially productive forest areas which only includes a part of Labrador, because most of Labrador part of the Province is inaccessible for commercial forestry. Looking primarily at the Island portion, which we are now in, we have 9.3 million acres of commercially productive forest area with 90 million cunits of wood. Our annual allowable cut at the present time is 1.6 million cunits per year from the Island and accessible parts of Labrador. We are presently using only 900 000 cunits of wood which shows that we are relatively in a good position. We are not using all we have. But it is not all that bright either. We have a number of shortfalls. And that is where this kind of convention is so important to us. One of the facts we are faced with is that we have a very major imbalance of age distribution. We have substantially mature and overmature tree stands in this Province. And, of course, although nature is very kind to us in regeneration of cutover areas, we will need artificial restocking. That is one of the problems that we are tackling today as a government. As I said before, policy briefs from groups like yourselves and advice from groups like yourselves are quite useful to us in tackling the silvicultural problems. We have a lack of regeneration in many areas. On the other hand, we have an overabundance of natural regeneration in some areas. And I said, we are embarking on an aggressive silvicultural program. To give an indication of what we are talking about we are looking at providing between $6 and $7 million each year over the next five years in a five-year program of regeneration, and other silvicultural operations. It might not sound like a major program to some, but it is in my opinion, a good and substantial program. It is a good start. With the new administration in the Province and I as the Minister responsible for forestry policies, we intend to embark on this aggressive program. We have major nursery operations approximately 60 miles from here in the Grand Falls-Bishop Falls area. Approximately six million seedlings are planned for production next year and from then on we are planning for utilizing 15 million tree seedlings from that nursery each year. That will provide for planting of approximately 10 to 12 thousand acres per year over the next number of years. We are using primarily black spruce for a number of reasons. Out of all the silvicultural investments, I think the best investment that a government can make today is the investment in tree seed improvement.

We are fortunate somewhat in this Province, I have to say sincerely, in having the assistance and cooperation of the specialists and experts available to us from the Canadian Forestry Service. We appreciate that and we are looking forward to working in a spirit of cooperation with them. In fact, this year a working group was formed in

the Province, with representatives from our Department and from the Canadian Forestry Service with regard to establishing tree seed improvement programs. We are looking at black spruce as the main species and also some white spruce. The ultimate aim of government, of course, is to eventually see the establishment of seed orchards which will give an adequate supply of genetically improved seed. In the meantime, we are establishing seed production areas around the Province where principally black spruce will be the species selected.

Despite the fact, that we have an overabundant total wood supply we are not utilizing the allowable cut fully.

Another problem of concern to us, and of concern to a number of other provinces, is the infestation of forests with spruce budworm. It appeared first in the Island portion of our Province in 1971-72. We carried out a fairly large spraying program in 1978. Approximately one million acres were sprayed in 1977-78. This year we are not carrying out such a large spraying program primarily because of the fact that the assessment of the situation last year showed an overall general decline in the budworm infestation in the Province. Watch is being currently maintained very closely by the agencies of the Federal Government and our own and I am expecting to have a report available to the Newfoundland Government some time in the near future indicating the present status. The present status may warrant us to take action in 1980, next year. It is a problem that has caused a lot of concern. The effects of budworm are obvious as we fly over the areas of the Province. Only a few days ago I did that and I saw many of the areas which were green forests now consist of dead and dying timber stands. When we have heavy tree mortality in young stands it is indeed a very serious problem. For all of us it is a very big challenge - for us as a government, and I would say this morning sincerely, a challenge to you as scientists and experts in your field. First of all, it is a challenge to us with regard to a major reforestation program. It is a challenge that somewhat presents an opportunity as well - an opportunity I feel, which you will recognize and allow us to select trees of our own choosing, allow us to select and choose desirable tree characteristics as well and thereby enable us to select our own growth conditions. So, these are the opportunities in accepting the challenge to deal with the problems we have. I feel, this is a challenge to you as well. A government counts on opinions and views and policies from groups like yourselves. You could devote some of the deliberations of your meeting in our Province and future meetings in guiding us with regard to tree species and varieties which will be resistant to insect infestation and meet our other objectives.

I would like to see some of your deliberations devoted to this very aspect of reforestation - of finding suitable species and varieties which would be somewhat resistant to insect infestation.

My comments this morning as to what we are doing as a Province may seem very modest to many of you here. But it is an indication of the importance this Province places on the forest industry and

the importance we are now attaching to investment in silviculture and forest management that we are determined to manage and reinvest in the vital forest resources and industry of this Province. As I said earlier, in doing so, we are counting on you and your deliberations made at various meetings to assist us. I am convinced your meeting is going to be of benefit to us. People from our Department, including today the Deputy Minister and myself, will be staying this morning to listen to the reports from the various parts of the country and from the U.S.A. We will have officials here throughout your conference who will listen intently. We shall get reports from these officials as to what took place in your meetings here and the results from them.

I shall say in closing that your contribution to the renewable resources is vital not only to this Province, but to all of Canada and is highly recognized by government. And I am sure I can speak for governments throughout Canada. I wish you well in your deliberations in the next number of days and I sincerely hope you enjoy yourself in our Province of Newfoundland and Labrador.

INTRODUCTION

M.A.K. Khalil

Chairman Symposium on: Tree Improvement in the Boreal Forest: Today and Tomorrow

Ladies and Gentlemen:

I have the honour and the privilege of welcoming this distinguished gathering of scientists, forest managers and leaders in government and industry to this symposium the theme of which is "Tree Improvement in the Boreal Forest: Today and Tomorrow".

Forest genetics aims at improving quantitative characters, which are controlled partly by the genotype and partly by the environment. The long process of evolution of forest tree species has resulted in variation among as well as within species and populations. Detection and exploitation of this variation is the mainstay of forest tree improvement. This is achieved by one or more of the following approaches:

1. Identification and testing of the best existing populations and genotypes by selection. This is done by provenance tests and trial of exotics, which identify superior populations and by progeny tests which assess the genetic worth of the superiority.

2. Improvement of existing genotypes by hybridization, polyploidy and mutation.

3. Establishment of seed orchards and clone banks for mass production of genetically improved seed and other planting material.

All these methods require long periods of time, which may be as long as 40-50 years, for yielding finally acceptable results. At present we are living in a dynamic age where fast change is the law of life. Concepts of and criteria for superior trees have changed during the last 40 years resulting from the changes in the use of wood and fibre and in the technology of wood processing. I am sure that during the next 40 years these changes in the uses of wood as well as in the technology of wood and fibre processing will undergo even more dramatic changes than have occurred in the last 40 years. The industry's concepts of and criteria for "superior" trees will certainly change in the next 40 years. The "superior" trees which we are aiming at today may no longer qualify for being superior 40 years hence.

Speeding up our pace in tree improvement is the call of the hour and if we do not respond to this call forest genetics and tree improvement as a science and as a profession may cease to exist. This is particularly applicable to boreal forests which have slower growth and longer periods to sexual and economic maturity than other forest regions. Fortunately, however, biologists and biochemists have developed techniques in recent years which have enormous potential for detecting

variation and for selecting superior populations as well as for mass propagation of superior genotypes within a very short period of time. Isozymes, chemosystematics and physiological techniques are now available for detecting variation and for isolating superior populations and individuals. Techniques of clonal propagation and tissue culture are now available for mass production of superior genotypes.

I have the good fortune of being able to assemble today, under one roof, the best talent on all the methods of detecting variation and for isolating superior populations as well as for mass production of accepted superior genotypes - both conventional and new. I am grateful to all the specialists who have agreed to discuss their specialties for the common cause of tree improvement. This has caused personal inconvenience to all of them for which I am sincerely grateful.

I do not want to intervene between you and the specialists for long and proceed to the commencement of the proceedings.

PROGENY AND CLONE TESTING IN BOREAL BREEDING PROGRAMS

E.K. Morgenstern

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ABSTRACT

The role of tests, principles of test design, and the types of tests currently used in boreal Canada are discussed. Recent ideas on clonal selection and testing by means of rooted cuttings so far are applied to conifers only in Ontario. Yet the particular difficulties of boreal breeding programs (low intensity of silviculture, scarcity of specialized staff and facilities) could be alleviated if simplified testing based on the clonal approach is used more widely, particularly in the first generation. It is concluded that we need comparative experiments which demonstrate savings in test area and improved selection efficiency.

RÉSUMÉ

Nous allons étudier le rôle des analyses, les principes de leur conception, et les différents types d'analyses actuellement utilisées en Canada Boréal. Jusqu'à présent les récents procédés de sélection clonale et e'analyse par implantation de boutures n'ont été appliqués aux conifères qu'en Ontario. Cependant on pourrait alléger les difficultés spécifiques aux programmes de reproduction boréaux (basse intensité de la sylviculture, pénurie de personnel et d'équipement) si on procédait plus souvent à des analyses simplifiées basées sur une approche clonale, en particulier pour la première génération. En conclusion, nous avancons que nous avons besoin d'études comparatives qui mettent à jour une économie dans le domaine de l'analyse et qui réhausse l'efficacité des méthodes de sélection.

INTRODUCTION

Progeny testing is defined as the evaluation of parents by comparing the performance of their offspring. Progeny tests rank the parents and provide estimates of additive and in some cases dominance genetic variance. In contrast, clone testing is the direct evaluation of individual genotypes (which may be used as parents) since all members of a clone are genetically identical. Clone tests provide estimates of total genetic variance without a breakdown of the types of variances involved.

The two types of tests therefore have somewhat different functions and possibilities in selection programs (Shelbourne 1969; Synder 1972).

Progeny tests are widely used in boreal breeding programs and the basic ideas that guide them have been well described (Klein 1968; Yeatman 1979). Clone tests applied to the major conifer species (Rauter 1974) are few in number but should receive more attention in view of their potential. It could well be that they will make it possible to accelerate breeding programs in boreal regions. These regions present particular problems: large clear-cut areas and seed requirements (e.g. for direct seeding), continental climates with their extremes of temperature and drought, and a less-than-intensive silviculture. These conditions call for programs of great simplicity (low input of specialized skills and facilities) and low cost per unit area of regenerated land.

This review will therefore deal with both types of tests. It will discuss their role, survey their application in Canada, and point out some of the advantages of clone testing that have not been sufficiently recognized. Because of similar silvicultural problems, the review will occasionally include programs in montane and subalpine regions in British Columbia and Alberta, and temperate regions in Ontario, Quebec, and the Maritime Provinces.

THE ROLE OF TESTS

Breeding programs consist of a series of activities ranging from mass selection in wild stands to seed production in orchards, and repetition of selection, testing and seed production over several generations. In such programs, progeny and clone testing serve two main functions.

First, tests are designed to increase genetic gain by identifying parents of high breeding value. When plus-tree selection is initiated in wild stands (the common situation in boreal regions), the genetic gain expected from this step is usually small, particularly for growth and yield. This is so because selection for weakly inherited characteristics in heterogenous environments is difficult. Progeny testing then verifies the accuracy of initial selection (Morgenstern et al. 1975).

It has been suggested that open-pollinated progeny tests established as soon as possible after seed collection could be assessed at about age 10 years from seed and the results be used to rogue an orchard before it comes into full production (Franklin 1972). This idea is applicable both to grafted clonal orchards and also to seedling seed orchards of black spruce (*Picea mariana* Mill. B.S.P.) and jack pine (*Pinus banksiana* Lamb.) (Morgenstern et al. 1975; Yeatman 1979).

Genetic gain can also be increased by looking at a progeny test as an opportunity for selection rather than as a test of parents. It is then more correctly referred to as a family test, particularly

if the parents have not been preserved. This applies both to open-pollinated progeny tests and to tests with progenies from controlled pollination. The latter are particularly valuable in this respect and could achieve twice as much genetic gain as open-pollinated tests (Wright 1976). Often both can be used in successive stages of a program.

In contrast to progeny tests, clone tests are a direct test of the parental genotypes. Thus a breeding program based on clonal selection would require neither preservation of original parents (ortets) nor establishment of clone banks to retain their genotypes. However, this assumes that grafts or rooted cuttings behave in predictable fashion. Unfortunately this is not always the case because of cyclophysis (physiological age effects) and topophysis (position effects in the crown) (Klaehn 1963; Roulund 1974).

The second function of tests is to measure genetic gain and genetic parameters. Such parameters as genetic variances, heritabilities, and genetic correlations are needed for the planning and further refinement of breeding programs and their economic justification. They are particularly important in the early stages of programs, particularly for new species and regions where such information is not available. Even results from pilot tests will then be very valuable (Khalil 1978a).

PRINCIPLES OF SAMPLING AND TEST DESIGN

Degree of Subdivision

To be efficient and to produce reliable results, progeny tests must be organized on the basis of both genetic and ecological information.

The degree of subdivision of natural populations indicates genetic population structure. If this has been obtained earlier from provenance or combined provenance-progeny tests (Morgenstern 1969), sampling of the populations for progeny testing is easier. For example, if the distribution of genetic variances is known at the stand, subregional and regional level, sampling can be concentrated where the greatest variation exists. Good examples of the types of experiments involved have been given by Yeatman (1974) and Dhir (1976).

Breeding Regions

Breeding regions are a necessary framework for all tests. The general principle is that progenies are tested over the range of sites where they would be normally grown in silvicultural practice (Dudley and Moll 1969), i.e. only native progenies would be grown in one region. This is common sense for if these progenies are not adapted to the climates, soils and biotic factors of the region in question, survival will be poor and the results incomplete. Another more elusive reason is that even if the trees survive and grow, the reaction of introduced populations to the different photoperiods and temperature regimes in the test region will not be normal and the resulting genetic variances biased (Stern 1962; Morgen-

stern 1973). Both the white spruce (*Picea glauca* (Moench) Voss) progeny test program initiated in British Columbia by Kiss (1976) and the jack pine program in Manitoba and Saskatchewan (Klein 1968) have carefully applied this principle of testing within regions.

Statistical Design

Lattice-square and incomplete-block designs have often been recommended for progeny tests but Wright (1976) emphasized the advantages of flexibility in layout and analysis offered by randomized blocks. Some grouping into sets of progenies is usually required to reduce the sizable error inevitable in large tests.

Locations and Replications

Reliable estimates of genetic parameters can only be obtained if there is adequate sampling at each level. In an open-pollinated progeny test, if the trees are in half-sib families with $\underline{n}$ to a plot and with $\underline{r}$ blocks in $\underline{e}$ environments, the phenotypic variance of family means $\sigma^2_{\bar{p}}$ for a given character consists of:

$$\sigma^2_{\bar{p}} = \sigma^2_f + \frac{\sigma^2_t}{nre} + \frac{\sigma^2_b}{re} + \frac{\sigma^2_{fe}}{e}$$

where $\underline{f}$ refers to families, $\underline{t}$ to trees within plots and $\underline{b}$ to blocks or replicates. The criteria of statistical efficiency would be low sampling (standard) errors of the variance components to estimate heritability, and a low variance of the family means to rank progenies for selection (Namkoong et al. 1966; Morgenstern 1972).

Of course all design factors are interrelated. For example, statistical efficiency depends in one way upon the total number of replications, but these can be distributed over few or many locations (environments). There should be more than one location to arrive at an estimate of the family-location interaction (σ^2_{fe}). The number of locations depends generally upon the geographic dimension of the breeding region and its ecological heterogeneity. In practice, it has often been more efficient to plant few replicates in more locations than vice versa, but obviously management cost also enters the picture (Rundfeldt 1957; Wright 1976).

Family and Plot Size and Shape

Family size is another aspect of design which is related to those just mentioned. Wright (1976) presents a table for optimum family size in relation to the total number of trees in a test. For example, with 20 000 trees in a test and a heritability of 0.1, only 80 trees need to be included per family. With 4 locations, only 20 trees should be at each. The calculations involved also consider selection intensity but this aspect will not be discussed here.

Plot size is also related to other than statistical questions. With low survival, small plots will be of doubtful value. They are also less useful in long-term tests when thinning is anticipated or when influenced by plot-to-plot competition after crown closure. Experiments in Scotland showed that 5- to 10-tree plots are needed up to age 10 years for *Larix* spp. and *Picea sitchensis* (Bong.) Carr. (Johnstone and Samuel 1974). Johnsson (1974) in Sweden showed that in *Pinus sylvestris* L., 36-tree plots were needed when 2 thinnings were made before age 18.

Multiple-tree plots are also needed to predict volume production. It is not possible to assume that individual genotypes when grown together will perform as when grown individually because of different degrees of competition (Stern 1960; Johnsson 1974).

Because of competition, square plots are preferable to row plots (Hühn 1970).

Clone Tests

The experience with clone tests is limited, particularly for boreal conifer species. Theoretically the total number of ramets of a clone (e.g. rooted cuttings) needed to obtain genetic, environmental, and interaction variances should be small because the genetic correlation between the ramets equals 1. Therefore the total test area should be smaller than for families. But the most efficient distribution of ramets to plots, replications and sites has not been investigated.

For other species, e.g. *Populus* spp., small plots are common. For example, a test of *Populus deltoides* Bartr. consisted of 2-ramet plots with 4 replicates on each of 3 sites (Randall and Cooper 1973).

A SURVEY OF TESTS IN BOREAL CANADA

Controlled Crosses

While progeny tests based on controlled pollination have been well described from many breeding programs particularly in Europe and the southern United States, they have made relatively little impact in boreal Canada. So far, only the programs in Ontario are using controlled crosses in white spruce and black spruce to rogue grafted clonal seed orchards during the first selection cycle (Rauter 1978). In other provinces the orchards have not yet reached that stage or controlled crosses will be made in clone banks for the second selection cycle, after results have been obtained from open-pollinated tests (Klein 1968, 1979; Kiss 1976; Yeatman 1976; Wheeler 1978).

The impression is that, under current conditions, the levels of skills, professional manpower, and facilities required are too great and costs too high to cover the vast area of boreal regions within a reasonable length of time.

Open Pollination

Progeny tests following open pollination were recommended as early as 50 years ago (Fabricius 1922) and have become very popular in many countries because of their simplicity and the possibility to demonstrate results and obtain genetic parameters rapidly. The method is promising if the proportion of additive genetic variance is relatively high and this has been found to hold true for the species studied to date. The progeny tests could be derived from open-pollinated clones in a grafted clonal seed orchard, or open-pollinated trees in wild or planted forests. Only the latter method is commonly used. The advantage of open pollination has sometimes been seen in the additional benefit of converting progeny tests into seedling seed orchards (Wright and Bull 1963), but to combine two functions in the same plantation may be difficult or impossible (van Buijtenen et al. 1971).

A number of programs in boreal regions are using open pollination. In British Columbia the work of Kiss (1976) with white spruce largely relies on this method initially although controlled crosses can later be made in clone banks. In Alberta, sizable programs with lodgepole pine (*Pinus contorta* Dougl.) and white spruce have been initiated (Dhir and Vincent 1978). In Manitoba and Saskatchewan a federal program with jack pine was initiated in 1966 (Klein 1968) based on family tests from open pollination and preservation of scions from parent trees in clone banks. In Ontario, black spruce family tests have been initiated in three boreal regions (Morgenstern 1978). Open pollination is also used as a rapid screening method for seed production areas of white spruce (Rauter 1978), and in a jack pine breeding program (Yeatman 1979). In Quebec, an open-pollinated test program with jack pine was initiated in cooperation among several provincial and federal organizations (Yeatman 1976; Lamontagne 1978). In New Brunswick and Nova Scotia combined seedling seed orchards - family tests with white spruce were started by the Canadian Forestry Service and provincial and industrial cooperators (Fowler and Coles 1977). In Newfoundland the Canadian Forestry Service has started progeny tests both with white and black spruce (Khalil 1978b).

Obviously, open pollination is commonly applied but most programs have been initiated by research organizations and so far only a few of many regions have been covered. Therefore even this method is too laborious. Most forest districts are unable to establish large test areas with thousands of plots under current financial conditions. A reduction and simplification of test programs is desirable.

Clones

A breeding program using clonal propagation and testing was initiated by Rauter (1974) for both black spruce and white spruce in Ontario. It is based on selection of "superseedlings" in the nursery at which stage rooting is easy. This program uses special rooting facilities developed at the Orono Nursery in eastern Ontario and is progressing well.

Rooting of cuttings is also undertaken in several other species in Canada but more commonly to study rooting techniques than for direct application in tree improvement, at least at this stage (Girouard 1970; Brix and van den Driessche 1977).

THE POTENTIAL OF CLONE TESTING BY ROOTING

The advantages of clonal testing and selection have been recognized even by the first tree breeders (Schreiner 1939; Larsen 1956) but large-scale application was limited to the genus *Populus* until recently. Problems with cyclophysis and topophysis were the principal reasons why the method was not applied.

During the last two decades there was a marked change. Incompatibilities between scion and rootstock in grafted clonal orchards in New Zealand and western North America awakened interest in rooting as an alternative to grafting while a basic comparison of selection methods and genetic gain by Libby (1964) reaffirmed the advantages of the clonal approach. Since the mid-sixties much new work has been initiated, both in the development of rooting methods, as well as in its application in selection programs. In addition, symposia have been held and review papers published (Brix and van den Driessche 1977; Lindgren 1977; Zsuffa 1979).

Some of the basic ideas and experiences from such recent research are summarized below.

1. When family selection and clonal selection are compared, the efficiency of clonal selection is greater because of the higher genetic correlation among clone members. Clonal testing and selection makes rapid genetic improvement possible particularly during the first selection cycle (Libby 1964; Shelbourne 1969).

2. For a given degree of test precision, far fewer ramets per clone are needed than seedlings per family, giving a more compact and economical experiment. If heritability is low, the clone test will be much more efficient than the family test provided the non-additive genetic variance is small and there is no topophysis (Burdon and Shelbourne 1974). These conditions appear to apply to growth and yield of black spruce, for example, which has low dominance variance and where topophysis is no problem when young seedlings are rooted (Morgenstern 1974; Rauter 1979).

3. Since clone tests estimate only total genetic variance, i.e. heritability in the broad sense, it is necessary to derive at least additive genetic variance from other associated progeny tests. The combined use of data from clonal and progeny tests has been demonstrated by Corriveau (1976).

4. Clone tests can also be used to refine breeding strategy. In a series of tests replicated over several sites, clone tests will

estimate clone-site interactions more efficiently than family tests will estimate family-site interactions. Part of the reason is the high sensitivity of clones to environmental change; they are buffered less than families. Clone testing could therefore be beneficial if interactions are used to subdivide breeding regions (Burdon and Shelbourne 1974; Shelbourne and Campbell 1976).

5. Another advantage of clone tests is that they circumvent the problems of flowering and seed production, e.g. delays of testing occasioned by infrequent flowering years. Related to this is the problem of a possible negative genetic correlation between flowering and growth (fast growing trees which do not flower or flower rarely). To test them vegetatively will then overcome this problem (Weissgerber 1973; Lindgren 1977).

6. In general, the younger the ortets, the easier it is to root them. The problems of cyclophysis and topophysis have been avoided to a great extent if the tests were based on young seedlings selected in nursery beds. Both the programs in Ontario (Rauter 1974) and Lower Saxony in Germany (Kleinschmit 1974) use this approach. Subsequent rooting is done in greenhouses, and this is followed by nursery and field testing. The field tests can be used for seed production and as base populations for future generations (Kleinschmit and Schmidt 1977).

7. Concern has been expressed that selection of juvenile plants may favour genotypes that will not maintain fast growth over a complete rotation period (Lindgren 1977). In white spruce, there is evidence that individual trees and families with superior height growth during their first decade maintained their lead at least during the second decade (King et al. 1965; Ying and Morgenstern 1979).

CONCLUSIONS

This review indicates that most breeding programs in boreal Canada are evolving along traditional lines and are based predominantly on control- and open-pollinated testing. The impression prevails that the potential of clonal selection and testing should receive more attention. A corollary is that the Ontario program should be examined more closely by breeders in other provinces.

Therefore, particularly to those of us working in research, there is a great challenge here. Dr. Zsuffa mentioned during the Ontario Tree Improvement Symposium in Toronto in 1978 that we should intensify our efforts to catch up with developments elsewhere (Zsuffa 1979). In relation to clonal testing, it is obvious that theory is available but that convincing examples are lacking. There is a need for comparative experiments that will show differences between tests based on sexual and asexual propagation in terms of plot sizes, numbers of replicates, total test area, and genetic gain per year - at least during the first selection cycle. Research of this kind should have high priority.

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DISCUSSION

Dr. J.I. Klein. I have a comment. There is a Research Associate at the University of Alberta who has been producing clones of Ponderosa pine seedlings.

Dr. E.K. Morgenstern. I know that work of this kind has been done. Dr. Zsuffa also has worked on jack pine.

Dr. L. Zsuffa. I welcome Kris' discussion on clones. In Ontario, at Maple Station we have tried to develop clones of coniferous species in addition to poplars in the last 10 years and, in addition to what is mentioned, for many years we rooted pine species also. In my experience many of the pine species are not more difficult to root than spruce. We have selected white pine clones for rooting ability. In addition to what Kris said about the role of clones in progeny testing we should not forget that the greatest advantage of clones is in preserving the superior genotype.

Dr. J.L. Farrar. I did not quite understand how you propose to use the clones after you have tested them.

Dr. E.K. Morgenstern. Well, my ideas on the subject are relatively recent. I have developed them during the last year or so. None of our programs has gone far enough to show actually what can be done. In Germany, the programs I saw last week are using simple tests to reduce the number of clones, and after two screenings in the greenhouse and nursery they are going to a field test. I believe the more advanced field tests are used for seed production. My time was too brief to find out exactly how they anticipate to continue their programs. But the success is great and the number of clones successfully rooted goes into the thousands. In one state forest service several provisional selected Norway spruce clones are available. The total forest area in that state (Lower Saxony) is only 800 000 ha, i.e. it is smaller than one district in northern Ontario.

Dr. F.C. Yeh. I personally find it extremely difficult to compare the efficiencies of different testing procedures. For example, take the open-pollinated testing. What are you going to do with the more advanced generations, because you have lost control of the ancestry in your populations? Personally I feel that using open-pollinated testing you have certain advantages, like you can start a program right away because you can test a large number of initial selections. But for estimating genetic components and building up breeding populations for the future it does not have advantages over other mating designs. One of the problems we

face in British Columbia is that no matter what kind of mating design you use you are going to have to plant the stuff and the cost of planting is the major concern in our programs.

Dr. E.K. Morgenstern. Well, I see the advantage in the initial stages, but I do not know what happens later on. I cannot discuss the whole breeding program here. I believe the cost of clone testing is significantly lower than in other tests because one can work with fewer trees and replicates. The cost should be much lower than for open-pollinated tests or control-pollinated tests, because one is testing the genotype and not its descendants.

THE ROLE OF PROVENANCE TESTS IN TREE IMPROVEMENT

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ABSTRACT

Provenance test methodology, objectives and findings are briefly reviewed. Test objectives are: (1) To delineate regions in which provenances having acceptable adaptation and productivity within the breeding zones are to be found. (2) To find sub-regions containing provenances having superior productivity. (3) To determine the relative magnitude of variation among provenances and among families from within provenances.

Item (1) yields information for the genecologist and ecologist. In breeding a native species, information on (3) is of highest priority. The advantages of provenance selection, item (2), would become insignificant after a few generations of breeding.

A breeding program without prior provenance testing is outlined as follows:

1. Delineation of seed zones and breeding zones (limited to two or three seed zones).

2. Selection of 400-600 parent trees representing 40-50 stands -- the majority from the breeding zone but including some from adjacent seed zones.

3. Progeny testing at two or three sites in the breeding zone and one test each in adjacent colder and milder seed zones.

This program should allow adjustment in the breeding zone if necessary, and provide a base breeding population that with rapid generation turnover would reach and surpass the gains that could be achieved with initial investment in provenance testing and selection.

Exotic species must be provenance tested.

RÉSUMÉ

Nous allons étudier brievèment la méthologie des tests de

provenance, les objectifs à atteindre et les découvertes. Les objectifs des tests sont les suivants: (1) Délimiter des régions dans lesquelles il est possible de trouver des provenances ayant des qualités acceptables d'adaptation et de productivité à l'intérieur des zones de reproduction. (2) Trouver des sous-régions offrant des provenances de productivité supérieure. (3) Déterminer la magnitude relative de variation parmi les provenances et entre les familles à l'intérieur de mêmes provenances.

L'article (1) fournit des informations aux généticiens et écologistes. Les données contenues dans l'article (3) intéressent en priorité la reproduction d'une espèce indigène. Les avantages de la sélection de provenances, article (2), deviendraient insignifants après quelques générations de reproduction.

Nous exposons les grandes lignes d'un programme de reproduction sans test de provenance préalable comme ci-indiqué:

1. Détermination de régions de graines et de régions de reproduction (ceci se limitera à 2 ou 3 zones).

2. Sélection de 400 à 200 arbres parents représentant 40 à 50 peuplements -- la majorité provenant de la zone de reproduction et certains provenant également de zones de graines adjacentes.

3. Analyse de descendance sur 2 ou 3 sites de la zone de reproduction et analyse dans chaque zone de graines adjacente, plus froide et moins froide.

Ce programme devrait permettre un éventuel adjustement dans la zone de reproduction, et fournir une population de reproduction de base qui, avec un rendement rapide de reproduction, atteindrait et surpasserait même les gains que l'on pourrait réaliser grâce à un investissement initial dans l'analyse de la provenance et de la sélection.

Les espèces exotiques doivent subir l'analyse de provenance.

INTRODUCTION

Provenance testing goes back more than 200 years to the Scotch pine (*Pinus sylvestris* L.) inventory provenance research of Duhamel du Monceau (Langlet 1971). For more than 150 years after Monceau's early efforts, provenance tests were conducted by silviculturists and foresters for forest management purposes. The objective was to find good reproductive materials for artificial regeneration of European forests as they gradually were brought under intensive forest management.

In the last 40 to 50 years, provenance testing has become an adjunct to tree improvement and is now considered a pre-requisite to any serious tree breeding effort.

My objectives today are: (1) To outline some of the methods that have been employed in provenance testing programs, (2) To summarize the purposes of provenance testing vis-a-vis tree breeding, as well as some of the benefits derived from such tests, (3) To make some generalized observations on what we have learned from past studies; and with that as a background, (4) To explore the future role of provenance testing as part of tree breeding.

PROVENANCE TESTING PROGRAMS

Types of Provenance Testing

The older tests of a century ago were limited in scope. Often they involve a few selected provenances recommended by colleague foresters; larger efforts were broad rangewide survey-type studies. The rangewide studies were valuable as methods of getting an overview of general patterns of variation within species. But they were not considered adequate to identify outstanding provenances, and they therefore led to new larger studies often of limited portions of species ranges -- in other words, to studies that had the selection of superior strains as the main objective. The result has been that today many authors recommend that provenance trials should be divided into two phases (Campbell 1974; Lines 1967; and Burley 1969). Lines (1967) for example, stated that the objectives of first stage studies should be to identify broad regions in which provenances having acceptable adaptation and productivity can be found. While the objectives of second stage studies should be to find sub-regions and provenances having the greatest productivity.

More recently, it has been recognized that adequate planning of breeding strategies requires a knowledge of the relative amounts of variation among provenances as well as the amounts of variation within provenances. The result is that a third phase now is considered important by some authors (Lines 1967). In this phase progenies representing a restricted number of provenances are tested. Some authors have suggested that second stage limited range testing be eliminated and replaced by the combined provenance-progeny tests.

Provenance Test as a Source of Breeding Material

Occasionally during the course of Phase 1 studies, as described above, superior provenances exceeding local seed sources in growth are identified. This, however, would be a chance event. By design the objective of the more detailed second stage of study is the selection of breeding material.

Using selected provenances, the breeder has two options. Either the breeding population can be developed from the population of a single superior provenance, or the breeding population can be selected from several provenances. In the latter case the breeder hopes to achieve added gains through heterosis resulting from provenance hybridization. At present, what might be achieved from hybridization is not clear. Some authors do not recommend it (Shelbourne and Campbell 1976). Some of the

best studies have been conducted in Sweden; they don't give an unequivocal answer. In Norway spruce, generally, crosses between Swedish spruce and Central and Eastern European spruce have greater yields than the provenance material of the northern hybrid parent. However, certain hybrid combinations show higher yields than either parent on northern sites; in other words, the combining ability of the individual parent tree selection overrides the provenance effect (Nilsson 1973).

The results with Scotch pine are in some respects similar. In terms of survival, parent phenotype (superior tree progeny-better) and planting site (hybrids not the best on severe sites) determine the results. Similarly, yields of hybrids tend to be greater than the yield of within-population crosses but the genotype of the individual parent tree can completely override the provenance effect. It is clear that mass-hybridization of populations representing different provenances would be an inefficient approach (Ehrenberg 1975).

Information Derived from Provenance Research

Broadly speaking, we obtain three kinds of information from provenance tests. First, we are able to compare the performance of the provenances represented in the study, and if the study is established on several different sites -- as good provenance tests should be -- we gain information on the genotype x environment interaction. Secondly, we get an understanding of the patterns of variation over the part of the range of the species that is being studied. By relating such patterns of variation to the environmental conditions of provenance origins, we get pertinent genecological information. Finally, we get a considerable amount of information that I think of as technique-facilitating information. Phenology of flowering, seed production potentials, information on juvenile-mature correlations, character correlations, etc. all belong in this category. It is information of great value in the planning phases of breeding programs.

Use of Provenance Test Information

Some outstanding provenances have indeed been identified in a number of species, and the identified populations have furnished selections for the breeding programs. The knowledge of variation patterns has been used in a number of ways to delineate seed zones and breeding zones (for example Rudolf 1956; Miller and Murphy 1976). And more recently, seed transfer models have been developed for some species (for example, Campbell 1974).

Technique-facilitating information such as juvenile-mature correlation has enabled us to shorten generation turnover through early selections. Character correlations have uncovered cases in which selections in one characteristic may be completely negated by simultaneous negative selection in another important character. In this way we have been able to avoid unprofitable approaches in our programs.

Patterns of Variation - What have we Learned?

In terms of variation patterns, we have identified a number of common relationships. Northern provenances are slow growers, flush early in the spring, start growth early in the growing season, are most susceptible to late spring frosts, lose their leaves early in the fall, and are less susceptible to early fall frosts. They also show more intense fall coloration, particularly during their juvenile phase, and are most resistant to extreme winter cold. Similar patterns can be described when provenances from wet sites are compared with provenances from dry sites. Trees from the wetter sites grow faster, have smaller seeds, are more shallow-rooted, and have greener foliage (Wright 1976).

However, such patterns are not universal. In a number of species, for example, southern provenances or provenances from milder climates flush earlier rather than later than their more northern or colder counterparts. Black walnut (*Juglans nigra* L.), yellow-poplar (*Leriodendron tubipifera* L.), sweetgum (*Liquidamber styraciflua* L.) and American sycamore (*Platanus occidentalis* L.) have early flushing southern seed sources (Nienstaedt 1974).

Some common types of genotype x environment interactions have been identified. They include: (1) Rank reversals over climatic gradients, (2) Changes in magnitude of provenance differences without changes in rank over climatic gradients, and (3) Unsystematic changes with no apparent relations to climatic factors.

Numerous tests have demonstrated that local provenances are among the best adapted to the prevailing climatic conditions within the area. Often, however, they are not the best. It is common to find the provenances from somewhat milder climates (i.e., southern or lower elevation provenances) are better than local sources. Jack pine is an example of this (Jeffers and Jensen 1979), but again, this is not an immutable rule. In northern Sweden per hectare volume production in Scotch pine is greater in stands reproduced from seed originating up to 2 degrees north of the planting site (Remrod 1976).

THE ROLE OF PROVENANCE TESTING: DO WE NEED IT?

With this brief overview of what provenance testing is, how tests are conducted, and what we have learned from them, I hope I have set the stage for the subject area that I want to concentrate on. Do we need provenance tests?

First, a distinction must be made between exotic and native species. We have some examples of successful introduction and breeding programs without provenance testing. In New Zealand, breeding of *Pinus radiata* D. Don has yielded improved types that exceed in growth the best provenances in more recently established tests. And similarly, in white spruce, a landrace adapted to windbreak conditions on the outwashed sand plains in Jutland, Denmark, may have developed in the course of a few

generations of selection.[1/]

Nevertheless, there is no question that any introduction and/or breeding of an exotic species without provenance testing is too risky. Predicting adaptive responses on the basis of climatic similarity between the region of origin and the new region of planting is simply not satisfactory for exotics. However, climatic similarity should be used to limit the native region to be sampled for testing.

But, if it is certain that provenances must be tested if the introduction and breeding of exotics are to be profitable, it is equally certain that it is possible to proceed breeding native species without provenance tests. Using the axiom that local seed sources are adapted, we understand enough of forest tree population structures to delineate reasonable preliminary breeding zones without any provenance testing, and proceed with the selection of parent trees for the breeding population. If we pay sufficient attention to sampling and the origin of the parent trees and their subsequent performance during the evaluation testing, we should be able to simultaneously eliminate the less desirable genotypes and make adjustments in the initial breeding zones if necessary.

However, there is a wide gap between the three-phase provenance testing programs and proceeding without any provenance testing whatsoever. Since we cannot generalize and apply variation patterns from one species to several other species, and since we have demonstrated that variation patterns -- i.e., genotype responses -- are environment determined, we do need some equivalent to provenance test information. In order to focus on the problem, let me again state the three testing phases and their objectives.

Phase 1: Broad rangewide testing with the object of delineating regions in which provenances having acceptable adaptation and productivity within the breeding zones are to be found.

Phase 2: Regional provenance tests in which the main objective is to find sub-regions containing provenances having superior productivity.

Phase 3: Testing of progenies from a limited number of provenances to determine the relative magnitude of variation among provenances and among families from within provenances.

The Phase 3 objective has highest priority, in my opinion. An answer to that question will have a major bearing on the development of the breeding population.

[1/] Results of provenance tests at Holtum and Kragshov Heathes in Jutland on file FSL, Rhinelander, WI.

Understanding the variation patterns within the species is second in importance. But we have obtained enough information on our far ranging north temperate species to make it clear the rangewide tests are of limited importance from a breeding point of view. We know that if we want to breed white spruce for southern Canada and adjacent United States, we don't have to sample the Alaska-Northwest Territory or Labrador ranges of the species. I believe we can generalize with confidence and say that we don't need the rangewide tests for the other North American species. We can limit our studies of variation patterns to selected regions within the species distribution.

I would place the selection of superior provenances third in priority. Obviously, it is an advantage to be able to initiate a breeding program with a breeding population representing the "best" provenance. Early gains would be greater. However, in advanced generations the differences would become insignificant.

Considering these objectives and their priorities, it is clear that we need testing of progenies from the start. Our emphasis should be on Phase 3 type testing. We need to test progenies from stands within defined environments. This means that we have scaled down provenance testing to the point where it can be undertaken as the first step in the breeding program.

Designing Provenance Tests as a First Step in the Breeding Program

The first step in developing a program requires the delineation of seed zones and breeding zones for the region under consideration. Zoning based on environmental conditions and in mountainous terrain on elevation has been developed for the United States, and is adequate for the purpose. Breeding zones will be made up of two to three of the basic units of seed zones.

Secondly, natural stands must be sampled to establish the breeding population. For smaller programs in which ownership is entirely confined within the breeding zone selection is simple -- the main concern should be to include a sufficient genetic base in the initial population. For larger cooperative or state and federal programs, the breeding zone will be the area to be most intensively sampled. However, considering that seed transfer from milder to somewhat more severe conditions in many cases is successful and leads to some degree of improvement, adjacent seed zone(s) representing milder conditions should also be sampled in an effort to identify adapted more rapidly growing genotypes. Also, in an effort to increase hardiness and if possible broaden adaptation, I suggest sampling on a less intensive scale in an adjacent seed zone representing more severe conditions.

I suggest that a total of between 40 and 50 stands be sampled in the three areas. Fifty percent of the stands could represent the breeding zone itself, 30 percent the stands in the seed zone milder than the

breeding zone, and 20 percent the stands in the seed zones with the more severe climatic conditions. Ten to 15 trees per stand should be sampled. The selection of the parent trees is beyond the scope of this paper; suffice it to say, dominant trees without disease or defect should be satisfactory. For most species, complicated, intensive, and costly phenotype selection in natural stands will not be worthwhile.

The initial test will involve 400 to 600 individuals. Two to three tests should be established in the breeding zone, and at least one in each of the adjacent seed zones.

A test of this design would provide information on provenance (stand) variation, family variation, and genotype x environment interactions both at the provenance and family level. Heritabilities using family means could be computed and it would be possible to predict gains resulting from either provenance, family selection, or both. After roguing, the tests could be used as production populations and provide genetically improved seed for reforestation at an early age.

Breeding strategy is beyond the scope of this paper and is being discussed elsewhere in these Proceedings (Kang 1980); however, let me emphasize that the pay-off in tree improvement is achieved through advanced generation breeding. Breeding strategy and the adequacy of the tests as breeding populations will to a large extent depend on the variations encountered. Significant interactions at the provenance level resulting from changes in ranking at the different test sites would suggest the necessity of re-defining the breeding zone. Smaller sub-zones would be required unless one or more broadly adapted provenances could be identified in the test. If sub-division of the initial breeding zone were necessary, the initial number of selections in the new zone could be reduced to the point where it could become necessary to augment the breeding populations through new selections.

The test should indicate provenances to be favoured in the re-selection for the establishment of the final breeding population. If it should become necessary to broaden the genetic base beyond the provenances involved in the test, some general guidelines for selection have been suggested by Shelbourne and Campbell (1976). Populations near the centre of the zone should provide genotypes more stable than selections from populations near the edges of the zone. Selections from near the boundary of the more severe climatic zone should provide more hardy genotypes with less growth potential, while selections near the edge of the milder climatic zone may provide greater growth potential at the cost of some degree of hardiness.

The amount of genotype x environment interaction would also have an impact on the production population derived through roguing from the test. If interaction is not significant, a single orchard would suffice; a large interaction would, of course, necessitate that two or more tests be developed into orchards made up of selections adapted to the specific environments of the new breeding zones. Secondly, it could reduce the genetic diversity of the orchards. In a production population, however,

this is less important. The orchards would be expected to produce seed for only a limited period of time. They would be replaced as soon as more advanced generation orchards came on line. Another possibility would be to use the "advancing" front orchard design suggested by Libby (1969), and add new selections to the orchard as new genotypes were identified in later tests.

CONCLUSION

In conclusion, let us go back and look at the broad subject of provenance testing -- provenance testing not only as it related to breeding. Let me make clear that I feel the broad rangewide survey type provenance testing should be continued. They are necessary if we are to understand fully the overall genetic makeup of our species, and if we are to understand the genecology and adaptive capabilities of our trees. Much research is needed if we are to understand how our species interact with the complex factors of the environment; the type of narrow sampling I have described will not be suitable for that type of research. On the other hand, with tree improvement through breeding as the goal, broad range provenance testing and great efforts to identify the best superior provenance will be costly, time-consuming waste. Rather we should invest our time and money in intensive examination of progenies from within the region that is of direct interest to the breeder.

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DISCUSSION

Dr. C. Heimburger. On page 9 of the manual you said something about the variation in site traits over a very small area. That I think is a very important thing. It is found all over the boreal forest. From personal observations yesterday and before that for many years I think it is found all over the boreal forest and we have to live with it. We have to include these variations in our tests. I think it is a valuable attribute. The text books on statistics always say that your test area has to be as uniform as possible. I do not think that is necessary. I think that a progeny or a provenance that mopes all over both good sites and poor microsites in the same area is no good. The one that is flexible and the one that adapts itself and shows variation over the test area is good. That is what we want in our breeding material and that is a favourable attribute for the conditions in the north.

Dr. Hans Nienstaedt. I agree with you. We have sometimes been fortunate and have found adaptable provenances, but I am sure that we can achieve the same thing by maintaining sufficient variation in our breeding populations and production populations. I think I would interpret what you are saying to mean that we must maintain a large amount of genetic variation in our breeding populations. We must accept the fact that we are not going to get absolutely uniform production all over our sites. You are going to have a situation where you will not achieve the maximum improvement that you could on the very (Interrupted by Dr. Heimburger).

Dr. C. Heimburger. No, we do not understand each other. The area that I talk about is between 25 and 100 ft^2 and has variation, and if you have a forested area a certain genotype planted over such an area would respond to such small differences in site. And on the scale which you are setting for your pre-commercial thinning as we do on the west coast it would also eventually help natural expression of dominance. It will produce more yield, more wood.

Dr. Hans Nienstaedt. O.K. now I understand. No disagreement.

Dr. C. Heimburger. No disagreement. Good.

Dr. P.P. Feret. It comes to mind that specific or general combining ability may override provenance effects. Would you care to make an extension of the same argument against plus tree selection, looking at it from the point of view of what happens if you just breed true lines.

Dr. Hans Nienstaedt. We now feel very dubious about plus tree selection. I would not say that we would use simple random picking of the parent trees. I think that if there is anything to genetics some degree of selection is worthwhile, but I would not spend a big effort on phenotypic selection. Our approach now is that where we have test results that identify superior stands we select good dominant trees in such stands without any measurements for comparison with adjacent trees. Lacking test information we pick our parent trees in good stands. That is how we establish our first generation breeding population. And I think the sooner we get to that the better off we are. In the past we have spent much too much time on the phenotypic selection of plus trees. From my personal experience, the only time I have phenotypically selected a white spruce that was genetically superior was in a plantation of unknown provenance growing on a fairly uniform site. It was 26 years old and I selected eight trees. Six of them are persistently among the top 15 in our progeny tests. That is in our situation; it may be different in a different situation. I was thinking of the lodgepole pine. I saw the other day photos of trees with tops, God knows how many feet above the canopy. I find it hard to accept that that would not be a very important type of selection, if that is possible.

THE ROLE OF SEED ORCHARDS IN FOREST TREE IMPROVEMENT

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ABSTRACT

Today tree improvement programs are receiving greater support across Canada as the need for genetically improved seed is recognized. This paper deals with the rationale behind the development of seed orchards. It describes the basic concept of seed orchards and divides them into two systems; the production orchard and the breeding orchard. Orchards can be either clonal or seedling in nature. An example of program development for a seedling seed orchard of black spruce to supply a planting program of one million seedlings per year is outlined. The use of rooted cuttings appears to be a promising technique for the near future in progeny testing the full-sib families from seed orchards and in mass producing these good families, particularly when genetically improved seed is in short supply.

RÉSUMÉ

Aujourd'hui l'on soutient de plus en plus les programmes d'amélioration des arbres à travers le Canada, à un moment où l'on devient conscient de la nécessité d'utiliser des semences génétiquement perfectionnées. Cette communication concerne la raison d'être du développement des vergers de semence. Nous évoquerons le concept de base de verger de semis que nous divisions en deux systèmes; le verger de reproduction et le verger de production. En ce qui concerne les vergers, il peut s'agir soit de clones ou de plantes de semis. Nous proposons un programme de développement pour un verger de semis composé d'épinette noire qui recouvre la plantation d' I million de graines par an. Il semble que la technique d'implantation de boutures soit prometteuse dans le futur proche pour l'analyse de la descendance des géniteurs de même famille provenant de vergers de semence, et pour la reproduction massive de ces bonnes familles, tout particulièrement quand les graines génétiquement améliorées sont en quantité insuffisante.

INTRODUCTION

WHY SEED ORCHARDS?

Emphasis on forest regeneration is increasing dramatically in Canada due to the anticipated growing demand for wood, predicted wood shortages and a reduced land base available for forest production. To meet future requirements, the area to be artifically regenerated must increase; and yield per unit area per unit time must rise, not only through greater silvicultural management, but also through the use of genetically improved seed. It is expected that the area to be artificially regenerated will increase from the 240 000 ha of 1977 to 420 000 ha by 1987 and seed requirements will rise from 4.1 billion to 7.3 billion (Morgenstern and Carlson 1979). Programs to obtain the improved seed are currently receiving increased support across the country in funds as well as manpower.

Improved seed can be obtained through proper provenance selection, superior stand and superior tree selection. Increasingly the management forester is questioning the planting of 'green junk' that originates with seed from uncontrolled collections. In some parts of Canada, seed is still bought 'across the counter' from contract pickers. There is no assurance that this seed was collected from superior stands or indeed that it was picked from the same site region or seed source area. Provenance trials have shown the importance of using the proper seed source. Morgenstern (1975a) stated that 'our native species have differentiated and adapted to the regional climates in which they occur'. In spruce (*Picea* A. Dietr.) a movement of 3 degrees latitude and 300 m elevation from the place of origin can result in losses of 6-17% in survival and height growth (Morgenstern and Roche 1969). Progeny and clonal trials have also shown that there is a significant variation among stands and among trees respectively, thus supporting the need for selection programs to obtain genetically improved seed. In jack pine (*Pinus banksiana* Lamb.), there is a potential difference of 5-15% in height growth of 10- to 20-year-old stands with seed selected from different areas within the same site region (Yeatman 1979). Clonal tests of pines have shown relatively high heritabilities for form characteristics and moderate heritabilities for growth (Yeatman 1974, Zsuffa 1975). Selected stands for seed collection and seed production areas will serve as an interim measure for some improvement, but material from superior trees when placed into a seed orchard will ultimately provide the greatest genetic gain. Carlisle and Teich (1971) showed that for as little as a 2-5% gain, intensive tree improvement programs such as seed orchard development could be justified; however, gains of up to 40% in volume have been obtained from orchard material (Wright 1976).

Due to the long-term nature of seed orchards and the time, money and effort required, detailed planning for the development and subsequent management is a necessity. It is essential that the most effective, most efficient procedures be used in determining the selection of material to enter the seed orchard program, the type of orchard to be established, the best design and spacing of the orchard, the best method of testing the material in the orchard and the type of management procedures that will provide the greatest volume and best quality seed. The benefits to be

derived from seed orchards are directly dependent upon all of these factors. If the procedures used are not efficient and effective, all efforts will be wasted; if they are efficient and effective, considerable gain can be realized.

BASIC CONCEPT OF SEED ORCHARDS

The premise for establishing seed orchards is that above-average material, when brought together, will produce seed that is superior to that from the original stand from which the selections originated. This premise assumes that the desired characteristics are inherited and that the offspring will exhibit the same favourable traits, but different characteristics have different heritabilities (Yeatman 1974, Zsuffa 1975). The lower the heritability, the greater the need for progeny testing to verify the superiority of the orchard material.

The orchard itself can be a production orchard with the prime purpose of producing large quantities of good seed in the shortest possible time or it may be a breeding orchard where experimental controlled pollinations are carried out to determine the best interspecific and intraspecific hybrids for future regeneration programs. Ideally in a complete tree improvement program, both orchards are present. The material in either of these orchards can consist of one species from within a given provenance or site region selected for features such as superior growth and form, or trees with resistance to a given insect or disease, or selections of two or more species for the production of species hybrids. Production orchards may be either seedling or clonal orchards, whereas breeding orchards are normally clonal.

THE PRODUCTION SEED ORCHARD

The production seed orchard is established to produce genetically improved seed that will fulfil the requirements of an artificial regeneration program. The quality and the quantity of seed produced is primarily dependent upon the genotypes in the orchards; however, the seed can be improved by isolating the orchard from outside wild populations thus reducing the contamination of seed with foreign pollen, by selecting an area that has a favourable climate, soil and moisture conditions; by designing the orchard to minimize inbreeding; and by intensive cultural practices such as fertilization, irrigation and pest and weed control (Morgenstern 1975b). The size of the orchard is dependent upon anticipated seed requirements.

Seed from the orchard should produce seedlings that are suitable to the environment in which they will be growing. Most orchards are established with material selected from within the same geographic source or site region. For example, in Ontario, production orchards are being established for each site region that has a large enough regeneration program to merit such an orchard. Conceivably, some site regions should be further subdivided and orchards established within the subdivisions.

In most cases, the material selected for a first generation

orchard originates from good natural stands, but in some parts of the country the good stands have been removed or so degraded that selections must be made from either plantations or remnant stands. Unfortunately, many of the plantations do not have good seed source identification and may come from an adjacent site region or from a collection of a small number of trees. Remnant stands often consist of only a few trees that remained after a cutting operation and are probably genetically inferior to the quality of the original stand. If possible, selections should not be taken in these types of stands due to potential problems of improper seed source, inbreeding and/or genetically inferior stock. It is most desirable to select plus trees from natural stands as the offspring will be adaptable to the same environment and will have the capability of performing at least as well as their parents.

The amount of genetic gain obtained from a production orchard is dependent upon the selection procedures used for obtaining material, the characteristics of the species in question and the method of propagation for establishing the seed orchard. Two common methods of selecting plus trees are the absolute standards or intensive method of selection and the comparison tree or extensive method of selection (Morgenstern 1975b, Rauter 1979). The first is based on a site-index curve and is particularly useful for species such as white pine (*P. strobus* L.) and white spruce (*Picea glauca* (Moench) Voss) that occur in mixedwood and/or unevenaged stands. With this method, a high selection differential is used and relatively few specimens are selected. The specimens are propagated by grafting scions and establishing a clonal seed orchard. Although cost of selection and establishment is high, potential gains are also high. In the second method, the candidate tree is compared to trees in the vicinity and is most effective with species such as black spruce (*P. mariana* (Mill.) B.S.P.) and jack pine that occur in pure, evenaged stands. With this method, a relatively low selection differential is used and many specimens are selected. Seed is collected from each specimen and the resultant seedlings established into a seedling seed orchard. Cost of selection and establishment is relatively low and large areas of orchard can be established over a very short period of time. When calclating total gain and rate of gain for both clonal seed orchards and for seedling seed orchards considering variations in heritabilities, selection intensities and other factors, neither one is superior in all instances (Namkoong et al 1966).

Clonal or Seedling Seed Orchard?

Both clonal and seedling seed orchards have a role to play in an improvement program. Each type of orchard has advantages and disadvantages that must be weighed against the characteristics of the species for which the orchard is being designed.

The original concept of seedling seed orchards as proposed by such authors as Wright (1959) and Goddard (1964) was received, for valid reasons, with much skepticism (Zobel 1964, Toda 1964). The major advantage of the concept put forth was that the progeny test and the seedling seed orchard could be combined into one plantation. The progeny test was established in the normal manner with a replicated randomized complete block design and with a small number of trees per plot. The test was evaluated

several years after establishment and then thinned to the best trees in the best families and converted to a seedling seed orchard. The major concerns were that the optimal site for a seed orchard is considerably different from that of a normal planting in an artificial regeneration program; that management for seed production is different than that for optimal growth; and that spacing, even if one- to four-tree plots are used, becomes very erratic after thinning. Figure 1 illustrates the spacing of 15 families in one replication of a progeny test cum seedling seed orchard before and after thinning (Goddard 1964).

Two of the major concerns can be overcome by simultaneously establishing seedling seed orchards and progeny tests on sites suitable to both and with appropriate spacing, design and management methods for each (Morgenstern 1975b, Rauter 1979). Different layouts and designs have been proposed specifically for seedling seed orchards (Klein 1974, Rauter 1979). The one proposed by Rauter overcomes the problem of erratic spacing after the first thinning by planting clusters of four individuals from four randomly selected families. Figure 2 illustrates the spacing before and after thinning using the same number of families and the same number of trees as Goddard in Figure 1, but using a different planting design. When using this method in practice, a much larger number of families would be incorporated into the orchard. This design allows for 75% removal of material on the basis of progeny tests, yet it retains relatively good spatial arrangement of the remaining trees. The major shortcoming of simultaneous progeny test - seedling seed orchard establishment is that families rather than individual genotypes are being evaluated.

Several species characteristics can favour the establishment of seedling seed orchards. Those that occur over large tracts of land as relatively pure, evenaged stands lend themselves to the selection procedures of choosing large numbers of trees at relatively low selection intensities and low cost. Species that start to flower at an early age can take advantage of a rapid turnover of generations for subsequent selection and improvement. Since large areas of orchard can be established at minimal cost, this method suits species that require large volumes of seed to satisfy the needs of a regeneration program. Black spruce and jack pine are two species that have all of these features and are best suited to the seedling seed orchard approach in the first generation of improvement. Other species, such as Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), have problems with graft-stock incompatibility and are also suitable for seedling propagation.

Many seedling seed orchards are already established in Canada (Morgenstern and Carlson 1979). One of the earliest is a Douglas-fir orchard planted in 1963 in combination with clonal material. Orchards for other species include white spruce, black spruce, jack pine and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.).

Most of the orchards in Canada consist of selected phenotypes brought together into a clonal orchard. Although the cost of establishment is high, several factors favour the development of this type of

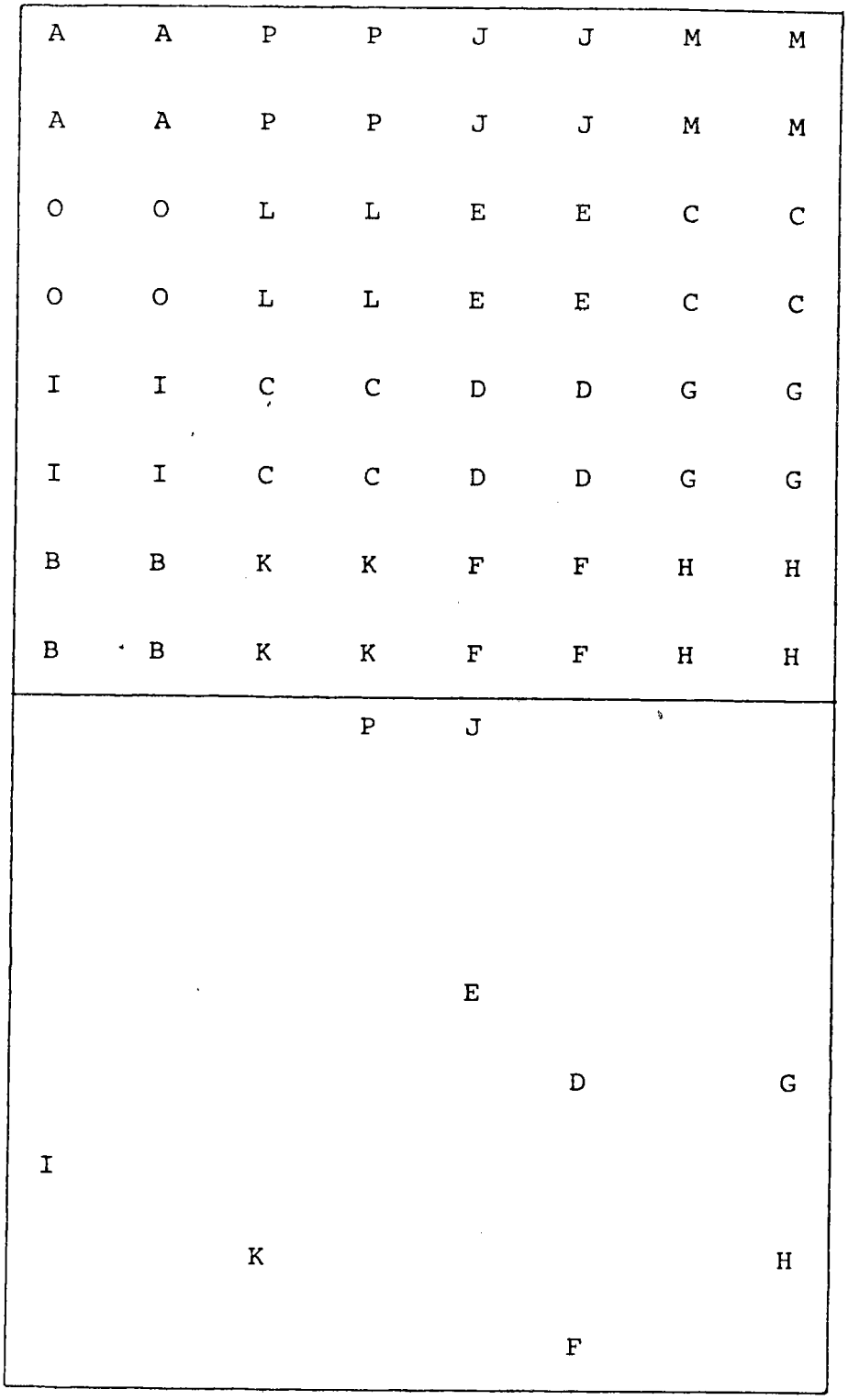

Fig. 1. One replication of a progeny test converted to a seedling seed orchard before and after thinning. (Goddard 1964).

A	P	J	M	A	B	C	D
O	F	F	C	E	F	G	H
I	C	D	G	I	J	K	L
B	K	L	H	M	O	P	C

D	A	O	C	K	P	C	F
P	J	F	I	D	A	G	L
B	M	C	E	B	C	E	H
G	L	H	K	I	M	J	O

	P	J					D
					F		
		D			J		
	K					P	

					P		F
P		F					
G			K	I		J	

Fig. 2. Seedling seed orchard before and after thinning using same number of families and trees as Goddard (1963), but different design and spacing.

orchard. Since each tree is vegetatively reproduced normally through grafting, each ramet is genetically identical to the original selection, and greater gains are attainable than with seedlings that result from wind-pollinated collections. For most species, the time between plus tree selection and flower production in the orchard is much shorter in grafted clonal orchards. Through the progeny testing of clones, the combining ability is determined for each parent; and the genotype, rather than the phenotype, becomes known. If full-sib progeny tests are established, then the material can be used for advanced generation selections as well as for roguing the orchard to the best clones. A major problem with grafting has been the incompatibility of scion and rootstock in such species as Douglas-fir and red pine (*P. resinosa* Ait.). If alternative methods to standard grafting techniques can be found such as the paired-rooting technique for Douglas-fir (Brix and Barker 1971), clonal orchards may still be practical, otherwise the approach for improvement should be changed. Some of the graft incompatibility in other species is due to the use of poor quality scions or scions collected from overmature trees. This problem can largely be overcome by careful treatment of scions during shipment and storage and by selecting younger, more vigorous trees.

Some species such as white pine and white spruce are particularly suited to clonal seed orchard establishment as they often occur in uneven-aged, mixedwood stands where the intensive plus tree selection method is favoured. Since the selection cost per tree is high, the number of selections low, and since maximum genetic gain is through clonal orchards, this approach is favoured. Cone crop periodicity for some species is several years, thus if the trees are in a stand scheduled for harvesting, they may be destroyed before a good seed crop is produced. Further, even if a good cone crop is present, some species shed their seed within a few days of maturity and timing of collections is critical. Species with these characteristics are more suitable in a grafting program as scions can be collected over several months throughout the winter. Flowering maturity for some species is 20 years or more from seed, but can be reduced to less than 10 through grafting scions from mature trees.

Even though the seedling seed orchard is recommended for species such as black spruce and jack pine in the first generation of improvement, selections can be made in the progeny tests and scions collected for clonal orchard establishment for advanced generation selection and breeding.

Site selection is an important factor in the establishment of any orchard. Clonal orchards are often planted south of the area of intended use as the milder climate induces larger seed yields in fewer years, but care must be exercised to isolate the orchards from all species that may hybridize with the material in the orchard thus causing serious contamination problems. Seedling seed orchards are normally planted in the site region of use. Although isolation from surrounding stands of the same species is maintained when possible, it is not as critical a problem as when the orchard is established in a different site region.

THE BREEDING ORCHARD

The breeding orchard is not designed to produce large quantities of seed for regeneration programs, but rather to provide a foundation for obtaining new combinations and new genotypes that will be suitable for future programs. The selections in the breeding orchard are of more diverse origin than the material in the production orchard. They may be representative of 1) the clonal production orchard for preservation of genotypes in case of destruction by such agencies as wind or fire, or for ease of controlled pollinations for the purpose of progeny testing, 2) genotypes that are not currently desirable in the production orchard, but have traits that could be useful in the future, 3) plus tree selections of species that do not currently have a sufficiently large regeneration program to merit an orchard, 4) several provenances for inter-provenance hybridization and 5) exotic species for interspecific hybridization to breed trees for specific environmental conditions or with resistance to a given pest.

This orchard must be established in an area remote from the production orchard to avoid possible contamination of the production orchard. It should also be intensively managed to encourage early flower production, since the sooner crosses can be made, the sooner new genotypes are available for testing.

AN EXAMPLE OF PROGRAM DEVELOPMENT FOR A SEEDLING SEED ORCHARD

To effectively determine the size of the program required, the time and manpower needed and the anticipated funding throughout the years, detailed plans must be prepared in advance. Programs are currently being drafted in Ontario for each of the major coniferous species incorporating the different systems that can be used, including seed collection areas, seed production areas and seedling and clonal orchards. Table 1 is a preliminary draft of an annual work program that is required to sustain an annual planting program of one million black spruce seedlings with seed from a seedling seed orchard. The steps required for the program are identified in advance so that preplanning for land, labour, equipment and funding is possible. As first generation seed orchards come into production, seed collection and production areas will be phased out; and as advanced generation orchards start producing, the first generation orchards will be eliminated. However, the program must be a continuing one that constantly adds new material and removes poor material so as to keep a broad genetic base and to obtain constant genetic gain.

Due to a lack of concrete data in such areas as seed yields for individual trees for different age classes and different stand densities, changes in nursery procedures (i.e. conversion from bare root to container stock) and advances in seed orchard management (i.e. increased cone yields through use of gibberelins), this draft program is based on a number of assumptions using the averages of data currently available. Following are some of the assumptions made in calculating the figures for Table 1. Assuming seven seeds are required to produce

Table 1. Seedling seed orchard development (first generation orchard)

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Collection Series	#1			#2		#3																		
Selections required	400			400		400																		
No. cones collected (M) (200 cones/tree)	80			80		80																		
Seed extracted (M) (13 viable seed/cone)		1040			1040			1040																
Seed sown in containers (M) (700 seed/tree)			280			280			280															
Tot. no. seedlings to ship (M)					140			140			140													
No. seedlings for S.S.O. (M) (50 trees/family)					20			20			20													
No. seedlings for progeny test (M) (100 trees/family)					40			40			40													
Seedlings for regen. prog. (M)					80			80			80													
Total S.S.O. area required (ha)			22.5																					
S.S.O. area to site prep (ha)				7.5			7.5			7.5														
S.S.O. area to be planted (ha)					7.5			7.5			7.5													
No. trees planted in S.S.O. (M)					20			20			20													
Total S.S.O. area to be managed (ha)					7.5	7.5	7.5	15	15	15	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
No. trees thinned from S.S.O. (M)													15			15			15					
No. trees remaining in S.S.O. (M)													5			10			15					
Selection of plus trees for advanced generation orchard													#1			#2			#3					
Seed coll. from S.S.O. (M) (13 v.s./cone, 125 cones/tree)															4400*			11700		19000				22000
Progeny test area to site prep (ha)				16			16			16														
Progeny test area to plant (ha)					16			16			16													
No. trees planted in progeny test (M)					40			40			40													
Survival evaluation					#1			#2			#2													
Measurement and evaluation									#1			#1&2			#2&3			#3						

* 1st cone collection based on 75 cones per tree and 13 viable seed per cone

one shippable seedling and that the seed crop periodicity is three years, 21 million seeds are required every seed year to sustain a one-million-tree planting program. It is assumed that the seedlings in the orchard will produce 125 cones sustaining 13 viable seed per cone (Morgenstern 1975b) every seed year, thus producing 1 625 seeds per tree. If the thinned orchard as shown in Figure 2 contains 665 trees per ha and if 90% of the trees flower in the seed year, the number of seed produced is 972 500 per ha. Thus, the total area required to supply the volume of seed is approximately 22 ha. If some of these assumptions are changed, the size of the orchard program should change accordingly. For example, if nursery procedures are altered and the ratio of seed to shippable seedling drops from 7:1 to 3:1, the size of the orchard program would drop considerably. If the periodicity could be reduced from three to two years by advance in seed orchard management, then the size of the orchard would drop by one-third. If these two modified factors are used, then the number of plus tree selections required per seed year would drop from 400 to 115, the number of seedling selections for the seed orchard from 20 000 to 5 700, the area of seed orchard established from 7.5 ha to 2.1 ha and the number of seedlings for progeny tests from 80 000 to 23 000. Given this information, it is up to the production manager to determine how money and manpower can best be used in optimizing the quantity and quality of seed and the subsequent use of the seed produced from a seed orchard.

FUTURE DIRECTIONS

Zobel (1964) stated that since improved trees are developed for future use, those concerned with tree improvement and genetics must be the most forward looking of all. Researchers must be the creators of new ideas and new ways to obtain the gains that are required. Often, they will have to take calculated risks to obtain material suitable for future regeneration programs. Production managers must be willing to accept the changes based on the recommendations of the researcher. Both must work closely together to take advantage of information as it becomes available and to obtain the greatest gain with minimal effort and minimal duplication.

An example of a proposed change from that of current practice is an alternative scheme to the conventional means of progeny testing a clonal seed orchard. Normally, wind-pollinated or controlled-pollinated seed is collected from individual clones and seedling progeny tests are started to evaluate the parent material. By means of vegetative propagation through the rooting of cuttings, it is possible to multiply each seedling and to establish clonal trials rather than seedling trials. This method is advantageous when insufficient numbers of seed per family are available to carry out the conventional progeny trials. Since fewer seeds are required, the size of the pollination program can be reduced and the start of the testing program hastened. Also, since rooted cuttings are genetically identical to the cutting donor, full advantage can be taken of genetic gain and the best clones mass propagated for the regeneration program. Figures 3 to 5 illustrate the greenhouse facilities and the rooting development in a black spruce cutting.

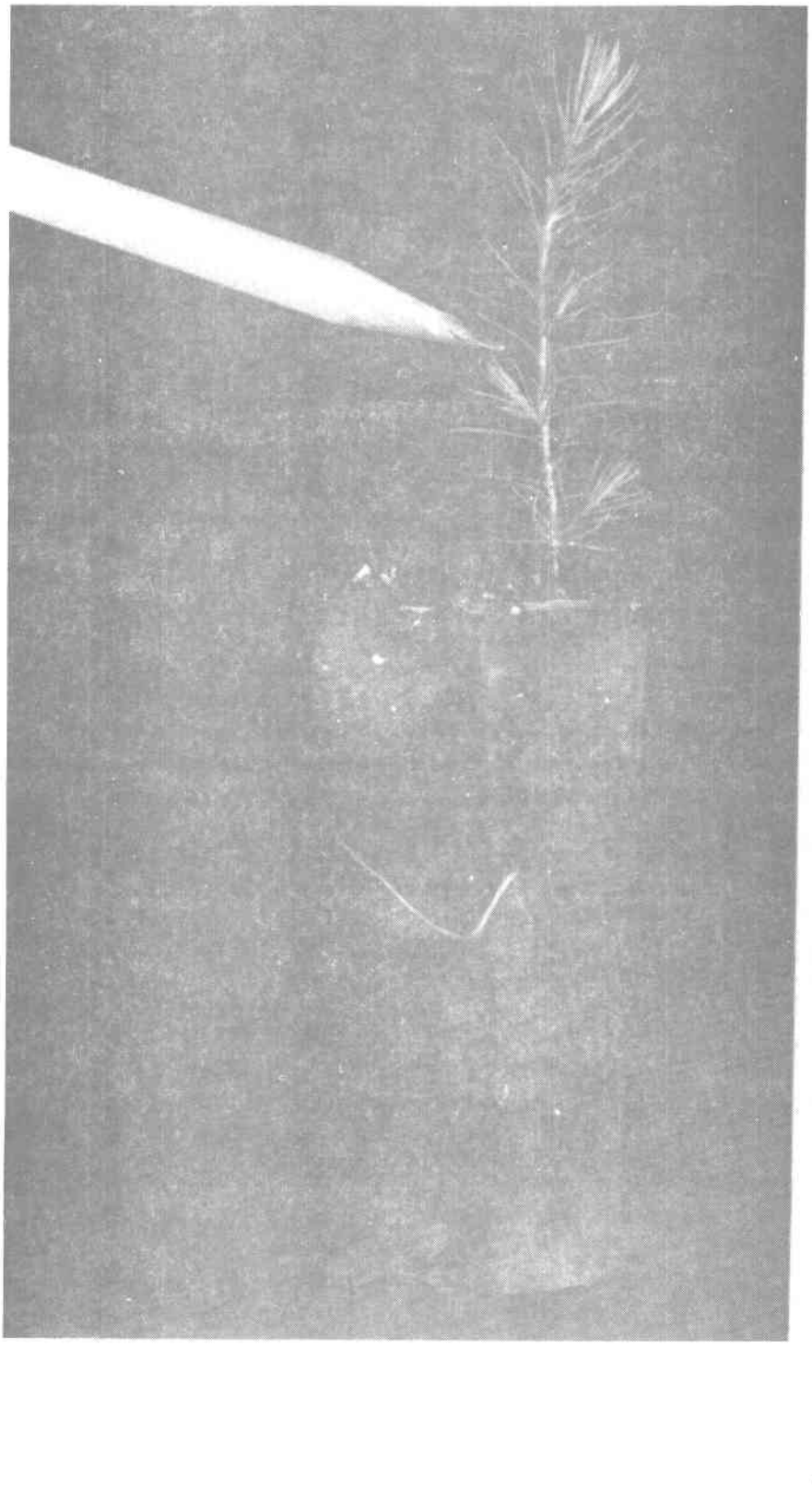

Fig. 3. 10-week-old black spruce seedling. Pencil tip indicates point where seedling is to be cut.

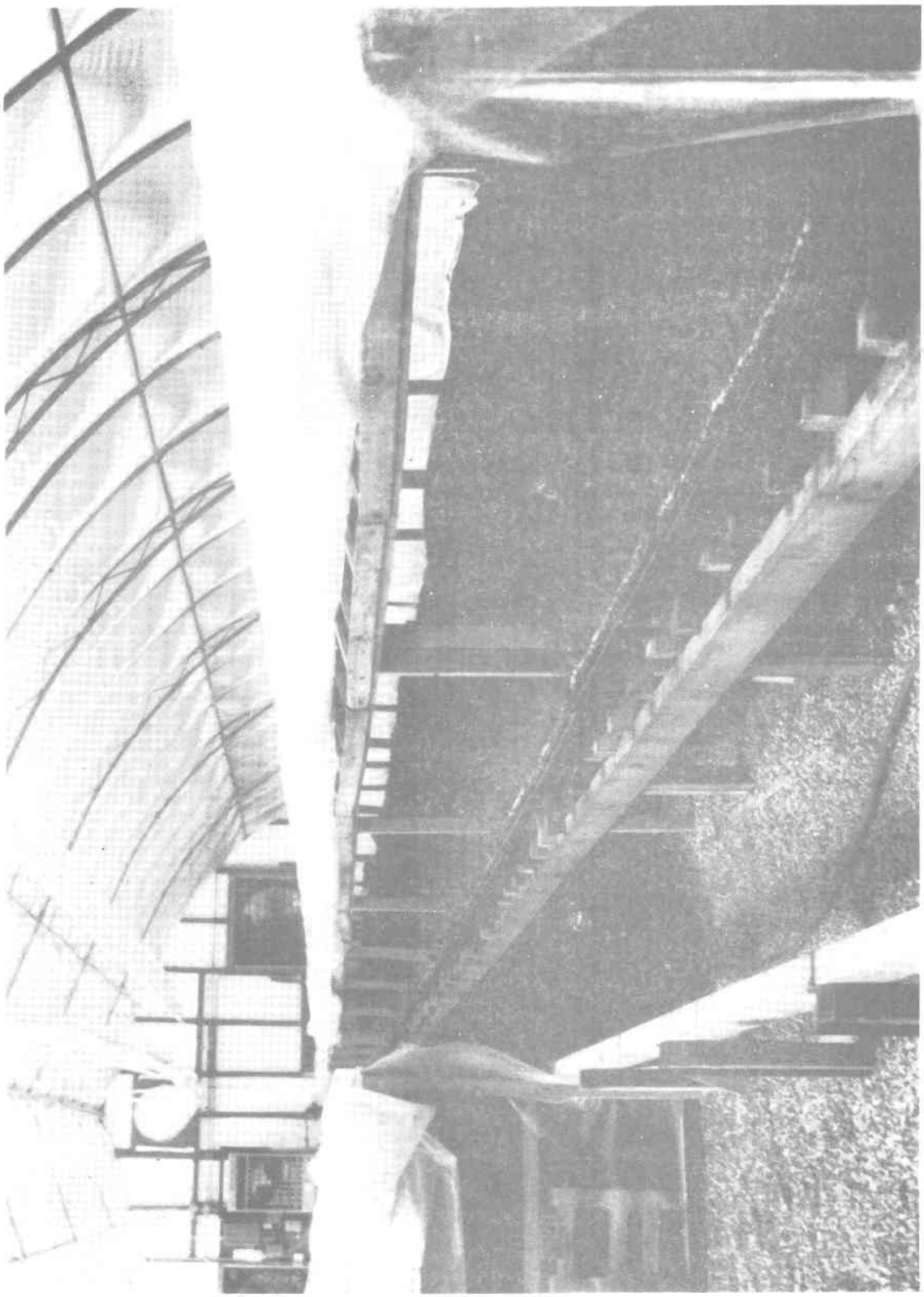

Fig. 4. Rooting beds.

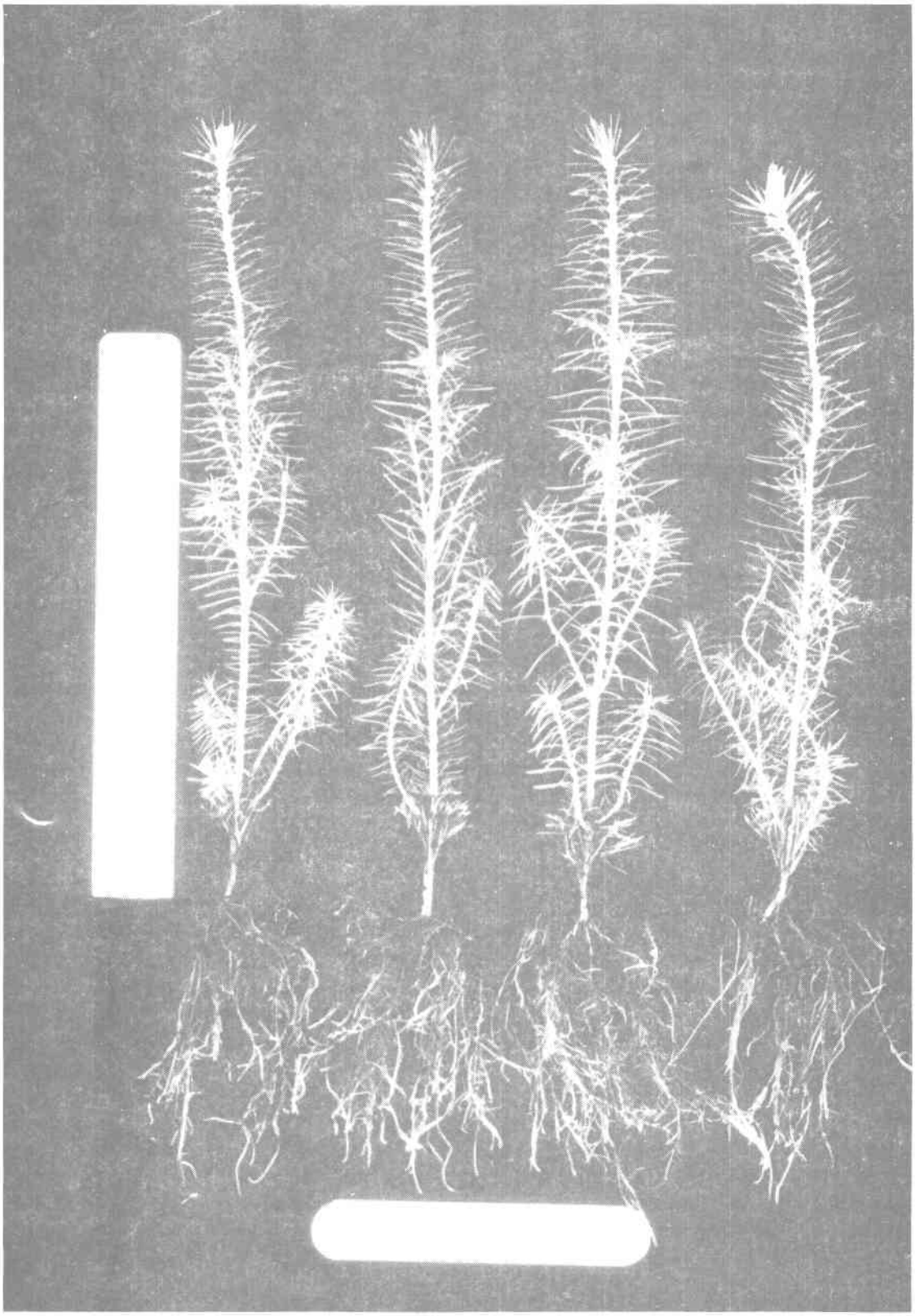

Fig. 5. 15-week-old rooted cutting.

This alternative scheme is proposed initially for black and white spruce. In Ontario, the vegetative propagation program for the rooting of spruce cuttings was initiated in the 1960s (Rauter 1971). Techniques and rooting conditions have been constantly changing and rooting averages improving. Results from the 1979 program of rooting cuttings for juvenile seedlings (seedlings less than 1-year-old) (Armson et al. 1975) indicate 98.5% rooting from 85 000 seedlings (Fung pers. comm.). As a result of the success of this program, the 1980 material will consist of seed from controlled pollinations of two clonal seed orchards. The current proposal is that 25 seedlings from each of 25 families for each orchard will be raised, thus producing a total of 1 250 clones. It is anticipated that each seed will yield 100 rooted cuttings within 12 months of germination (Perez de la Garza 1977). Trials will be established not only to evaluate the parent material in the orchard, but also to select superior individuals from the clonal trials for mass vegetative propagation for the artificial regeneration program.

Modifications in techniques such as that described above can provide significant genetic gain with a reduced input and a shorter time period. These 'short cuts' become crucial when one realizes the staggering work load that is still ahead of all of us in any of the tree improvement programs currently underway or under development in Canada. During the past few months, I have spent much of my time trying to sort out how the Ontario Ministry of Natural Resources can best obtain genetically improved seed for the future. I am quickly realizing the size of the program that is required to produce the volume of seed needed. It is up to us, the researcher, the geneticist, to provide management with the most effective procedures that will ultimately supply the artificial regeneration program with the best seed in the shortest possible time.

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DISCUSSION

Mr. W. Burry. It seems that what you have accomplished to this point in your research on super-seedlings is not a large scale cloning of individual seedlings but rather a developmental stage in rooting techniques for scions of seedling origin. From your slide presentation you are not actually cloning this material yet, as you have made only a single copy of each seedling.

Miss R.M. Rauter. Yes, we have made a genetic copy of the seedlings. In this program we used a bulk seed lot. One of the reasons we received support for this program was that we ran into a desperate shortage of black spruce seed in one of our major clay-belt site regions. We showed that the rooting program worked. Recently, the Ministry hired a young forester who works only on this program. He does vegetative propagation. He got a 98.5% take the first time around. We have taken a second group of cuttings. These are coming along and it looks like rooting is over 90%.

Next year we will take some of our control-pollinated seed and put that out to germinate. The material will be rooted, cloned and tested. The best clones can be continually reproduced through vegetative propagation. Based on an estimate of family performance and based on the number of clones we are testing per family, we are then able to return to the original orchard and evaluate some of the parents. We can say that one particular parent when combined with different males did well and we will keep the parent; or that it did poorly and we would like to remove it from the orchard program. We may also want to do some selection within that particular family for our subsequent orchard. We will continue to select our best material and reproduce it through the rooting of cuttings. The results described here were for run-of-the-mill material to see if the program worked. It seems to have worked very well. From now on, we will be using only superior material identified by families.

Dr. H.S.D. Swan. May I hope that all this material you have so kindly shown us this morning is going to be in your paper because it could be invaluable to anyone who is waiting to follow along the same lines.

Miss R.M. Rauter. I was not planning to put the pictures in but I will have the tables. Would you like to include the pictures?

Dr. H.S.D. Swan. Please include the tables and the techniques. They will be valuable to all these people who want to work on these lines.

Miss R.M. Rauter. O.K.

THE ROLE OF EXOTIC AND HYBRID POPLARS IN THE POPLAR IMPROVEMENT PROGRAM OF CANADA

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ABSTRACT

Exotic species and hybrids have a prominent place in Canadian breeding programs. Exotic species have expanded the range which native species offer in site adaptation, pest resistance, rooting ability of cuttings and biomass production. These attributes, together with hybrid vigour (heterosis) have led to the development of many good hybrid combinations of exotic and native species from which excellent clones have been selected and widely propagated for forest production.

Exotic and hybrid poplars have been planted in Canada for more than two centuries. Some of these have spontaneously hybridized with native species. However, the influence of this introgression on the genetic material of native poplar is local and minimal.

RÉSUMÉ

Les espèces de peupliers exotiques et hybrides occupent une place de premier rang dans les programmes canadiens de reproduction de peupliers. Les espèces exotiques ont élargi les possibilités que les espèces indigènes offraient dans leur adaptation au terrain, leur défense contre les insectes, les capacités d'acclimatation des boutres, et les qualités des matières organiques. Ces espèces, ainsi que la vigeur des hybrides reproducteurs (hétérosis) ont donné naissance à de nombreuses espèces hybrides exotiques ou indigènes de bonne qualité et ont débouché sur des clones excellents et très largement utilisés.

Des peupliers exotiques et hybrides ont été plantés au Canada depuis plus de deux siècles. Certains d'entre eux se sont spontanément hybridés avec des espèces indigènes. Toutefois, l'influence de cet apport dans la nature génétique de peuplier n'est que locale et minime.

INTRODUCTION

Exotic and hybrid poplars have been used as ornamental trees and grown for their timber and other qualities for many centuries and in many countries around the world. The reasons for their almost universal

spread are: ease of cloning by rooting of cuttings, ease of hybridization, fecundity, fast and ubiquitous growth and versatility.

The oldest exotic poplars known are the fastigiate varieties. Lombardy poplar (*P. nigra* L. var. *italica* Duroi) originated in central Asia, and was known in Italy and France as an ornamental tree prior to 1750. Presently, it is cultivated on all continents (Zsuffa 1974). *P. nigra* L. var. *thevestina* Dode and *P. alba* L. var. *bolleana* Lauche also originated in Central Asia and were spread in Middle Ages by Arabs, Moghuls and Turks through West Asia, the near East, North Africa and southeastern Europe. In addition to their ornamental qualities they are also important timber trees (Allegri, 1962).

The interspecific hybrids of poplars, created and cultivated by man, are a first among forest trees. According to Muhle Larsen (1960) the history of *P. deltoides* Marsh x *nigra* hybrids goes back 300 years, to the time when the first trees of eastern cottonwood (*P. deltoides*) were imported into France from southeastern Canada. There they freely interbred with the native black poplar (*P. nigra*) and gave rise to hybrid seedlings noted for fast growth, good form and ease of vegetative propagation by cuttings. The description of the earliest cultivar of this hybrid (*P. serotina* Hartig) dates back to 1775. Soon, the descriptions of others followed and in 1795 the collective name of *P.* x *canadensis* Moench was given to *P. deltoides* x *nigra* hybrids (Zsuffa 1975). They spread rapidly through many countries and were used as ornamental and timber trees. As the interest in poplars increased, species and varieties were studied and breeding programs initiated.

In this paper the role and significance of exotic and hybrid poplars will be discussed in the light of poplar breeding objectives in Canada, and examples of Canadian programs will be outlined.

EXOTIC AND HYBRID POPLARS IN CANADA

Exotic and hybrid poplar varieties have been known in Canada for more than two centuries. The oldest exotic poplars planted are Lombardy poplar and silver poplar (*P. alba* L., chiefly a female variety with irregular branching and a large crown). The oldest known hybrid is the "Balm of Gilead: or "Ontario Poplar" (*P. Ontariensis*, Desf. syn. *P. candicans*, Ait, a selection from *P.* x *Jackii*, Sar., *P. balsamifera* L. x *P. deltoides*, hybrid introgression). It has been cultivated since 1755 (Rehder, 1940) and planted on many homesteads. Carolina poplar (*P.* x *euramericana* (Dode) Guinier cl. *Eugenii* (Simon-Louis) Schelle), an important hybrid, has been in Canada for more than a century (Zsuffa and Calvert 1976) and is widely spread as an ornamental and timber tree.

Settlers used native poplars for shelterbelts and amenity purposes on the prairies of Manitoba, Saskatchewan and Alberta since before the turn of the century. However, these lacked the essential characteristics for protection from strong winds and drifting snow, such as low branching crowns and drought resistance. Pioneer horticulturists and tree

geneticists embarked on importing and developing more suitable varieties. Efforts were concentrated at the Agriculture Canada Research Station at Morden, Manitoba (Anon. 1974) and at the Federal Government's Tree Nursery at Indian Head, Saskatchewan (Cram, 1968). Skinner (Roller 1968) directed most of the research in Manitoba and Cram (Cram 1968) in Saskatchewan. Clones of imported and newly developed hybrids have been in test plantings at Indian Head Nursery since 1942, and large quantities of cuttings have been produced and distributed annually. Commonly planted varieties have been Berlin poplar (*P.* x *berolinensis* Dipp, a *P. laurifolia* Ledebour x *P. nigra* var. *italica* hybrid), Russian poplar (*P.* x *Petrowstryana* Schneider, a *P. laurifolia* x *deltoides* hybrid), Dutch poplar (*P.* x *euramericana* cv. *Gelrica*), tristis poplar (*P. tristis* Fischer), Brooks poplars (*P. deltoides* x *Petrowskyana*) as well as others.

Natural hybridization exists between many related introduced and native poplars that flower simultaneously. Lombardy poplar and Carolina poplar have likely hybridized with eastern cottonwood and balsam poplar. Vallee (1979) found trees of such spontaneous hybrid origin in naturally grown poplars, especially around old settled areas. Heimburger (1936) found natural hybrids of *P. alba* x *grandidentata* Michx. in places where native *P. grandidentata* (largetooth aspen) had grown near planted silver poplar. Ronald and Steele (1974) found evidence of natural hybridization between the Russian poplar, the native plains cottonwood (*P. deltoides* var. *occidentalis*, Rydb.) and balsam poplar. This has added a new dimension to the already complex spontaneous hybridization of poplar on the Prairies.

Poplar seed is short-lived and the germinants do not survive in weeds and on dry soils. Thus the chances of spontaneous development of large groups of hybrids between native and introduced poplars are minimal. However, on bare, wet soils, such as found in gravel pits, road cuts and embankments, poplar seeds occasionally grow into plants.

Environmental concerns and fears of changing the germ-plasm of native poplar species exist due to spontaneous hybridization of introduced exotics and hybrids. However we believe that (a) the influence of the exotics on the natural gene-pool will remain localized, restricted to urban and plantation areas, and therefore non-significant; (b) such hybridization can no longer be avoided, because it has already happened with the earlier introduced exotic ornamental trees; and (c) poplar breeders will often find excellent selections to fit their needs in spontaneous hybrids. Such has been the situation in some European countries with long histories of planting exotics, where spontaneous hybrids gave rise to numerous new clones of recognized value.

THE SIGNIFICANCE OF EXOTICS IN POPLAR BREEDING

Exotic species, combined with native species through interspecific breeding, have a significant role in producing poplar strains and clones of vigorous growth, improved disease resistance and better rooting ability of stem cuttings. By interspecific breeding, hybrids can also be produced for different ecological environments and with new biomass qualities.

Heterosis in Interspecific Poplar Hybrids

Related poplar species, when crossed, have often produced offsprings exhibiting hybrid vigor or "heterosis". The parental species that came from geographically distant areas or from regions otherwise isolated produced heterotic hybrids most frequently. Heterosis also occurred in hybrids of the parental species adapted to different ecological environments.

Many crosses between species of section *Leuce* Duby, which evolved in well-separated areas, have been noted for vigorous growth. The *P. tremula* L. x *tremuloides* Michx. hybrids drew attention on account of heterosis more than 40 years ago (Wettstein 1938, Johnson 1956). Hybrid vigor has also been commonly observed with *P. alba* x *grandidentata* and others (Heimburger 1968).

The *P. deltoides* x *nigra* hybrid of North American and European poplars is well known for its heterotic growth. The clonal varieties of this hybrid have spread all over the world and have given rise to a new timber culture. An International Poplar Commission of FAO of the United Nations was formed after the Second World War to deal with the different problems of breeding, management and utilization of the wood of what was essentially this hybrid, that seemed promising for solving the pronounced shortage of timber in many countries of the world. In 1950 the name *P.* x *euramericana* (Dode) Guinier, followed by the name of the cultivar, was accepted by the International Poplar Commission for *P. deltoides* x *nigra* and its advanced crosses and backcrosses.

Many of the hybrids between balsam poplar species (section *Tacamahaca* Spach) and intersectional cottonwood (section *Aigeiros* Duby) and balsam poplar species hybrids have shown vigorous growth (Stout and Schreiner 1933, Steenackers 1970). However, here, as well as with the other interspecific hybrids, the vigour of growth depended not only on the parental species, but also on the selection of parent trees and on the filial generations of the hybrids.

Disease Resistance in Interspecific Poplar Hybrids

Different poplar species show various degrees of resistance to diseases. According to Steenackers (1972a) *P. deltoides* is known to be resistant to diseases caused by *Marssonina* spp., *Melampsora* spp., *Septoria* spp., and *Dothichiza populea*. However, about 90% of the individuals in tested populations of *P. deltoides* are susceptible to bacterial canker (*Aplanobacter populi*). *P. nigra* is generally susceptible to *Melampsora* spp. and to *D. populea*, but it is extremely resistant to bacterial canker. Thus the *P. deltoides* x *nigra* hybrids, if derived from properly selected parent trees, can unite the resistance of both parent species and give rise to clonal varieties of multiple resistance. Another example, showing the potential of interspecific breeding of poplars for inducing disease resistance, is in *Aigeiros* x *Tacamahaca* crosses. The balsam poplars are known to be highly susceptible to *Septoria* canker (*Septoria musiva*), to which *P. deltoides* and *P. nigra* are resistant. The crosses of balsam poplars with

either one of these *Aigeiros* species thus offer a possibility for the selection and cloning of resistant individuals.

Most poplar hybrids are developed into clones. By cloning, resistant genotypes can be readily copied. However, the ability of pests to overcome the resistance of clones by evolving new races is sometimes mentioned. This will depend on the nature of resistance, biology and genetics of the pest, as well as on the availability of unimproved strains of host trees in the area. Evidence of clones remaining resistant for a long period of time exists in poplar. Clones selected 40 years ago in Italy for resistance to spring time defoliation (*Venturia populina* (Vuill) Fabr.) have remained resistant to the present. Similarly, clones selected in western Europe for leaf rust resistance have remained virtually immune for many years (Steenackers, 1972b).

Improving the Rooting Ability of Poplars by Interspecific Breeding

Some poplar species of the sections *Aigeiros* and *Tacamahaca* show very good rooting ability from stem cuttings. However, the rooting ability of *P. deltoides* varies and sometimes, under unfavorable field conditions, large numbers of its cuttings perish (Zsuffa 1977). *P. nigra* cuttings root more readily. This species transmits its rooting ability to hybrids with *P. deltoides*. In fact, cuttings of *P.* x *euramericana* clones commonly root even under adverse field conditions.

Within the poplars of the *Leuce* section, *P. alba* is the only species easy to propagate by cuttings. It creates fertile and vigorously growing hybrids with several *Leuce* species and at the same time transmits its rooting ability to the hybrids, as reported for example by Heimburger (1968) for crosses of *P. alba* with *P. grandidentata*, *P. tremuloides* and others. By backcrossing the hybrids to *P. alba* and by producing multiple hybrids of unrelated parents, such as *P. canescens* Sm. x (*alba* x *grandidentata*), Heimburger was able to produce heterogenous progenies with outstanding individuals in which the rooting ability was strengthened.

POPLAR EXOTICS AND HYBRIDS IN CANADIAN BREEDING PROGRAMS

In Canada, active poplar breeding programs exist in Saskatchewan, Manitoba, Ontario, Quebec and Newfoundland. In addition, selection and studies of native poplar are in progress in most provinces.

In Saskatchewan, poplars planted for shelter belts tend to be short-lived due to adverse climatic conditions and susceptibility to disease. Imported clones, such as Russian poplar and Berlin poplar, and their spontaneous hybrids with native plains cottonwood and balsam poplar have shown the best resistance and growth to date (Lindquist et al., 1979).

In Manitoba, Ronald[1] maintains and studies large collections of

[1] Ronald, W.G., Agriculture Canada, Research Station, Box 3001, Morden, Manitoba, ROG 1JO, personal communication, 1979.

clones of exotic, hybrid and native poplars. Some of the best clones, such as *P.* x *euramericana* cv. *Serotina de Selys*, *P. tremula* cv. *erecta*, and *P. canescens* x (*alba* x *grandidentata*) cl. CAG 135, are exotics and their hybrids. Successful breeding projects include recently produced complex intersectional hybrids, such as *P.* (*deltoides* x *balsamifera*) cl. Northwest x *P. canescens* x (*alba* x *grandidentata*). This complex hybrid possesses promising combinations of different parent species and may become a valuable source for new clones with desirable, hard-to-find characteristics.

In Ontario (Zsuffa 1979) large poplar clonal and sib collections are maintained, bred and tested. Exotics and their hybrids have a prominent place. Many successful crosses are between native and exotic species. Good results have been obtained with hybrids such as *P. deltoides* x *nigra*, *P. alba* x *grandidentata*, *P. alba* x *davidiana* (Dode) Schneid., *P. alba* x *sieboldii* Miq., *P. deltoides* x *trichocarpa* Hook, *P. balsamifera* x *nigra* and *P. deltoides* x *Maximowiczii* Henry.

In Quebec, (Popovich 1977, Vallee 1979) a collection of almost 800 clones, one-third exotics, is being studied and tested. In addition, rich genetic material exists in provenance planting. These consist of one native species - (eastern cottonwood) and two exotics (*P. trichocarpa* and *P. nigra*). The latter two species, when crossed with native balsam poplar and eastern cottonwood, have shown much promise in clonal trials. Hybridization programs are active, and the best families produced are of native and exotic species percentage, such as *P. balsamifera* x *nigra* cv. *italica*, *P. deltoides* x *nigra* cv. *italica*, and *P. deltoides* x *Simonii* Carr. cv. *fastigiata* C. Schneid.

In Newfoundland (Khalil 1978) a collection of clones is being maintained and tested. These have been selected from native species, from exotic and hybrid poplar amenity trees found in the province and imported. In nursery trials clones of exotics and their hybrids have shown much promise.

CONCLUSIONS

Exotic species and hybrids have a prominent place in Canadian breeding programs. Exotic species have expanded the range which native species offer in site adaptation, pest resistance, rooting ability of cuttings and biomass production. These attributes, together with hybrid vigour (heterosis) have led to the development of many good hybrid combinations of exotic and native species from which excellent clones have been selected and widely propagated for forest production.

Exotic and hybrid poplars have been planted in Canada for more than two centuries. Some of these have spontaneously hybridized with native species. However the influence of this introgression on the genetic material of native poplar is local and minimal.

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DESIGNING A TREE BREEDING SYSTEM

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ABSTRACT

A tree breeding system is the collection of efforts and resources invested to develop improved tree seeds. To make a tree breeding system successful it is important to plan it well and incorporate flexibility and versatility in the design. As an example, a jack pine breeding system is presented that features the coordinated population concept, which includes production, breeding, and index populations.

RESUME

Tout système de reproduction des arbres incorpore les divers efforts et ressources consacrés au développement de graines d'arbre améliorées. Il est nécessaire de bien organiser un tel système de reproduction et d'incorporer une certaine flexibilité et souplesse dans sa conception afin d'en garantir le succés. A titre, indicatif, nous présentons un système de reproduction pour le pin de Banks, qui comporte le concept de population coordonée qui inclut la production, la reproduction et les indices de population.

INTRODUCTION

At first tree breeders were primarily interested in determining the extent of genetic variance existing in natural tree populations because the existence of genetic variance was considered to be fundamental basis of breeding. They found that genetic variance existed in most tree species, and on the average 15% genetic gain in volume plus an equal gain in quality would be obtained through breeding efforts. So it is obvious that breeding is an important way to increase the productivity of forest land.

Despite past accomplishments, tree breeders are uncertain about the direction of future breeding (Thielges, 1975; IUFRO, 1976). The objectives of this paper are to discuss the causes of uncertainties and to present a concept that will help plan future tree breeding activities.

CAUSES OF UNCERTAINTIES

In recent years, an increasing number of tree breeders have pondered over multi-generation tree breeding techniques because many of their tree populations have now entered the second generation. Unfortunately, most of the techniques used by tree breeders to determine the genetic structure of breeding populations are not suitable for projecting multi-generation breeding programs (Kang 1979). Because breeders originally emphasized single generation tree breeding, there was little incentive to develop techniques that could be used for multi-generation forest tree breeding. As a result the pool of information on long-term tree breeding techniques is small.

Another cause for uncertainty is the fact that the public awareness of the importance of environment has grown only slowly, though steadily. People are especially interested in the influence of logging practices on the ecosystem. Tree breeders must explain how the new improved tree populations will harmonize with uncertain future ecosystems.

A third reason is that tree breeders' concern about gene conservation has increased since the epidemics of corn leaf blight in 1970 (NRC, 1972). Tree breeders also have seen the near extinction of American elms and American chestnut by disease and realize a species could disappear from the earth within a short time span. We are not clear on what gene conservation is, but the lack of understanding on the matter is sufficient reason for tree breeders to take the issue seriously.

Breeding objectives also change as industrial and financial needs change. "Wood for energy" is being considered for the energy shortage so if trees are proven as an energy substitute, they would have to be bred for energy as well as timber and pulp.

Thirty years ago these problems either did not exist or were not considered serious. There is no way of knowing how many more problems of this kind will occur in the coming generations, but the techniques that tree breeders have used for the first generation tree breeding cannot solve most current problems. Therefore, instead of enjoying their accomplishments, breeders are asking themselves "what methods should I use in the future?"

BREEDING SYSTEM FLEXIBILITY AND VERSATILITY

Some tree breeders have attempted to look at the overall problems that have arisen and to list the factors that tree breeders should consider in developing long range tree breeding strategies (van Buijtenen 1975; Libby 1976). A review of the lists immediately indicates that many of these factors are unanswered. The authors did not imply that their lists were complete, and it is certain that many new problems will arise with time. It is not my intention to offer answers for any of the unknowns or to add more factors to the already existing lists. I am interested in discussing the the possibility of defining an ideal structure that can be used to solve problems that will continuously arise.

The structure that I call the "tree breeding system" is the collection of efforts and resources invested to develop improved tree seeds. The breeding system represents a physical structure that corresponds to the broad conceptual term "tree breeding". Important components of the tree breeding system would be breeding stocks, land, and human resources.

A tree breeding system may be considered ideal if it functions like the human hand. In his presidential address to the 1978 Annual Meeting of American Statistical Association, George Box (1979) said:

> "Look at the human hand, for example. I doubt if there is any single thing that it does that could not be done better by some instrument, but it is very good at doing a very large number of things that come up in facing the world as it actually is."

Tree breeders who take advantage of genetic principles to improve trees may look at the hand concept from an evolutionary point of view. It is obvious that the hand existed before most of the things that we do today using hands, were developed. Contrary to eyes and nose that evolved for the specific functions of seeing and smelling, the hand did not evolve for a closed set of specific functions. The natural and social environments within which we breed trees change continuously, and we need a tree breeding system that functions in an analogous manner as the human hand; a system that is flexible and versatile.

Little effort was made in the past to recognize the designing of breeding systems as a science. It is thus not possible to present a set of methods that can be used to develop flexible and versatile tree breeding systems. Although a design does not generate any basic information on the components of the blueprints, designing is a science of optimization and can be studied scientifically. Following is an example of a breeding system that was developed with flexibility and versatility in mind. The example is presented to serve as a base for future discussion and development of system designing and should not be viewed as the final answer to an optimum breeding system.

JACK PINE BREEDING SYSTEM DESIGN: AN EXAMPLE

The basic hardwares of the system are: production population, breeding population, index population, and a jack pine breeding cooperative (Table 1). The three different types of populations will be referred to as coordinated populations. The following guidelines have been used to develop the system. (1) The hardwares should be structured so that the system may be used for multi-generation breeding. (2) The system description should include a minimum amount of technical decisions on improvement activities of each generation because it is undesirable for breeders of today to make decisions for breeders of tomorrow. (3) The system should include an element that will continuously check the alignment

of the breeding directions with the changing socio-economic demands and evaluate the structure of the system for its appropriateness to meet the demands.

Table 1. The physical components of the proposed jack pine breeding system.

(Lake States Region: Michigan, Minnesota, Wisconsin)

Hardwares	Note
Production population (seed orchard)	No specification
Breeding populations	Overall population size: 400 families Population structure: 20 random locations with 20 random families at each location. Standard selection method: Within population selection.
Index populations	A breeding population located near each breeding research organization -- Universities of Michigan, Minnesota, and Wisconsin, and the Forestry Sciences Laboratory at Rhinelander, Wisconsin.
Jack pine breeding cooperative	This could be replaced by a regional tree improvement cooperative.

Production Populations (Seed Orchard)

Seed orchards are created to produce the highest quality seed at the least cost. Each orchard is a specific group of selections from the breeding population designed to achieve this goal. Therefore, good breeding populations are needed for good seed orchards. Tree breeders would discard the orchards when their usefulness had ended and would not use them for further breeding.

Breeding Populations

Breeding populations are the backbone of the system, and the initial size and structure of the breeding populations will influence the flexibility and overall success of the system.

The necessary breeding population size has been discussed elsewhere (Kang 1979) so only the structure will be considered here. Given the overall breeding population of 400 families, a large number of different breeding population structures are possible. For example, the breeder could put all 400 families at one location (single population breeding approach) or put one family at each of 400 different locations. We chose to use the combination of 20 locations with 20 families each but there is no scientific reason that any other combination is better or worse. In the following discussions the term "multiple population" will be used to represent the 20-20 combination and each of the 20 tree groups will be referred to as a subpopulation of the 400-tree population.

Genetic Considerations (under the Assumption of no Genotype x Environment Interaction)*

The multiple population approach (MP) is a form of an inbreeding system at the group level because the selection and matings are made within each subpopulation. In MP the rate of inbreeding within a subpopulation is likely to be more rapid than in a single population breeding approach (SP). Yet, MP is superior to SP in respect to inbreeding in operational seed production. Under SP the amount of inbreeding depression existing in the breeding population will be reflected in the production operation, but under MP it is possible to take advantage of heterosis by crossing individuals from different subpopulations in the seed orchards.

The heterosis expressed under directional dominance gene action is a function of the inbreeding coefficient and the variance in gene frequencies among the subpopulations. The variance in gene frequency is caused by the small size of the subpopulations (genetic drift). When the subpopulations are crossed, excessive amounts of heterozygotes are obtained in the progeny population. The larger the variance, the greater the excessive amount of heterozygote genotypes in the progeny population (for given average gene frequency of the entire population). The excessive amount of heterozygote genotypes will increase the progeny population averages.

Inbreeding increases the heritability of the breeding population, which will help breeders identify superior genotypes. Inbreeding also exposes the deleterious alleles of the populations faster. It will be easier to clean up the breeding populations by removing deleterious alleles in MP than in SP. An important fact to keep in mind is that it takes many generations to develop inbred populations while it takes only one crossing to create an outcross population. When the average gene frequency is the same, MP is considered better than SP.

Genetic Consideration (under Genotype x Environment Interaction)

The single population breeding approach (SP) necessarily assumes that either genotype x environment (GxE) interaction does not exist or that environments within the breeding region are homogeneous. This assumption is not realistic and is one of the reasons why SP is not a flexible approach.

*Some ideas presented in this section are found in van Buijtenen and Lowe (1979) and Namkoong (1976).

The existence of GxE interaction further increases the variation in gene frequencies of the subpopulations so the variance is more cumbersome to interpret under GxE interaction. If similar gene complexes are selected in the subpopulations at different locations, the gene frequency variance caused by GxE interaction could be desirable in respect to outcrossing (heterosis). If different gene complexes were selected in different subpopulations, it is difficult to predict the outcome of the outcrossing. At least, breeders may expect to obtain broadly adapted progeny populations through outcrossing.

The selection for different gene complexes in different subpopulations is a source of the flexibility of MP. Because the selection will improve the phenotypic performance of genetic stocks regardless of the specific gene complex involved at a given location, the genetic diversity among the subpopulations can be maintained while also improving the overall performance of the entire population.* Furthermore, the sources of diversity (different subpopulations) are classified according to environments and this could be a handy feature. For example, if the breeding environment of a location suddenly changes, the breeder could review the existing environments at different locations before developing the optimum gene complex for the new environment. If an environment that was similar to the new environment existed, the breeder could simply transfer the population to the new environment.

The effort to take advantage of GxE interaction may be viewed as an effort to replace time with space. Naturalists deduce the evolutionary processes that occurred in the past by observing the species variability at some given time. Tree breeders can use the same idea in an opposite manner. They can take care of some of the unknown future problems by spreading their genetic stocks over a large area and letting the genetic variability among the subpopulations occur.

Management Considerations

The multiple population approach (MP) is not a good approach for the operation of a single organization but is very useful under a multiple organization cooperative operation. Because there are 15 potential tree breeding organizations in the region of our example, each participating organization will take care of less than two breeding subpopulations. The small size of a breeding population does not require much extra investment for the establishment. Initially the breeding population could be placed in a corner of a plantation or a seed orchard. Any participating organization may develop its own large scale breeding program while maintaining its share in the cooperative as an insurance policy and a source of information exchange.

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*This argument is correct for earlier generations. In the long run MP may not prove to be much better, but it will be at least 400 years (or 20 generations) before the long-term value of MP can be judged in respect to probability of gene fixation. Even at that time the outcrossing possibility of MP will make the system attractive.

Even under the single population approach (SP), the breeding population is replicated for additional breeding activities. Depending on the situation the entire population may have to be replicated, but usually only a small portion of the entire population should be replicated. Because the smaller unit of replication in SP is not defined it is difficult to determine the relations among the activities occurring at different replicates and that of the main breeding population. This problem is most serious when breeders want to coordinate their research results.

Research Considerations

Statistically, it is reasonable to sample a small portion of the population. In most tree breeding research (especially in multi-generation breeding), the sample size itself is one of the most important factors influencing the performance of populations. Therefore, under SP, unless the entire breeding population is assessed, the test information becomes irrelevant to the breeding population. In the MP system, four to five subpopulations may be randomly chosen for research. Because the size of the subpopulations is equal, the information obtained from the test subpopulations can be safely used to approximate the average performance of the entire breeding population.

The repeated use of the same test subpopulations for studying different tree characteristics is economic and efficient because the research efforts can be concentrated and coordinated within a small set of subpopulations. These sample research subpopulations are referred to as index populations.

Index Populations

The concept of the index population originated because of the assumption that research results would become increasingly difficult to apply in breeding practice as the breeding generations advanced. For example, in the first generation the information obtained from a research population can be used in breeding populations whose founders are not necessarily the same as those of the research populations. One may assume that both research and breeding populations are random samples of a base population that covers large geographic areas. As the generations progress, the relation between research and breeding populations becomes weaker unless they are descendents of the same or equivalent founders that have undergone similar breeding practices.

The index populations are used to assess the progress of the breeding activities by maintaining some controls within the index populations. They are also used to diagnose the average performance of the breeding populations and to develop the general prescriptions for the breeding activities in the following generations. Note that breeding prescriptions based on the information obtained from index populations are not specific. In order to write the breeding prescriptions for a population -- determination of trees to be selected, crosses, crossing

pattern, etc. -- administrative studies will have to be made using the materials in the populations.*

Because jack pine flowers early, it is an attractive species for developing tree breeding techniques using juvenile traits. Studies of inbreeding or traits that are selectively neutral are ideal. For example, breeders are interested in learning how many generations of selfing would be necessary to eliminate the majority of lethal genes from the breeding populations. Conversely, one might want to know the rate of change in the fraction of filled seeds, seed germinations, and the changes in the correlation structure of these traits and early performance of trees with time (over generations).

Much selection research can be performed using index populations. One of the most important parts of juvenile-mature correlation research is the determination of the genetic gain per unit time. Frequently, the unit time is defined as a segment of a single regular selection cycle (for example, one year). A slightly different approach to the problem is possible by defining the entire regular selection cycle as the unit time of interest (Fig. 1). The objective of the research is to determine how many cycles (short) per unit time would result in the maximum gain. The underlying hypothesis of this system is that as long as the juvenile-mature correlation stays positive and reasonably high, say $r \geq .4$, then shorter selection cycles would be more advantageous than the regular cycle.

Another use of index populations is to breed for juvenile traits only (Table 2). If we set our long-term breeding goal at shortening the generation turnover time instead of increasing the yield at matuity, we can bypass the problem of juvenile-mature correlation. For example, we could set the breeding objective as obtaining the current 20-year tree height in 10 years. In this case we are dealing with 10-year traits and the correlations between 10- and 20-year tree height are irrelevant to our breeding efforts.

Much of the research shown above is exploratory, and it is too risky to do those studies using the entire breeding population. Because index populations are samples of breeding populations, any information obtained in index populations are directly applicable to breeding populations. Therefore, a minimum amount of information will be lost as the result of concentrated research efforts in index populations.

Kang and Namkoong (1979a,b) found that different balanced mating designs are the same in respect to the ultimate probability of gene fixation. Their finding was based on the single locus theory and further generalization of the theory is necessary. But if it proves to be true in general, it will

*It is assumed that as the generations progress, the current research tools such as analysis of variance, estimation of heritability, prediction of genetic gains, etc., will become management tools. The researchers will put more of their research efforts on evaluating the average performance of populations with time.

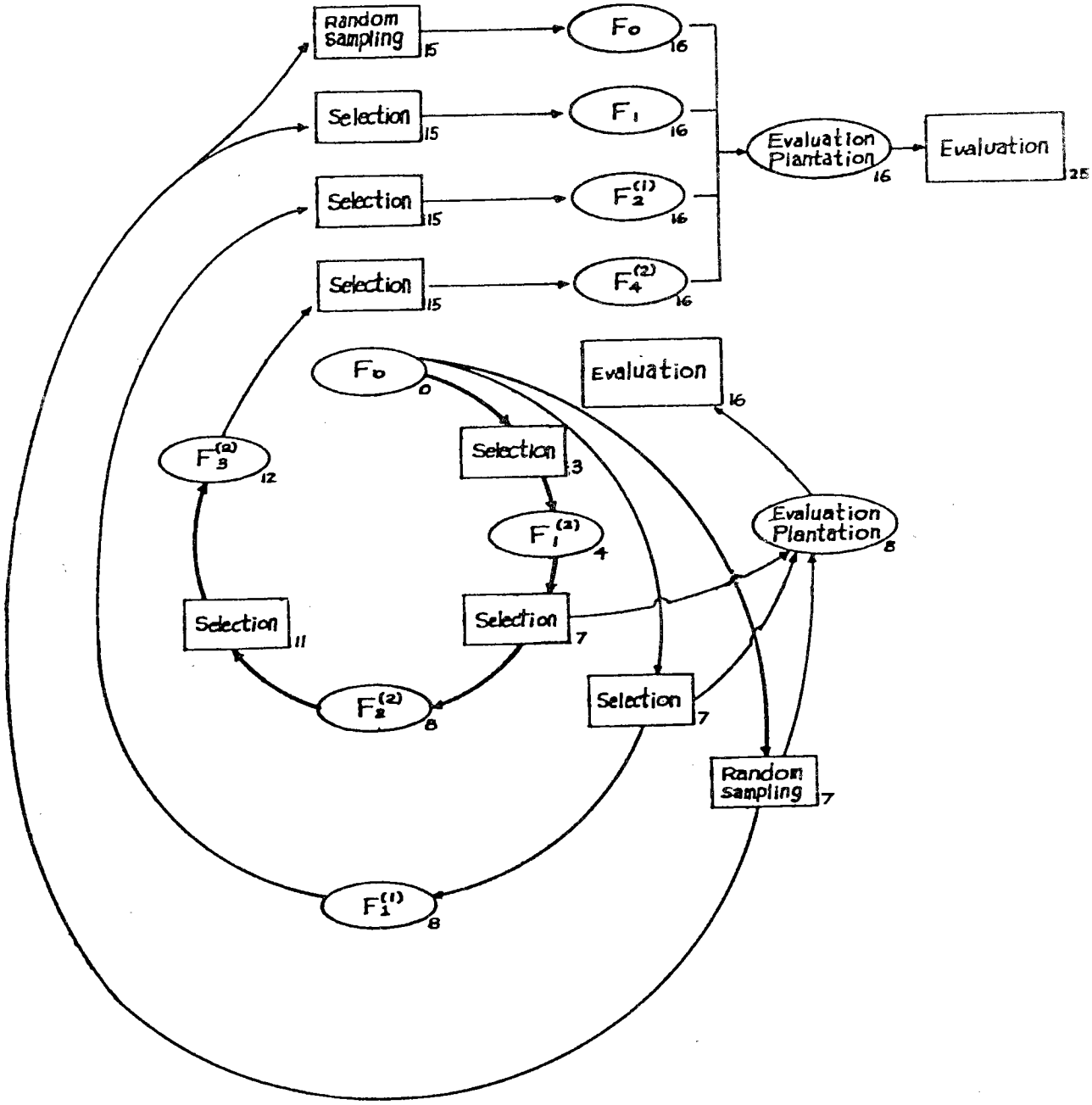


Fig. 1. Selection at different ages.
The numbers represent the years.
The numbers in parentheses designate different cycles.

Table 2. A classification of studies that can be made on the index populations using fast generation turn-over cycles.

INTRA-POPULATION STUDIES

Single trait

Mating

Selfing
Relative mating
Random mating
Mating designs

Selection

Mass
Family
Within family
Combined
Selection intensity and population size

Multiple traits

Tandem selection
Independent culling
Index selection

INTER-POPULATION STUDIES

Reciprocal recurrent selection
Within population selection

. . .
. . .
. . .

OTHERS

Genotype x Environment interaction

. . .
. . .
. . .

be possible to use complex mating designs, such as the partial diallel design, in index populations while using single pair mating in the breeding population. The use of different mating designs in different types of subpopulations will save a great deal of the cost of crossing.

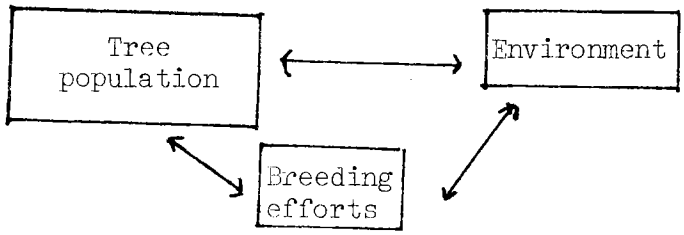
Jack Pine Breeding Cooperative

The primary function of the cooperative is to coordinate the flow of breeding information and the distribution of materials and efforts. The cooperative includes the entire spectrum of research and breeding activities -- from population genetics theory to seed orchard development (Fig. 2).

The secondary function of the cooperative is to support a tree breeding system design and analysis function (SDA). The concept of SDA is similar to that of systems analysis suggested by Namkoong et al. (1971). SDA is responsible for guiding the evolution of the system. To maintain the system flexibility, SDA will continuously review and improve the system design itself. The activities performed by SDA would also include predicting changes in social, environmental, and economic trends.

CONCLUSIONS

Until men began interfering with nature the evolution of tree populations was directed only by the environment. Today, both tree breeders and nature are co-directors of the evolutionary process and their objectives do not necessarily coincide. So a tree breeding situation may be represented as:



Because both tree populations and environment are dynamic, a fixed set of breeding efforts cannot solve all the problems occurring in the triangle. The efforts of breeders should evolve in such a way that a balance exists among the components of the triangle. To make their efforts effective, tree breeders should develop a breeding system that is flexible and versatile.

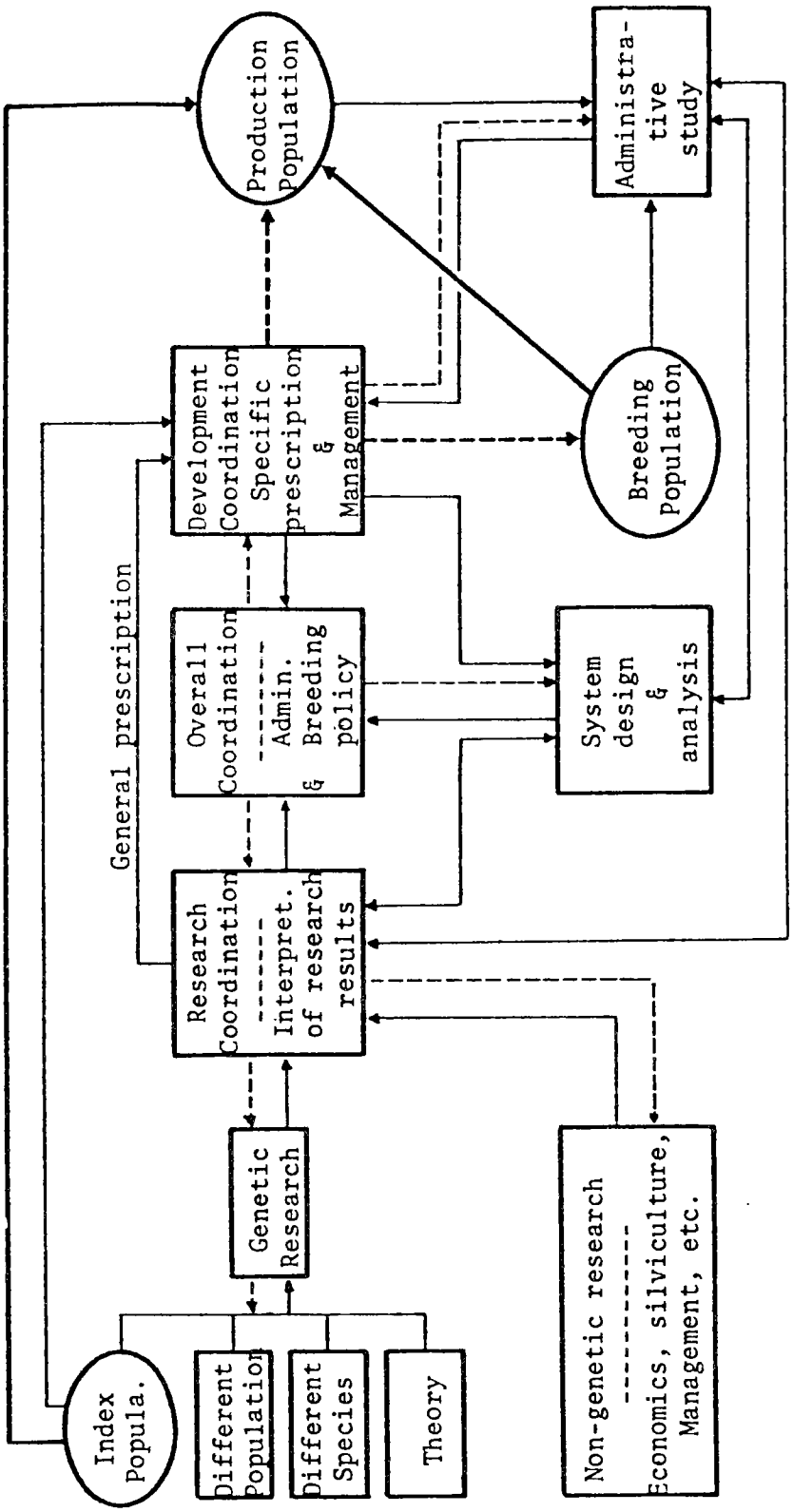


Fig. 2. Functional structure of a jack pine co-operative.

——— Information flow

- - - - - - - Allocation of resources

═══ Material transfer

═ ═ ═ ═ ═ Development and operation

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DISCUSSION

Dr. J.I. Klein. In case of separate populations I think you would lose some opportunity for recombination. Should this not be overcome by taking one such population just for recombination.

Dr. H. Kang. Theoretically there is no difference.

Dr. F.C. Yeh. I have two questions, first: are we going to establish, let us say 20 seed orchards?

Dr. H. Kang. They are not seed orchards but breeding populations.

Dr. F.C. Yeh. I know there are breeding populations. But based on breeding populations results are we going to be fixed with 20 seed orchards, or do we have the alternative of setting up smaller number of seed orchards?

Dr. H. Kang. You have all kinds of alternatives. I did not want to go into details.

Dr. F.C. Yeh. It looks to me as 20 populations to 20 sites in a way. How are you going to extrapolate the information to reduce it to a smaller number of seed orchards in a way?

Dr. H. Kang. You will have to do research on that over a few generations. I have placed more emphasis on time than on space. So, I have deliberately minimized discussing what will happen at given generations.

Dr. F.C. Yeh. Second question. Have you looked at different mating designs for each of those breeding populations?

Dr. H. Kang. I suggested a little bit about that and I did not have time to go more into those in detail. So I think we could discuss it later.

Dr. E.K. Morgenstern. I do not think you can define your situation as you do. The basic argument is that you have to look at your environment first, which you can subdivide into ecological or breeding regions. I think your environment pretty well simplifies breeding for you. I think your argument is not well adapted to your actual situation.

Dr. H. Kang. I do not know if you can proceed without considering environment. There is no such thing as a phenotype without the corresponding environment.

Dr. E.K. Morgenstern. I would say that you have to look at the environment first and also ask the silviculturist who will tell you at what level of environmental subdivision he can work. And then the breeding program can proceed.

Dr. H. Kang. Are you talking about management problems or breeding problems?

Dr. E.K. Morgenstern. Both need to be integrated. On the basis of this silvicultural information your breeding program will have to be developed.

Dr. H. Kang. I think you ought to read the paper. I just touched on the management problem. All I could say was about genetic considerations. I did not go into management considerations.

Dr. E.K. Morgenstern. That is the basis of the whole approach. You can come up with as many mating designs as you want but if the silviculturist cannot use the material this will not work.

Dr. H. Kang. This is not a mating design. This is a breeding system plan or design.

Dr. E.K. Morgenstern. But the breeding system or plan should evolve very closely on the basis of silvicultural possibility.

Dr. H. Kang. Yes, indeed. That is why I did not discuss in detail as to what happens in each generation because I cannot forecast what is going to happen 1,000 years from now.

Dr. Hans Nienstaedt. Why don't you very briefly explain how this fits into the zones in the Lake States and how the cooperators will handle it?

Dr. H. Kang. Why don't you do it?

Dr. Hans Nienstaedt. All right. First, this particular program is fitted into the Lake States on the basis of the provenance tests. We are fortunate to have meaningful provenance tests in the Lake States. On the basis of the tests, the 400 families represent one breeding zone. Secondly, we will be working with a number of cooperators; each cooperator will handle a minimum of two of the sub-populations and develop them in connection with their program. So I think that we are meeting the kind of requirements that you are talking about. We have the additional advantage that we can go from one subset to another in the program, since they all fit into the breeding zone. Hyun has minimized the amount of work that the individual tree breeders will have to do and yet they have retained the advantage of the large base population.

Miss R.M. Rauter. If you have that many breeding populations where are you planting the material then so as to get isolation from the surrounding stands, because that will be a critical problem?

Dr. H. Kang. Even if you have foreign contamination the gain you would get would be about half of what you would expect under controlled crossing. And, if it is the most economical way to go (in other words, crossing is very expensive), then you would like to be content with half the gain. I cannot say what to do but I suggest that you think of your own way of tackling the problem.

Dr. D.P. Fowler. In your initial population you are just taking 20 random trees as your sub-population. I realize you are trying to keep your options open, but I certainly would recommend starting with at least a low level of selection for desired traits.

Dr. H. Kang. I think it is more of a philosophical matter. When you talk about selection from natural populations you have to ask what kind of heritability you would expect to have in the natural population. If it is less than say 0.05 you can see that the gain you get by making plus tree selection would not be great but you would be spending a lot of time and money to search through the woods to find good trees. On the other hand a tree with good form might be worth while to look for because tree forms are likely to have higher heritability even in nature. I am presenting this plan as a basis for discussion rather than as a proven breeding system. I do not think that such a thing (proven system) exists.

Dr. C.W. Yeatman. I have no quarrel with your plan. I think it is an excellent one. It is absolutely essential that the breeding population be developed within the breeding zone. In the boreal forest breeding zones are much narrower than at the southern end of the species range as in the Lake States. Each zone requires a unique breeding population. Your concept could be expressed in terms of 20 unit breeding programs from each of which 20 proven genotypes will be developed. These will subsequently contribute to a larger consolidated breeding population within the zone.

Dr. H. Kang. Actually we have got all of this under way and the reasons I chose the random sampling of trees for base population were several. First the seed trees we had at hand were generally good ones.

Dr. Hans Nienstaedt. In respect of Dr. Fowler's question, actually a good share of that seed was selected from stands that, on the basis of provenance tests, produce superior material.

Dr. H. Kang. We had talked about the advantages and disadvantages of going different ways. For instance, we could have selected trees from separate natural populations which were adjacent to the locations and where the breeding populations were to be established. We thought it would take us five to six years to be able to come up with a reasonably good complete job. Compared to that, if we started (i.e. establish breeding populations) right away and turned over generations as fast as we could we might come out better over all. We were more concerned about being able to select immediately. I mean sooner in the testing situation rather than matching environment and initial breeding populations more precisely than we did. We will be able to match them eventually with time.

THE MAKING OF A COOPERATIVE FORESTRY PROGRAM

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ABSTRACT

Forestry cooperatives have the objective of accomplishing a goal by group rather than by individual effort. Ingredients for a successful cooperative include enthusiastic leadership and committed clientele. For the Cooperative Programs at North Carolina State University, all cooperators are treated equitably, regardless of size, age or state of knowledge, and all are assessed the same fee for membership. All members are expected to operate at a defined threshold level of activity, and all are required to share research results and plant materials with other cooperators. Research results are made available to the public with minimal delay. Continuing education is an essential part of any successful forestry cooperative.

RÉSUMÉ

Les coopératives de sylviculture se sont fixé d'atteindre leur but grâce à une impulsion plus collective qu'individuelle. Il est nécessaire dans le cadre de la coopérative que l'équipe de direction soit enthousiaste et que la clientèle se sente impliquée afin de garantir le succès. En ce qui concerne les Programmes de Coopérative de l'Université de l'Etat de la Caroline du Nord, tous les membres recoivent le même traitement quel que soit leur importance, âge, ou degré de connaissance, et doivent tous s'acquitter d'une cotisation de montant égal. Tous ces participants doivent opérer à un niveau d'activité défini, et l'on attend d'eux qu'ils mettent en commun les résultats de leurs recherches et leur équipement. Les résultats de ces études seront communiqués au public dans de brefs délais. La formation permanente constitue une condition essentielle du succès de toute coopérative sylvicole.

INTRODUCTION

Forest tree improvement programs are complicated by the long time required for the trees to reach sexual maturity, the long reproductive cycle which extends through two years for most conifers, and the long time required for the resultant progeny to reach financial maturity. In addition, the logistics of consummating controlled crosses and obtaining seed

and scion material from mature trees which range to 50 metres tall are restrictive. The time, effort and finances required to conduct such a program on a scale to produce improved plant material for operational use while maintaining a broad genetic base for future cycles of breeding are generally prohibitive for all but a few public organizations. Even those organizations lack the alacrity to accomplish both short- and long-run objectives. The alternative is to accomplish the job by group effort rather than by individual effort.

The group effort for genetic improvement of forest trees was envisioned by Dr. Bruce J. Zobel in 1951. Employed as a silviculturist by the Texas Forest Service, Dr. Zobel organized a coalition of southern forest industries to fund and conduct the necessary research for formation of an operational tree improvement program. Reliance on private industry to accomplish the job was as surprising then to the South as it is now to other regions within and outside the United States. Antagonists were convinced that public agencies were the only organizations suited to conduct long-range research, and they were equally convinced that the forest industry was too fickle for long-range commitments. How could private organizations of a strongly competitive commodity group organize to accomplish a common goal? The structure and accomplishments of the North Carolina State University-Industry Cooperative Tree Improvement Program, with frequent reference to other forestry cooperatives, are recounted to answer that question.

COOPERATIVE DEFINED

A cooperative is defined as a means of working together to achieve a common goal. That loose definition has allowed extension of the principle to mean involvement from the least to the greatest degree. Farmer alliances are examples of the lesser degree of cooperative involvement. For a fee, which is usually exacted from the selling price of the commodity, the farmer can deliver his tobacco, corn or cattle to the cooperative, which assumes all responsibility for selling to the processor. Better prices and greater assurance of selling are the rewards of the cooperative effort in which the farmer usually has no investment. He is involved only to the degree that he commits his crop to the venture.

The end opposite farmer alliances on the spectrum of cooperatives is total involvement of all members in all activities. Added stipulations are that all members be treated equitably, regardless of size, state of knowledge, or longevity, and each organization be required to perform at a threshold level. Anyone failing to meet these standards would be declined admission or purged. It is the latter type of organization to which we will address our attention.

COOPERATIVE PERCEIVED

A forestry cooperative works best when the need for the joint

effort is perceived by its potential members. The succeeding step is presentation of the proposal to a number of public agencies or foundations to determine who has superior capabilities for administering the project. Being involved in the decision to house the project within a specific agency induces harmony among the cooperators.

Successful forestry cooperatives also exist as a result of an enterprising person or group of people convincing the clientele of the value of a combined effort. In the beginning such cooperatives almost always attract fewer cooperators than anticipated. The result is to increase the fee structure of member organizations to form an acceptable operating budget or to operate on a reduced budget. Neither option is attractive; member organizations express lack of confidence in the first instance, and results are slowed in the second instance. The only way such a program can succeed is by accomplishment. A degree of respectability is gained when results are obtained; failure results when they go begging.

A variant of the joint effort perceived by potential members is additional funding of a project already in existence. Support by a couple of organizations which first recognize the value of the research often serves as a catalyst for contributions by other organizations until a full-fledged cooperative is formed. The Herbicide Cooperative at Auburn University, Alabama, is an example of such a success story.

COOPERATIVE INGREDIENTS

Successful forestry cooperatives need not be formed to the same mould. Greatest differences are in the authority vested in the directors. Some directors are given broad control, whereas others have to operate within the confines of committee action. I espouse the broad control method because it has been used successfully by the Tree Improvement, Hardwood Research, Forest Fertilization, Forest Equipment/Systems, and Tissue Culture Cooperatives at North Carolina State University. The method presupposes that the director is the expert on the subject and that his judgment is valued over that of a committee, each member of which knows relatively less about the subject than the director.

Justification

Major reasons for support of cooperative programs by forest industry in the South are diversity of land ownership, and time, cost and effort of conducting long-range research. Most organizations supportive of cooperatives own or control from 80 000 to 2 000 000 hectares of land. Within an ownership, the land extends across several geographic provinces, many states, and a multitude of site productive classes. The diversity prohibits the intensity of research needed for each classification, even for those organizations with a large support staff. The philosophy is that a coordinated effort by a group of organizations can accomplish more in a given time at a cheaper cost than can each of the organizations working separately.

Coordinator

To render impartial decisions it is imperative that the coordination of a cooperative forestry program be vested in an institution distinct from that of its members. Forestry schools within major universities have commonly filled these roles in the South, although the U.S. Forest Service has coordinated cooperative programs in pollen management, lightwood production, and introduction of *Eucalyptus* (Table 1).

Table 1. Cooperative forestry programs in the South, by state and institution.

State	Institution	Cooperative Type
Virginia	Virginia Polytechnic Institute and State University	Biometrics
North Carolina	North Carolina State University	Tree Improvement Hardwood Silviculture Forest Fertilization Forest Equipment/ Systems Tissue Culture
Georgia	University of Georgia	Biometrics
Florida	University of Florida	Tree Improvement Forest Fertilization
Alabama	Auburn University	Herbicide Evaluation
Mississippi-Louisiana	Mississippi State University and Louisiana State University	Harvesting Systems
Texas	Texas A & M University	Tree Improvement Hardwood Silviculture
---	U.S. Forest Service (Southeastern Forest Experiment Station)	Pollen Management Lightwood Induction *Eucalyptus* Introduction

Requirement

The North Carolina State Tree Improvement Program operates without a contract of any type. Initial verbal agreement was to support the program for five years, after which time the cooperators were free to withdraw if unsatisfied with results. The Director was also given authority to

terminate membership if a cooperator did not perform to a threshold level. Some cooperatives also discourage renewed membership of organizations who terminate participation at periodic intervals. Nothing is more damaging to a cooperative program than a member who benefits at the expense of other cooperators. An unqualified stand against such practices has resulted in the North Carolina State Tree Improvement Program's growing from 10 members in 1956 to 30 members in 1979 without a single casualty.

One of the greatest benefits of a tree improvement cooperative is the amassing of a genetic base that would be almost prohibitive for any one organization to amass. Free exchange of the plant material then becomes imperative if the cooperators are to benefit from the best genetic material. It is not uncommon in the Tree Improvement Program for a clone of outstanding genotype to be found in the seed orchards of a half-dozen cooperators, and progeny from an outstanding clone in Virginia is likely to be under test on lands of a separate cooperator in Mississippi.

Another major requirement of the Tree Improvement Program is that all information obtained through the auspices of that program will be made available to all other cooperators without delay and to the public as soon as the information can be disseminated in oral or narrative form. In the 23 years the Cooperative has been in existence, the rule has rarely been challenged. Most cooperatives follow the principle of the Tree Improvement Program in disseminating results. However, some programs delay dissemination of the information for a time not to exceed two years, to allow member organizations to profit from the results.

It is a requirement of the Tree Improvement Program that all trees grafted into seed orchards will have been graded by the Program staff. The other stipulation is that the experimental design of progeny tests and other region-wide field trials be common among all cooperators. The former requirement assures a common base for genetic improvement of the southern pines, and the latter one adds efficiency to data collection, analysis and interpretation. Most other cooperative organizations of a similar nature have similar requirements.

Although the absolute requirements of the Tree Improvement Program are few, advice and recommendations are freely given on topics ranging from orchard establishment to deployment of genetically improved plant material. The technical representative is free to reject our counsel but he and his superiors are reminded that we assume no responsibility for failure if our advice is rejected. We are quick to admit failure when we have given a wrong recommendation but are just as quick to disclaim responsibility when the fault lies with the cooperator.

Qualification

Cooperative forestry programs require long-term commitments of money and manpower. The amount of money contributed to the coordinating unit is small in comparison to the expense of establishing and maintaining research and operational trials on cooperator lands. The cost to a single cooperator in tree selection, orchard establishment, progeny testing, and

collection and deployment of plant material in the Tree Improvement Program is up to 40 times that allocated to program coordination. That consideration has caused us to recommend against membership of any organization controlling less than about 80 000 hectares of land. Such organizations are advised to support the programs of their respective state forest services, from which genetically improved plant material can be obtained.

The Tree Improvement Program was formed with the sole support of forest industry. That policy was subsequently changed to allow participation by state forest services. The Forest Service of Virginia, North Carolina, and South Carolina are now among the 30 members of that program in which all participants are treated equally. The forest services of these and other southern states as well as the U.S. Forest Service also support one or another of the various cooperatives listed in Table 1.

Trade associations and commercial enterprises without a land base, which would benefit directly from cooperative membership, are discouraged from joining except as a patron. Membership is generally decided on the recommendation of the director, with final approval being the responsibility of the advisory committee.

Administration

With the implicit approval of the cooperators, a director is appointed by the coordinating organization, such as the university where the cooperative is housed. The director is responsible for composing a staff of the quality and quantity needed to conduct the business of the cooperative.

Contact between the director and the cooperator is made at two levels for most cooperative programs. The administrative contact is made through the advisory committee, which is composed of one administrator from each cooperator. The administrator has sufficient authority to make policy decisions regarding cooperative matters. Contact is maintained with the advisory representative throughout the year, and an advisory committee meeting is held annually, at which time a report is made by the director on accomplishments and plans and on financial status of the cooperative. The advisory committee interacts with the director on these matters.

The second level of contact by the director and the cooperator is with the technical representative. The technical representative, generally a graduate forester with a baccalaureate or Master's Degree, is responsible for cooperative activities of the cooperator. Although an employee of the cooperator, his duties are largely influenced by directives of the cooperative. Annual meetings are commonly hosted by the cooperators on a rotating basis to allow the technical representative to show his accomplishments and to see the accomplishments of his peers. Superiors of the technical representatives are excluded from these meetings, to allow latitude in discussion.

Finances

Financing of a cooperative program is usually jointly funded by the cooperators and the coordinating unit. The cooperatives at North Carolina State University enjoy the use of the capital plant, inclusive of facilities without the cost of overhead. The salary for the director, or an equivalent amount of money, and costs for associated goods and services are borne by the university. Monies collected from the cooperators on a scheduled basis are used for salaries of the support staff and graduate students, and for goods and supplies for day-to-day operations. Cooperator fees are self-imposed at the annual meeting for the following year, based on the budgetary process.

All organizations are generally charged a single fee, regardless of their size or status. An exception to that rule occurs when an organization has separate operations at locations separated by more than about 500 kilometres. The policy is to charge the set fee for the base unit of that organization and to charge a reduced fee for each supplemental unit. The rationale for charging a constant fee for all base units is that a similar amount of time and effort is required to service one organization, regardless of its size. Smaller organizations are content to pay the common fee because it assures them of the same attention received by an organization several times their size.

Cooperator fees for program coordination are small compared to the expenses of tree selection, orchard establishment, orchard management, progeny testing and deployment of seed on cooperator lands. The annual fee for the base unit of many cooperatives does not exceed $5 000. However, the industrial contributions have served as a catalyst for obtaining other monies. Some granting agencies find expediency in awarding a grant to an organization having matching monies, especially when the matching monies are of industry origin. We at North Carolina State University have received sizable grants from National Institutes of Health, the National Science Foundation, and the U.S. Department of Energy, the Ford Foundation, the Rockefeller Foundation, and the National Space Administration. The monies are used to complement or extend cooperator funds.

Coordination

The major function of a cooperative program is coordination. The position is comparable to director of research for a large industrial concern. The key is to produce results today while planning for tomorrow. This task is difficult to accomplish during the maiden years of a cooperative, but it is one that nevertheless has to be accomplished. The study of wood among and within species of southern pines was chosen to fill the void in the Tree Improvement Program. That vocation melded well within the Tree Improvement Program when the larger effort began to pay dividends. For those initiating a cooperative program, many subjects allied to forestry and of equal importance to the study of wood properties await investigation.

In addition to coordination, psychology has to be practiced for development of a successful cooperative. A case in point for the Tree Improvement Program is the establishment of separate seed orchards on the land of each cooperator. A more efficient alternative would have been to establish one or a few orchards for production of genetically improved seed for all cooperators. The need for each to have separate orchards to show accomplishment and pride in their work was soon recognized. Some efficiency may have been lost but the public relations gained from the dispersed operation has paid dividends many times over.

Coordination of a cooperative program cannot easily be accomplished without knowing what is transpiring in the profession and on cooperator lands. That accomplishment requires a tremendous amount of travel for the staff of North Carolina State Cooperatives whose membership ranges throughout a 13-state area. Policy visits to each cooperator are made at least annually; and service visits for tree grading, grafting, pollination and progeny testing are made as needed. Participation in symposia within and outside the region also claims a significant amount of time. However, we are convinced that the contact maintained through the travel has been a large part of the success of the Cooperatives.

Continuing Education

The incentive to house the administration of a cooperative program within the forestry department of a major university is twofold in addition to the university's being independent of the cooperators. These incentives are (1) ability to draw on expertise from closely allied disciplines, and (2) involvement with graduate and undergraduate education in the field of interest. At North Carolina State University, close contact is maintained with the disciplines of botany, biochemistry, entomology, genetics, horticulture, pathology, physiology, soils and statistics, as these subjects interact with tree improvement objectives. From 12 to 15 graduate students pursuing Master of Science or Doctor of Philosophy Degrees in forest genetics are annually associated with our Tree Improvement Program. Research conducted by the candidates has been instrumental in successful development of the operational tree improvement program. Graduates of this program are found in positions of influence and authority throughout the world; many of them are supervising the maturation of a second generation of forest geneticists.

A necessary ingredient of a successful tree improvement program is emphasis on continuing education. In addition to one-on-one instruction given for tree grading, grafting and progeny testing, short courses of about three days' duration are given to the technical representatives at least biennially and more often if needed. The objective of the short courses is to demonstrate tree improvement techniques and the theory behind these techniques. This effort does not substitute for a basic education in forest genetics principles; it is supplemental to the basic education.

CONCLUSION

The melding of many ingredients is necessary for the successful development of a forestry cooperative. The case study described for the North Carolina State-Industry Cooperative Tree Improvement Program has been successful for conditions in the southern United States. The same type of success may not be claimed in other regions of the world where differences exist in objective, environment, personnel and political persuasion. A different melding of ingredients will probably be needed for each condition. Regardless of circumstances, however, two ingredients appear paramount to the success of any forestry cooperative. They are enthusiastic leadership and committed clientele. Without these attributes the cooperative venture is doomed to failure.

DISCUSSION

Dr. H.S.D. Swan. I am very much interested in the courses that you run. Would it be possible for Canadian companies or provinces to send key young men who are interested in forest genetics down to you to participate in one of your courses?

Dr. R.C. Kellison. Yes. But let me explain how they are conducted. The short courses are convened when there has been sufficient turn-over in personnel within cooperating organizations to warrant the effort, or when new information is to be presented to the cooperator. On the average a short course of about three days duration is held every other year. The content is on the applied phases of tree improvement, but enough theory is given for the participants to understand why a practice is included. The participants are generally employees of our cooperatives, but participants from other organizations are invited if space allows. On one occasion we conducted such a short course solely for the U.S. Forest Service. To answer your question specifically, there would be opportunity for participants from Canada to be involved in a scheduled short course, or for a short course to be planned specifically for Canadian participants.

Dr. H.S.D. Swan. These are just short three-day courses you said.

Dr. R.C. Kellison. Yes. The three-day short courses allow us to touch on the applied phases of tree improvement.

Dr. H.S.D. Swan. Well, I was told they were three-month courses.

Dr. R.C. Kellison. On one occasion we conducted a one-month short course in conjunction with FAO, for participants from developing countries who were initiating tree improvement programs. We found the experience to be very rewarding for the single participant from each of about 30 countries. We are contemplating a repeat of the exercise for specific regions of the world, such as for tropical forestry, temperate forestry and boreal forestry. If there is specific interest for a tree improvement short course for Canadians, contact

should be made with me and I shall pursue the matter. Our administration strongly favours our participation in such short courses.

Mr. D. Winston. Do you provide your cooperators with some sort of manual for managing seed orchards?

Dr. R.C. Kellison. Yes, we do. We have compiled a tree improvement handbook with chapters on tree selection, seed production areas, progeny testing, orchard management, etc. The handbook is loosely bound and is continuously revised as new information is available. Distribution of the handbook has been restricted to our cooperators because of policy constraints. However, much information is available in publications. That information includes equipment and supplies needed for tree improvement activities, procedures for tree selection, orchard establishment and management, progeny testing and development of genetically improved seed. The value of the handbook is that all the information is collected in one place which relieves the orchard manager from searching for a published procedure on every occasion.

THE ROLE OF PHYSIOLOGY IN TREE IMPROVEMENT

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ABSTRACT

Because both the interaction between genotype and environment is expressed through the physiology of the tree, physiology is a central key to unlocking many tree improvement possibilities. If properly used, physiology offers to be one of the more important tools for tree improvement in the future as exemplified by some of the successes it has produced to date. Physiological techniques are now available, and more will surely be developed, for dealing with the two major roadblocks to tree improvement: long evaluation times and restrictions in reproductive biology. Studies in photosynthesis have probably been the most popular physiological approach to evaluating the potential of tree selections. While some success has been achieved, a complex set of factors influence photosynthesis and care must be exercised in using them as selection criteria. Techniques for studying photosynthate utilization may prove to be more useful. In addition, other physiological phenomena need consideration. Two examples to be discussed are photoperiod response and nutritional relationships, especially as they relate to the joint selection of trees and their root symbionts (mycorrhizal fungi and nitrogen-fixing organisms). Physiological techniques for overcoming reproductive barriers are being developed. Important strides have been made in vegetative propagation, inducing early flowering, and removing some varriers to hybridization. To take advantage of physiology in tree improvement, we tree improvement specialists need a better understanding of physiology and we need physiologists on our tree improvement teams.

RÉSUMÉ

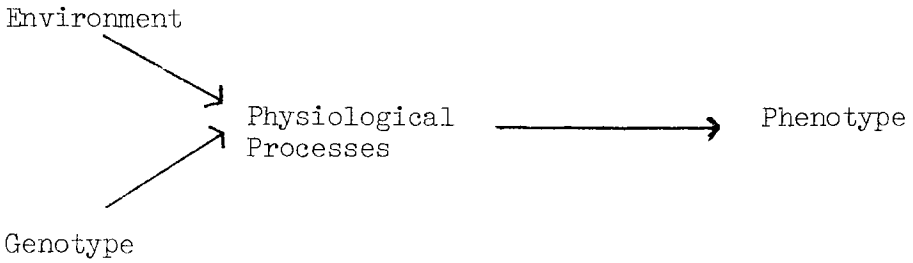
Puisque nous retrouvons dans la physiologie de l'arbre l'interaction entre le génotype et l'environnement, la physiologie doit offrir de grandes possibilités dans le domaine du sélectionement des arbres. Si on l'applique convenablement, la physiologie se trouve être l'un des outils les plus précieux dans la mise en valeur des arbres à l'avenir, comme le prouvent certains succès qu'elle a remportés à ce jour. Pour contourner les deux obstacles majeurs à la mise en valeur des arbres (longs délais d'évaluation et restrictions de la biologie reproductive), nous disposons maintenant de techniques physiologiques, et d'autres seront

encore certainement mises au point. Les études de photosynthèse ont probablement répresenté les approches physiologiques les plus populaires pour évaluer le potentiel des mises en valeur d'arbres. Tandis que certains succès ont été remportés, un ensemble complexe de facteurs influence la photosynthèse, et si l'on veut les utiliser comme critères de sélection, il faut procéder avec soin. L'étude de l'utilisation photosynthétique peut se révéler plus fructueuse. Il faut d'autre part prendre d'autres phénomèmes physiologiques en considération. Nous étudierons deux exemples: la réaction à la photopériode et les corrélations dans le domaine nutritionnel, surtout dans la mesure où elles se rattachent à la sélection combinée des arbres et de leurs symbiotes de racine (champignons mycorhizes et organismes fixant l'azote). Certaines techniques physiologiques visant à surmonter les barrières de la reproduction sont en voie de développement. Des progrès importants ont été réalisés dans le domaine de la propagation végétative, favorisant une fleuraison précoce, et s'attaquant à certains éléments défavorables à l'hybridation. Afin d'appliquer les avantages de la physiologie à la mise en valeur des arbres, nous qui sommes spécialistes dans ce dernier domaine, devons élargir nos connaissances en physiologie. Nous avons donc besoin de la participation de physiologues dans nos équipes de mise en valeur des arbres.

INTRODUCTION

Substantial progress has been made in tree improvement in the past without much resort to physiological tools, and perhaps this approach could be continued satisfactorily for some time into the future. However, we can much better consolidate the gains that we have made and proceed to long-term improvements by making physiological genetics a major part of our current and future program efforts.

Why is physiology so important? The complex set of physiological events that occur during the life of a tree gives rise to the phenotype that we harvest or select for our improvement programs. Both the genotypes that we try to assemble and the silvicultural environments that we try to provide influence the phenotype through the tree's physiology. That is,



There are several useful discussions in literature of the roles that physiology can play in tree improvement (e.g. Wareing 1964; Gatherum 1964; Ledig, 1975; and especially Cannell and Last, 1976). We will present a broad overview of the many ways that we believe that physiology

can be put to use for tree improvement, elaborating on a few systems that illustrate the potential and the pitfalls that can be involved. In treating the subject of physiological genetics, there is necessarily some use of morphological and biochemical concepts, but we will not attempt to review all the contributions that these latter two areas of study can make to tree improvement.

How Can It Help Thee - Let Us Count The Ways

There are two major bottlenecks to tree improvement progress:

1. The difficulties in evaluating potential selections.

2. The reproductive biology that controls the length of the breeding cycle and the propagation of our improved selections.

By understanding and manipulating physiological processes, we should be able to reduce both these problems.

PHYSIOLOGY AS A SELECTION TOOL

The conventional approach to evaluating tree selections is to observe their field performance over a period equivalent to at least 1/3 to 1/2 of a normal rotation. But a tree's performance over a long time period is really an integration of many physiological processes that can be measured over a much shorter time -- a matter of minutes or months rather than decades. The task is to identify key physiological processes that have a strong influence on ultimate performance and that are readily measured. Doing this can save more than just time in tree improvement work; it may well lead to greater total improvement than could ever be obtained by conventional selection techniques. Regular selection improves population performance by increasing the frequency of favourable alleles for a few major genes. But to capture the greatest total genetic gain may require a more complex set of favorable alleles (Wright 1932; Ledig 1975). If we understand the roles that a number of physiological processes play in determining final yield, then we can try to optimize these processes in our populations.

Because most of a tree's dry weight represents carbon fixed in the process of photosynthesis, that would seem to be the most important process contributing to plant performance. Unfortunately, experience in agricultural crop breeding has been disappointing in this respect. Little true improvement progress can be attributed to selection for photosynthetic efficiency in crop plants (Wilson 1979). Ledig (1974, 1976) reviews the studies of photosynthetic variation in trees. Success has been achieved in explaining site preferences for particular provenances and hybrids on the basis of environmental influences on photosynthetic rates. However, the correlations between growth rates and photosynthetic rates generally have not been good. Several reasons probably are responsible for this failure. Photosynthetic measurements taken on small trees do not adequate-

ly account for the mutual shading that takes place in the crowns of large trees. Large trees are impractical to study directly. Photosynthesis varies considerably with temperature, light flux, moisture stress (stomata influence), and season. Furthermore, rates of photosynthesis within a crown are influenced by "sink" demands -- the amount of new growing tissue that is placing demands on carbohydrate resources (Sweet and Wareing 1966; Wareing, et al. 1968; Kriedemann et al. 1976; Hansen 1977). A recent discussion among tree physiologists concluded that there is still an optimistic outlook for improving photosynthetic efficiency in trees (p. 520, Cannell and Last 1976), but until more is known about this process, it does not offer easy selection criteria.

The amount and duration of leaf display generally has proved a more useful selection tool (Wareing 1964; Ledig 1969; Wareing and Matthews 1971). Selection for rapid springtime expansion of new leaves, prolonged shoot elongation (leaf elaboration), and delayed photosynthetic senescence in leaves should all work toward greater tree yields. The growth of conifer shoots probably is composed of several heritable modes, each subject to improvement (Cannell et al. 1976). Pollard (1973) found differences in height growth of young *Picea mariana* (Mill.) B.S.P. seedlings to be closely related to their ability for "free" or lammas shoot growth. Work with *Populus* hybrids illustrates that there is genetic variation in leaf activity at different ages (Dickmann et al. 1975; Gjerstad 1975). Larson (1976) sounds one note of caution -- extending shoot growth and leaf expansion later into the season probably will affect wood quality, decreasing specific gravity and increasing vessel percentages. Therefore, it might be better to select for longer maintenance of leaf activity than for longer leaf production.

This concern about leaves might seem out of place since we foresters are interested primarily in stem wood production. Leaves are the investment that a tree makes in future wood growth and the compound interest principle applies. Genotypes that invest more carbohydrate capital in leaves often outgrow those types that hoard current production in the stem (see Ledig 1974). Carbohydrates aren't the only resource necessary for stem growth. Water and nutrients provided by the roots also are important. Therefore, allocations made for root growth will also have a feedback to the rest of the tree.

What is emerging is a regard for the tree as a system (Promnitz 1975). For stem growth to be maximal, there must be some optimal ratio of leaf to stem to root, which changes with time. Each component of the system bears a certain cost in terms of carbohydrate, water, and nutrient resources. At the same time, each component contributes benefit to the system, which we would like to measure as wood production. Models of photosynthate allocation and utilization have been proposed (Ledig 1969; Promnitz 1975; Ledig et al 1976). These models could account for genetic differences in tree growth rates on the basis of different budgeting of photosynthate. In other words, superior trees invest their carbohydrates where the greatest net gain can be realized. We tree breeders might realize greater net gains if we utilize these growth modeling concepts in our breeding programs.

As already stated, each component of the tree system bears a certain cost. This cost could be measured in terms of energy required or as carbon dioxide respired. The respiration of any plant part has essentially two components: a growth (constructive) component and a maintenance component, both of which can be estimated by appropriate techniques (Thornley 1970; Ledig et al. 1976; McCree and van Bavel 1977). Growth respiration used for the elaboration of new tissue is proportional to growth and to the gross carbohydrate supply. The conversion efficiency (plant dry weight produced/carbohydrate consumed) is relatively insensitive to temperature. Maintenance respiration is proportional to the living mass of plant tissue and is very sensitive to temperature. Different types of plant tissue have different construction and maintenance costs, and genotypes may vary in their efficiency for the two types of respiration. The point that we emphasize is that photosynthate allocation and respiration are as important to tree productivity as is photosynthesis. Respiration measurements should have fewer technical pitfalls than have plagued photosynthetic measures.

Another important physiological phenomenon is photoperiod response. We want to maximize growth activity during the favorable portion of the year and have growth cease in time for the plants to harden before winter. Thus, we can profit from a closer look at photoperiod response and its measurement (Ekberg et al. 1976). Our work with *Alnus glutinosa* (L.) Gaertn. (Robison et al. 1978) illustrates the need for such knowledge and techniques. In fitting the diverse germplasm available to new growing conditions in North America, we have only intuitive guidelines to follow. By combining short-term growth chamber tests with field observations on provenance tests in several locations, we hope to build a set of objective criteria for selecting the alder types to use in particular localities.

In breeding for stress tolerance and pest resistance, physiology has a major role to play. Periods of cold, drought, and/or pest incidence do not occur with regularity. Hence, straight field selection is very inefficient. If we understand the physiological basis of how trees avoid or tolerate these damaging agents, laboratory techniques can be developed that allow more rapid and certain screening of candidate trees. Examples of this type work for cold and drought stress can be found in chapters by Kozlowski, Tyree, van Buijtenen et al., Glerum, and Timmis in the book edited by Cannell and Last (1976). Illustrative examples of using physiology as a tool in selecting for pest resistance can be found in reviews by Bingham et al. (1971) and Hanover (1975, 1979).

The areas of biochemical and molecular genetics may have important uses in monitoring or modifying physiological processes related to tree improvement. Gordon and Promnitz (1976) reviewed the efforts made in our program to include measures of key enzyme levels (peroxidase and nitrate reductase) in physiological selection criteria for *Populus* clones. The cellular functions of repetitious DNA are still under investigation (Davidson and Britten 1979). However, it has been speculated that such important processes as protein synthesis chromosome pairing, gene regulation, genetic homeostasis, and crossability barriers are influenced

(Hall et al. 1972, 1976; Miksche and Hotta 1977).

The role of the root system in nutrition has been briefly mentioned already, and there certainly is evidence that nutrient efficiency varies within a species (Steinbeck 1971; Stern and Roche 1974; Goddard et al. 1976). What has been particularly intriguing to us is the role that symbiosis plays in tree nutrition. Considering that the normal tree root system is mycorrhizal, we should realize that joint selection of the host plant and its fungal associate probably is going to be important in improving nutrient uptake and growth. This adds an entire new layer of genetic variation and interaction. Physiological studies can best unravel this complexity and help us to select promising symbionts for field testing. An eloquent example of such studies is found in Mason's (1975) study of *Betula*.

The recent surge of interest in nitrogen-fixing tree species adds a third genetically variable component for consideration. In our studies of *Alnus*, we view the host, mycorrhizal fungi, actinomycete complex as a system that we want to understand and improve (Hall et al. 1979; Hall and Maynard 1979). Short-term studies of nutrient uptake and nitrogen fixation (Robison et al. 1978; Green et al. 1979; and Maynard, work in progress) are being used to select promising microbial symbionts. We are currently working on carbohydrate budgeting and respiration efficiences in the host plant as they relate to symbioses and tree growth potential.

The final, but most important, level of complexity to be considered in selection is performance of trees in stands or communities of other trees (Stern and Roche 1974). After all, our usual objective as foresters is to grow stands, not individual trees. Selection of individual tree superiority, our current practice, may not be the same as selecting for superior stand performance. Indeed, Ford (1976) suggests that tree breeders may just be spinning their wheels by trying to improve stand growth through selection on an individual tree basis. The alternative is to elucidate the morphological-physiological traits that control stand productivity and then select and breed for those traits in the seedlings or clones that we use to establish stands. Crown morphology, leaf display, root structure, nutrient efficiency, stress tolerance, pest resistance mechanisms, and growth analysis would all seem to be important considerations.

This section of the paper can best be summarized by referring to concepts presented in the closing discussion at the Edinburgh meeting on tree physiology and yield improvement (pp. 519-531, Cannell and Last, 1976). There are at least three roles that physiology can play in the evaluation phase of tree improvement:

1. Knowledge of physiological processes underlying economic traits can lead to the formulation of the ideotype needed to meet a specified use. It can help us define what we are after.

2. Measures of physiological processes offer hope of developing rapid selection criteria to shorten the evaluation process and/or increase the numbers of trees screened.

3. Understanding the physiological components of phenotypic traits can help us to avoid errors of judgement in making our selections and saving tree types for advanced generation improvement work.

REPRODUCTIVE PHYSIOLOGY

The other major contribution that physiology is making to tree improvement is in reproductive biology. Our improved trees are of little value until they are reproduced in commercial quantities. Even if evaluation time is not shortened, the ability to shorten the generation time would give us a head start on breeding some of the advanced selections that we would like to test.

Significant progress has been made in techniques for flower induction in the conifers, most notably by the R.P. Pharis group at the University of Calgary (Pharis 1974; 1976). Gibberellins sprayed on the foliage or injected into the vascular system have been used to shorten the non-flowering interval by 10 years or more in such important species as Douglas-fir and loblolly and lodgepole pines. Even more dramatic results have been achieved in the *Cupressaceae* and *Taxodiaceae* families in which flowering can be induced in the first growing season. Alternatively, accelerated growing conditions have been used to shorten the breeding cycle in spruces (Young and Hanover 1976). Less progress has been made with Angiospermous tree species. One exception is that continuous growth of birch under long photoperiods has produced breeding stock in less than one-year's time (Karki 1977).

These successes and the improvements that will be made on them in the future depend on advances in our knowledge of the physiology and biochemistry of flowering. Our most critical need for information concerns the nature of the juvenile and adult states in different tree species. Is there a certain stage of physiological maturity that must be attained before flowering can be induced? Or, do we need only to find the missing factor(s), such as the gibberellins in some conifers, to trigger flowering in trees of any age? The subject has been well reviewed (Zimmerman 1972; Jackson and Sweet 1972), but a clear pattern does not emerge. As important as the juvenile/adult transition is to flower production, propagation by cuttings, and wood quality, it should be receiving some major research attention.

Seed production in conventional seed orchards has become a matter of increasing concern (Matthews 1963; Puritch 1972; Sweet 1975). Economic analyses of tree breeding efforts indicate a direct payoff of increased seed production--each percentage increase in seed yield representing another percentage return on the tree improvement invest-

ment (Porterfield 1975). Recognizing the barrier that seed availability places on the use of its tree improvement results, the NC-99 Regional Tree Improvement Project in the U.S. has initiated flowering studies for *Pinus banksiana* Lamb. and *Picea glauca* (Moench) Voss at six locations across environmental gradients from Hastings, Nebraska, to Dryden, Ontario (Nienstaedt 1979). Future seed yield improvement will rest on increased understanding of the physiology of flower induction, pollination, and seed and strobili development.

A number of factors interact to influence seed production. A summary of past experience indicates that seed orchards should be located in areas with dry, warm summers, and an absence of spring frosts. The trees should be set at wide spacings and thinned periodically so that they can produce more photosynthate and growing points to support flowering. Fertilizers and irrigation should be applied, but timing probably is critical to avoid promoting vegetative growth at the time of floral differentiation (Sweet 1975). The application of gibberellins to some conifers can be used to increase the size of the flower crop, and auxins can be used to promote the production of female flowers and reduce abortion rates (Pharis 1976). And finally, it is known that genotype itself significantly influences the earliness and abundance of flowering (e.g. Johnsson 1949; Stern 1961; Teich and Holst 1969). The recommendation often has been made that we should select for flowering in the trees to be included in our seed orchards (Sweet 1975). Yet, the limited information that we have suggests that the energy that a tree spends in flowering subtracts substantially from potential vegetative growth (Fielding 1960). We face the dilemma of wanting to increase flowering in the orchard, but reduce it in the field. We need additional physiological studies on the "costs" of seed production in stands and non-genetic means of increasing seed production in orchards.

The influence of initial seed size on early tree growth (Perry 1976) seems to be an area of investigation that could pay extra dividends in conjunction with tree improvement. It certainly is a factor that must be considered in provenance and progeny tests (Perry 1976; Burdon and Sweet 1976). Physiological studies of seed development could lead to techniques for optimizing the nutritional status of the seed and, hence, early growth potential.

Vegetative propagation, the rooting of cuttings and tissue culture, is becoming one of our most valuable tools in tree improvement. No physiologist was needed to devise techniques for rooting cottonwood or willow cuttings, but physiological trickery is needed for other species (e.g. Libby et al. 1972; Shreve et al. 1974; van Buijtenen et al. 1975). We need to consider stages of juvenile-adult transition, rejuvenation techniques, hormone influences, internal nutrient/carbohydrate status, and development after rooting or plantlet formation.

Although controlled crossing has not played a very strong role in past tree improvement efforts, there is good reason to expect it will be a significant future activity in some programs (e.g. Schreiner 1970; Hall and Maynard 1979). Crosses between species and sometimes even within

species are often made difficult by physiological/biochemical incompatability barriers. These may affect the ability of pollen to germinate, the pollen tube to grow, or the capability of seed storage material to properly nourish the developing hybrid embryo (Hagman 1975; Willing and Pryor 1976). Through investigations of these processes, we can in some cases, manipulate floral physiology to allow the production of valuable hybrid offspring. For example, previously impossible crosses between the *Aegiros* and *Leuce* section poplars have been accomplished after chemical modification of the stigmatic surfaces (Willing and Pryor 1976). Kreibel (1972) suggests that research on the physiology of hybrid failure in *Pinus* section *Strobus* crosses be used as an aid for parental selection for crossability and a guide to embryo culture for producing viable hybrids. Future developments in cell and tissue culture may allow the construction of some truly unique physiological patterns as an aid to tree improvement (Carlson and Rice 1974; Hall 1977).

CONCLUSION

Some continued progress in tree improvement could be made without paying heed to physiology, and physiological genetics will not replace the need for the other tools of tree improvement. However, we can make substantial progress, we will be more sure that our progress is leading in the right directions, and we will put improvement into practice much more swiftly when we let physiology and physiologists have a strong influence on our tree improvement thinking and programs.

The future is now for the application of physiological genetics to tree improvement.

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THE ROLE OF BIOSYSTEMATICS IN TREE IMPROVEMENT

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ABSTRACT

The principles of biosystematics have been developed over the past several decades. The primary research thrust in biosystematics has been to demonstrate and elucidate phylogenetic affinities among plant taxa, in concert with traditional measurements of morphological characters. Current uses extend beyond taxonomic applications and it is these uses which hold particular promise for forest tree improvement. The purpose of this presentation is to introduce biochemical systematics and to outline its potential uses for tree improvement.

Potential uses are discussed within the framework of defining in situ genetic variation in natural populations and, in monitoring or developing selection indices for domestic populations. Potential uses are contrasted with potential difficulties in using biochemical parameters in applied programs.

RÉSUMÉ

Les principes de la biosystématique ont été élaborés au cours de ces dernières décades. La préoccupation essentielle des chercheurs a été de révéler et d'élucider les affinités phylogénétiques parmi les taxas de plantes, tout en continuant d'utiliser les méthodes traditionnelles pour mesurer les caractères morphologiques. Les techniques courantes dépassent le stade des applications taxonomiques, et ces techniques même sont tout a fait prometteuses pour l'amélioration des arbres de forêt. Le but de cette communication est d'introduire les systèmes biochimiques et de présenter des méthodes potentielles d'amélioration des arbres.

Nous envisageons l'application de ces méthodes potentielles en nous attachant à définir sur le terrain les variations génétiques dans les populations naturelles, en contrôlant ou en développant des indices de sélection pour les populations domestiques. Nous avons confronté les techniques potentielles aux difficultés potentielles au moyen de paramètres biochimiques dans les programmes appliqués.

PLANT BIOCHEMICAL SYSTEMATICS

Plant biochemical systematics is a general term used to describe the application of biochemical analyses to the study of the relationships among taxa. Comprehensive uses of the term include all comparative phytochemical investigations, irrespective of the objectives involved. For example, comparative investigations of chemical constituents of pine resin at any level of classification is rightly considered biochemical systematics, even if they include study of infraspecific variability.

Biochemical systematics exists as an area of study because plants show variation in secondary chemical compound composition. In contrast with primary compounds (i.e. chemical compounds "basic" to all plant life; e.g. chlorophyll, protein amino acids, DNA, etc.) secondary compounds show quantitative and qualitative variability. As an example, nearly 5 500 alkaloids, 100 monoterpenes, and 1 000 flavonoids are known to exist in plants (Harborne 1977). This variability serves as the focal point for biosystematic investigations, not just in a descriptive sense but because patterns of chemical variability can be interpreted to significantly aid in understanding plant population dynamics.

The plant compounds most usually studied fall into the categories listed in Table 1. These compounds ate studied because: 1) they are readily extracted from some plant tissue or organ; 2) methodology exists for their purification, identification and quantification; 3) they may play important physiological roles in plant metabolism, plant-animal or plant-plant interactions; and 4) they may be of pharmaceutical value. Thus, there is no mystery associated with choices of biochemical analytical techniques. Indeed, for many uses biochemical measures simply add an additional dimension to studies of morphologically or anatomically variable populations.

Plant compounds used for taxonomic study of quantitative biochemical differences among taxa must meet four criteria (Harborne 1967): 1) they must be variable; 2) they should be physiologically stable; 3) they should be ubiquitous in the taxa being studied; and 4) they should be easily extracted and identified. Qualitative analysis of plant compounds is also dependent on criteria 1, 2 and 4 above (von Rudloff 1975).

An often cited reason for utilization of secondary plant compounds for studies of plant variability is that biochemical characters are influenced quantitatively while morphological characteristics are often modified in cryptic ways (Alston and Turner 1963). Thus, while it might be argued that there is ample evidence illustrating non-genetic effects on biochemical traits (e.g. Feeny 1970) the magnitude of influence that non-genetic factors have on biochemical variation is probably far less than over morphological, anatomical or growth variation. It is generally agreed that the "closer" an analytical technique is to the actual DNA informational content of an individual, the less cryptic observed variability becomes. According to this argument morphological measurements are more affected by extrinsic factors than are biochemical measurements. Likewise, isozyme measures of genetic variation are less subject to

Table 1. An outline of two ways biochemical systematists often classify the organic compounds used for biochemical systematic investigations.

I. Classification by metabolic type (Lever and Burley 1974):

1. Primary metabolites-
   low molecular weight, fundamental to metabolic processes, not usually accumulated or stored, e.g., sugars, amino acids, etc.

2. Secondary metabolites-
   usually low molecular weight, often stored, often not metabolically well understood, e.g., alkaloids, terpenes, etc.

3. Macromolecules
   a. monomer &/or linkage invariant (e.g. cellulose)
   b. monomer &/or linkage variable (e.g. proteins)

II. Types of secondary plant organic chemicals (Alston and Turner, 1963):

1. Amino acids
2. Fatty acids
3. Carbohydrates
4. Alkaloids
5. Cyanogenetic substances
6. Phenolics
7. Quinones
8. Terpenoids
9. Serological variants

transient physiological or environmental influences than are more generalized measures of biochemical variability using organic compounds of metabolic processes to measure variability. Analyses of DNA content in cells (Miksche 1968) and DNA base composition analyses are probably little influenced by extrinsic effects (Miksche and Rollins 1971).

The cost of measuring plant chemicals to determine plant genetic variability, by measuring progressively closer to the DNA, is found in a loss in integration of the many systems influencing survival, growth, development and reproduction of individuals and populations. For example, knowledge of an allelic frequency for an isozyme allele, or knowledge of the presence or absence of a single phenolic compound may be useful as single information bits for defining a population of plants. However, knowledge of the number of stomates per unit of leaf surface may, as a single bit of information, tell more about a plant or set of plants. Thus at some point the chemosystematist must decide criteria for definition of different taxa; and the forest tree breeder must be cognizant of the fact that genotypes desirable from the standpoint of productivity probably will not be simply defined by some single biochemical indicator. Given these considerations, some of the applications biochemical systematics may have in tree improvement programs can be discussed.

Biochemical systematic investigations have progressed through several developmental stages. Early investigations (i.e. 1950's) emphasized research establishing biochemical "fingerprints" for a spectrum of plant taxa. From these taxonomists suggested new phylogenetic relationships or confirmed those previously hypothesized on the basis of morphology (i.e. von Rudloff 1975; Mirov 1961). A second stage of development focused on the comparative metabolism of plants and plant populations including studies of metabolic pathways, comparative enzymology, and DNA and protein structure and content. This stage of development, exemplified by the works of Fryer and Ledig (1972), Miksche (1967), Somero (1978), and Durzan and Chalupa (1968), among others, is scarcely illustrated in forestry literature. Finally, the third development stage is referred to as ecological biochemistry or phytochemical ecology. This subject area builds on previously described and newly discovered biochemical variation between taxa. Research in this area is reviewed by Horsley (1977), Harborne (1977) and Swain (1977).

IMPLICATION OF BIOCHEMICAL SYSTEMATICS FOR TREE IMPROVEMENT

A reading of the literature in biochemical systematics and ecological biochemistry suggests both proven and novel applications of biochemical techniques to tree improvement and to research in forest genetics. These investigative endeavours may be grouped according to the following categories:

a. Definition of interspecific and intraspecific variation

b. Utilization of biochemicals as unique markers of genetic variation in breeding programs, including seed source

identification

c. Use of biochemicals for development of indirect selection indices

d. Use of biochemical variations in helping to define seed zones and ecotypes

e. Breeding for insect and disease resistance

For most of the above applications ample literature discussions may be found (Lever and Burley 1974). Thus only brief development of the topics outlined above will be undertaken here. This paper should not be considered a review of literature.

For most forest tree species geneticists possess scant knowledge of the relationship between genetic variance between stands and provenances and among individuals in stands. The distribution of genetic variability within an individual (in terms of percent heterozygous loci) and between individuals (in terms of polymorphic loci) in a single stand is just now being actively investigated using isozyme analyses (see Conkle, these proceedings). Isozyme analysis is an extraordinarily useful tool for specifically and quantitatively defining genetic variance (Rudin 1976). However, other biochemicals may be as useful, particularly those to which a specific metabolic function or competitive advantage can be ascribed (Horsley 1977).

While basic research is greatly needed to detect relationships between secondary metabolites and adaptive strategies, continued study is needed to quantify biochemical variability among and within tree populations. Relative amounts of genetically controlled biochemical variation in central and marginal populations and in population samples from edaphic or climatic clines needs to be quantified completely to understand population genetics of trees (von Rudloff 1975).

For seed source studies, precursory studies of in situ biochemical variation may be particularly valuable for designing sampling procedures. For instance, chemical methods may be used to define tree races (Snajberk and Zavarin 1976), clines (Squillace 1976) and putative hybrid populations or zones of introgression (Zavarin, Snajberk and Critchfield 1977; von Rudloff 1977). Sampling and analysis of foliage from throughout the range of a species or provenance are relatively inexpensive. By providing a map of putative genetic clines, including potential zones of introgression or interspecific hybridization, the biosystematist could provide a priori evidence to suggest how provenance sampling might be stratified. This could greatly diminish the number of seed sources and trees within seed sources needed for yield trials. Additionally, the biosystematist could define populations possessing large amounts of variability, and population sets with similar biochemical profiles. By using non-random sampling techniques a limited number of population sets could be sampled for inclusion in breeding programs. This would reduce the number of plants needed for breeding

trials. The consequent cost efficiencies of being able to knowledgeably stratify large continuous tree populations are obvious.

The second major use of biochemical analyses in tree improvement is in the area of gene markers. Although the use of isozyme analyses (see Conkle, these proceedings) is likely the simplest approach to defining gene markers for use in breeding, there is no reason why terpenoids, phenolics and other biochemicals can not also be used alone or in concert with isozymes. Particular application of gene markers may be found in situations where pollen contamination is thought to be a problem in seed orchards. Analysis of expected and observed gene marker frequencies in seed orchard progeny can give precise measures of seed orchard pollen contamination. For analysing the amount of contamination, or the "take" of mist-blown pollen, biochemical markers should be of tremendous utility.

Often it is desirable to know the seed origin of a mature plantation for which no records exist. Because populations usually have unique biochemical profiles, it is probable that biochemical systematic techniques can be used to identify their genetic origins. This procedure would require in-depth knowledge of patterns of biochemical variation in natural populations. Similarly, biochemical fingerprinting can be a useful technique to monitor seed certification compliance (Bergmann 1972).

Biochemical gene markers may have use in following genotype frequency changes in improved populations when they are subjected to competition and suffer subsequent mortality. For example, using biochemical markers, gene frequencies could be calculated in maturing improved stands and the changes noted over time. Those seed orchard parents most likely to produce crop trees could thus be identified. This would be valuable in management of gene resources in a domestication program and would aid in the development of indirect selection techniques.

Indirect selection (Falconer 1960) is the procedure whereby a trait X is genetically selected for by applying a selection pressure to a genetically correlated trait Y. If it were possible to locate a biochemical marker genetically correlated to yield, then it would be relatively easy to select for yield. Although there are many difficulties in doing this in tree populations (von Weissenberg 1976), indirect selection for disease resistance, hence yield, may have a high probability of succeeding. There are indications in literature suggesting a rather direct relationship between secondary plant metabolites and disease (Levin 1976, Ehrlich and Raven 1964). For example, Franich et al. (1978) have discussed the possible relationship between needle epicuticular wax content and resistance to *Dothistroma*.

If high yield trees are high yielding because they are biochemically variable or if populations are high yielding because they are biochemically heterogeneous, then measurement of biochemical variance per se may be an indicator to use in indirect selection for yield. Improvement strategies may require selection of genotypes for specific environments. In contrast, natural populations may evolve strategies calling

for adaptability to many environments (Levins 1968, Rehfeldt and Lester 1968).

Biochemical analyses may be applied toward defining seed collection and transfer zones. It is possible to define seed zones on the basis of physiographic parameters and this has commonly been done both in the U.S. and Canada. Addition of biochemical information may in the future add a new dimension to the seed zone concept. Secondary plant chemical compounds play a role in conferring pathogenic and herbivore resistance (Levin 1976) and many may play an important role in control of metabolic functions (Seigler 1977). Subtle infraspecific variation in amounts of secondary compounds and/or in biochemical profiles may provide clues to environmental sources of selection not necessarily keyed to obvious edaphic or climatic influences. In other words, analysis of biochemical variability resulting from natural selection creating both effective and unique chemical blends may have potential for gaining greater understanding of seed zones. The biochemical niche may be an important concept in forest tree genetics - especially as it relates to disease and insect resistance. The genetics and physiological stability of the relative and absolute amount of secondary substances need considerable research if we are to understand the role of the biochemical niche in determining the fitness of tree populations and the relationship between adaptation and fitness to growth and yield (Levin 1976).

CONCLUSION

The principles of biochemical systematics have been developed over the past several decades. The primary research thrust has been to demonstrate and elucidate phylogenetic affinities among plant taxa in concert with traditional measurements of morphological characters. Biochemical systematics generally lends support to the contention that taxonomic organization in plant species is difficult and that diversity in plants is "idiosyncratic" (Levin 1979).

In a review of the applications of biochemical methods of forest genetics, Lever and Burley (1974) concluded that quantitative estimates of genetic variance in wild and domestic tree populations may be efficiently done using biochemical characteristics rather than growth and yield measurements. While that is true, it must not be overlooked that the tree improvement forester is primarily concerned with growth and yield. Biochemical systematics and genetics can assist the tree breeder in understanding the wild plants he is forced to deal with. However, before the tree improvement forester can understand the relationships between biochemical variability and growth and yield continued research in biochemical systematics is imperative. This research must be closely coupled with increased efforts in tree breeding, field testing, and long-term measures of growth and yield over varied environments and in populations composed of a variety of genotypic mixes.

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THE ROLE OF ISOZYME RESEARCH IN TREE IMPROVEMENT

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ABSTRACT

Gel electrophoresis is an efficient tool for large-scale genetics investigations. It is particularly valuable to tree breeders and forest geneticists since its unique features of simple inheritance and small environmental effects offer excellent opportunities to increase our knowledge of the genetic relationships between and within species of coniferous trees and to solve problems encountered in tree breeding and genetics research. These include identifying subpopulations, relatives and seed origin, determining the effectiveness of mass pollination techniques and the degree of selfing and wild pollen contamination in seed orchards, and aiding breeding programs through indirect selection.

RÉSUMÉ

L'électrophorèse sur gélose est un instrument d'une grande utilité pour les recherches génétiques de grande échelle. Cette méthode est particulièrement précieuse pour ceux qui s'occupent de la reproduction des arbres et pour les généticiens de la forêt. En effet, ses qualités uniques de descendance simple et ses effets environnementaux limités offrent une excellente possibilité d'elargir notre connaissance des relations génétiques entre et à l'intérieur des espèces de conifères et de résoudre les problèmes rencontrés dans la culture des arbres et la recherche génétique. Ceux-ci comprennent l'identification des sous-populations, de l'origine des parentés et des graines, l'evaluation de l'efficacité des techniques de pollinisation de masse et du degré d'auto-pollinisation et de contamination du pollen sauvage dans les vergers de semence, et la'aide aux programmes de reproduction grâce à une sélection indirecte.

INTRODUCTION

Hereditary variation, as reflected in the existence of multiple alleles in a population, is clearly a prerequisite for evolutionary change. The question of how much variation exists in natural populations of conifers is therefore of central interest to forest geneticists, since it determines to a large extent the evolutionary plasticity of a species. Yet until quite

recently the limitations of traditional genetic analysis on morphological traits (Yang et al. 1977) prevented investigators from determining precisely how much variation there is in forest tree populations.

Information on the distribution of allelic variation in natural populations can be obtained with the aid of electrophoretic analysis of enzymes. Gel electrophoresis separates enzymes on the basis of their molecular charge and their three-dimensional conformation. When this is combined with histochemical staining techniques, zymograms (banding patterns on gels) are generated. These are interpretable in terms of:

1. Multilocus structure of the enzyme (isozymes);

2. Allozyme variation (number of alleles present at a locus); and

3. Functional sub-unit structure of the enzyme (i.e. whether the enzyme is physiologically active as a monomer, dimer, etc.).

Allozymes which differ in electrophoretic mobility must differ in their primary structure (i.e. amino-acid sequence). The amino-acid sequence of allozyme is colinear to the nucleotide sequence of its gene locus. Consequently, by scoring allozyme variation, we are scoring the product of gene mutations. Screening single gene variation in population studies has numerous advantages over approaches such as provenance testing which are limited to analyzing morphological variation (Shaw 1965; Gottlieb 1971; Rudin 1976) because:

1. Genotypes are scored directly; there are, consequently, no problems with epistatic interactions or the environment mediating the expression of genotypes;

2. The expression of allozyme variants is generally codominant; under most circumstances, therefore, homozygous and heterozygous individuals can be differentiated from each other; and

3. The amount of genetic information studied is directly quantifiable (i.e. number of loci), whereas complex morphological traits are often controlled by an undetermined number of loci.

Analysis of enzyme variation, therefore, provides a relatively precise estimation of intra- and interspecific genetic variability in forest tree populations.

The use of gel electrophoresis in isozyme studies of genic variability in natural populations of conifers has permitted researchers to investigate many basic questions of evolutionary biology (Rudin 1976). These questions concern levels of heterozygosity within populations, distribution of genic variability within and between local populations, relative amounts of genic variability in central as opposed to marginal populations, and mating systems. Recently, isozymes have been used as genetic markers in seed orchards to determine and maintain the identity of individuals, crosses and breeding populations with greater accuracy

than previously possible (Rudin and Lindgren 1977). Information from studies in both natural and orchard populations will no doubt be extremely valuable in improving the efficiency of tree improvement programs.

In this paper are summarized areas of research where gel electrophoretic techniques can be used. Examples are drawn mainly from my working experience with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and lodgepole pine (*Pinus contorta* Dougl.). For more thorough reviews, including sampling and analytical techniques, see Feret and Bergmann (1976), Rudin (1976) and Morris and Spieth (1978).

APPLICATIONS

Amount of Genic Variability

The inherent variability within a conifer species is the raw material for its genetic improvement, whether it be by natural (evolutionary) or by artificial (selective breeding) processes. Recent studies (O'Malley et al. 1979; Yeh and El-Kassaby 1979; Yeh and O'Malley 1979) have shown that most conifers are highly heterozygous. If one considers that about 25 to 30 percent of codon differences are detectable by electrophoresis (King and Wilson 1975) and makes the correction for this factor, an individual is expected to be heterozygous for about 45 to 65 percent of its total genes. This rich gene pool within local populations is not surprising considering that most conifers are exceedingly variable in morphology, both across their native ranges and from tree to tree within stands.

The high level of detected gene diversity in conifers is probably due to a number of factors: divergent selection for macro-geographical adaptation (Allard et al. 1972), balancing selection for micro-geographical differentiation (Hamrick and Allard 1972; Mitton et al. 1977) and an open breeding system which facilitates gene flow within and between subpopulations. Heterosis might further promote the maintenance of genic variability (Lewontin et al. 1978). The notable exception to high heterozygosity in conifers is *Pinus resinosa* Ait. (Fowler and Morris 1977). The lack of genic variability within red pine, however, has been hypothesized to have resulted from a severe population bottleneck, probably during the Pleistocene, when red pine was reduced to small refugial population as a result of glaciation.

A striking feature of the most recent surveys has been the demonstration of a high degree of interlocus variation in heterozygosity within populations (Yeh 1979). Although no variation was apparent at some loci, at other loci more than one-half of the individuals were heterozygous.

Distribution of the loci relative to the frequency of heterozygotes was rather even for heterozygosities between 0.05 to 0.60;

however, between 38 to 56 percent of the loci analysed had heterozygosities lower than 0.05. The loci surveyed, therefore, do not seem to be equivalent in their contribution to the overall mean heterozygosity of the species. This broad range in heterozygosity, with a mode approaching zero, suggests that many isozyme loci should be surveyed to estimate reliably the amount of genic variability in conifers. Because of such heterogeneity, interpretations on the basis of differences in detected heterozygosity between species and subpopulations within a given species should be considered tentative until a large number of loci are studied.

Presence of large amount of genic variability would justify the cost of tree improvement in a given species. Knowledge of subpopulations within a given species containing large amount of genic variability would be useful for defining areas to be set aside for gene conservation, and could serve to define areas for selection of trees to be used for increasing the gene pool in previously selected breeding populations.

Distribution of Genic Variability and Geographic Pattern

Electrophoretic data on genic variability in a conifer could be summarized in terms of its hierarchical organization (O'Malley et al. 1979; Yeh and El-Kassaby 1979; Yeh and Layton 1979; Yeh and O'Malley 1979). This analysis (Nei 1973) enables genic variability to be partitioned among different hierarchical levels of population structure--within as opposed to between subpopulations.

Subpopulations were differentiated by 3 to 11 percent of the electrophoretically determined variability. Eighty nine to 97 percent of genic variability was maintained within subpopulations. That the majority of genic variability in conifers is maintained within subpopulations is, perhaps, a reflection of their ecological amplitude, their breeding system, and the lack of effective barriers to gene flow between subpopulations.

Use of isozyme variability for studying intra- and interspecific variation has been very fruitful. Isozyme variation from different biogeoclimatic regions greatly expanded our present knowledge of the genetic relationships among population types (Yeh and Layton 1979). Identification of continuous and discontinuous pattern of isozyme variability aided in the identification of ecotypes (Bonnet-Masimbert et al. 1978). Studies of introgression (Copes and Beckwith 1977) and mating system (Mitton et al. 1977) provided solutions to a number of basic and applied problems in forest tree genetics and tree breeding. Clearly, knowlege of distribution and pattern of genic variability greatly assisted in the understanding of ecological genetics of conifers. The acquisition of such knowledge would be of immense help in understanding and developing populations and in developing improvement strategies.

In coastal Douglas-fir (Yeh and O'Malley 1979) and interior Douglas-fir (Yeh-unpublished) geographic variation patterns were well known enough to identify seed sources. Possibility of using isozymes for seed source certification, therefore, has become apparent.

Analysis of Individuals

Isozymes that are simply inherited offer great potential as gene markers. Conifers are unique in that genetic control of specific isozymes could be determined in a single generation without extensive breeding experiments. The megagametophyte is haploid and has the same genetic constitution as the female gamete, the egg. Analysis of several megagametophytes from different seeds of a single tree would reveal the genotype of that tree. Genotype of the embryo could be determined, also, for isozymes. Knowing the genotype of an embryo and its adjacent megagametophyte, the pollen contribution of the embryo could be inferred. Inheritance of allelic variation could be inferred from Mendelian (1:1) segregation of alternative enzyme phenotypes when scoring numerous megagametophytes from a single tree heterozygous at a locus (Bartels 1971).

In coastal Douglas-fir, where the genetic control of many specific isozymes had been determined, we could identify relatives to the same extent that relatives in domestic animals could be identified through blood types. In this regard, we had used isozymes to detect labelling errors in both clonal orchards and progeny tests. Use of isozyme profile of individual trees may be extended to culling of potential selfers in orchards and to studying inbreeding and effective breeding sizes of orchards.

CONCLUSIONS

The potential uses of gel electrophoretic technique in the study of conifer genetics are considerable. Clearly, the technique can provide solutions to a number of basic and applied problems in conifer genetics and improvement. In the future, analyses of isozymes should significantly expand the body of knowledge in conifer genetics and aid in the planning of optimal breeding strategies for tree improvement and domestication.

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DISCUSSION

Dr. J.L. Klein. Have you tried any vegetative tissue for isozyme analysis or is there any vegetative tissue which might be so tried?

Dr. F.C. Yeh. Well, vegetative tissues have been used. I think we did not use them primarily because our laboratory is very small. When you have vegetative material you require a much more sophisticated laboratory, because you need to get rid of a lot of what we call phenolics which are junk material and we have much more success with seed and we are not going to change it unless we are forced to. Lately, we are forced to, because I have a project in which I have to look at incompatibility between root stocks and scions in which I will be forced to use vegetative material.

Dr. E.K. Morgenstern. You mentioned the point of using a number of isozyme systems at the same time to get the complete results. How would you integrate the results if there are many isozymes and you are looking at only two or three?

Dr. F.C. Yeh. It depends on the objective. For example, if you just want to talk about the common variation pattern you can pick up any one of those and you can see whether you have them or you do not. But of you want to see how much variation there is for a particular species then you will have to look at a large number of isozymes. For example, if you pick up esterase and you would say there is 50% variation in Douglas fir and this is to be multiplied by 3 or 4 which will become incredible. But personally I would like, because of the different classes of isozyme variation patterns, to look at the

average effect of genes.

Dr. N.C. Bhattacharya. My interest would be to know the basis for selecting a large number of isozymes. In my opinion there must be some basis for that particular type of tissue, because all isozymes are not going to be so effective in all the assay work.

Dr. F.C. Yeh. As I just said it depends on the special objective. I have not dealt with the indirect selection which I briefly pointed out in the paper that I am writing. For example, we have detected association between isozyme patterns and phenotypic traits of interest. Therefore, in that case that is all you are interested in and you are not worrying about whether the isozymes are directly selected for or they have been selected with the gene complexes responsible for them. The point is that you have detected the association and right now we are in the process of proving that type of association by creating artificial genotypes and look at them to see whether they can be subjected to treatment. In Sitka spruce frequency of certain Aconitase allele has been shown to increase with the flushing date and germination has been found to increase with the frequency of certain glucose 6-phosphatase dehydrogenase allele. One thing that I would like to mention is that you can set up an isozyme laboratory with about $1 000 or so like the set up that I have. When I first have students coming to our laboratory to work they first look at the laboratory and would not want to comment on the laboratory, because it looks so primitive compared to what they were used to at the university. They have really sophisticated equipment at universities. But once they get used to the system that we have they do not want to go to any other system, because it is very very productive. We can genotype about 3 000 genotypes a day in our laboratory. As I said each of these gels has 60 samples and each of these gels can be sliced into seven or eight slices and we can run eight of these gels. Therefore, 8 x 7 x 60 = 3 360 and that is the amount of genotypes that we are working on per day in our laboratory.

AMOUNT AND DISTRIBUTION OF ISOZYME VARIATION IN VARIOUS CONIFER SPECIES

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ABSTRACT

Estimation of the relative amount and the geographic distribution of genetically controlled variation is a central topic of tree resource management. Biochemical data from the analysis of forest tree enzyme variants provides a direct and precise measure of allele frequencies of tree genes.

The amount of genetic variation in several conifers; Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), loblolly pine (*Pinus taeda* L.), sugar pine (*P. lambertiana* Dougl.), knobcone pine *P. attenuata* Lemm.), lodgepole pine (*P. contorta* var. *latifolia* Engelm.), and Jeffrey pine (*P. Jeffreyi* Grove and Balfour) is assessed by determining the number of alleles per locus and species heterozygosities. Both measures indicate that native conifers are genetically variable and species differ in the kind and amount of variation they contain. Comparisons with herbaceous plants rank conifers among the most variable plants thus far studied.

The distribution of genetic variation measured by analysing stands and geographic samples is an active area of research. Published studies and current unpublished work leads to the conclusion that geographic trends can be identified that support the hypothesis that subsamples are significantly differentiated with respect to allele frequencies but the major amount of variation resides within the subsamples. Studies using isozyme data are providing data on breeding systems and comparisons of life history characteristics are showing consistent patterns which characterize the genetic strategies of forest trees.

RÉSUMÉ

L'évaluation de la qualité relative et de la distribution géographique de la variation faisant l'objet de contrôle génétique sont des problèmes de base dans l'organisation de l'exploitation des ressources en arbres. Les données biochimiques obtenues d'après l'analyse des variations d'enzymes des arbres de forêt donnent une indication directe et précise de la fréquence des allèles dans les gènes des arbres.

Le taux de variation génétique de plusieurs conifères; le sapin Douglas (*Pseudotsuga menziesii* (Mirb.) Franco), le pin à l'encens (*Pinus taeda* L.), le pin gigantesque (*P. lambertiana* Dougl.), le pin knobcone (*P. attenuata* Lemm.), le pin lodgepole (*P. contorta* var. *latifolia* Englem.), et le pin de Jeffrey (*P. Jeffreyi* Greve and Balfour) est trouvé en déterminant le nombre d'allèles par locus et les espèces hétérozygotes. Ces deux mesures indiquent que les conifères indigènes sont génétiquement variables et que les espèces diffèrent par la nature et le taux de variation qu'elles contiennent. Des études comparatives avec les plantes herbacées rangent les conifères parmi les végétaux les plus variables qui aient été étudiés jusqu'à présent.

La distribution de variation génétique mesurée sur des plants d'arbres et des échantillons géographiques représente une branche active de la recherche. Des études publiées et des travaux en cours non publiés nous poussent à conclure que l'on peut identifier des tendances géographiques qui affirment l'hypothèse selon laquelle les échantillons secondaires sont largement différenciés en ce qui concerne les fréquences d'allèles, mais que le plus grand taux de variation se trouve à l'intérieur des échantillons secondaires. Des recherches basées sur l'isozyme apportent des informations sur les systèmes de reproduction. Des comparaisons sur la nature du cycle de la vie présentent des modèles stables qui caractérisent les stratégies génétiques des arbres de forêt.

INTRODUCTION

A current challenge in forest research is to estimate the amount and distribution of genetic variation between and within taxa. This information is applied in assessing how significant improvement in the commercial products of forests can be realized while maintaining sufficient genetic variability to cope with uncertain future environments.

If a scale for measuring relative amounts of genetic variation was developed with natural populations representing maximum and cultivated varieties representing lesser values, most native North American forest trees would probably rank near the top. Cultivated varieties and introduced species of trees have not displaced native vegetation and most native populations have not shrunk to the extent that tree breeders are confronted with a severe reduction of genetic variability. Cautions from breeders working with animals and crop plants that have long histories of domestication, however, are influencing tree improvement plans. Many, perhaps most, domesticated species and varieties have severely restricted genetic resources (Harlin 1975). Breeders are actively searching for usable genetic variability among native populations (Harlin 1976, Brown et al. 1978). Forest tree breeders need to identify the amount and distribution of genetic variation in managed species to develop efficient tree improvement strategies. Isozyme analyses play an important role in the description of genetic variability.

My current research effort is to identify allelic forms of isozymes and characterize the genetic variability within six conifer species. This research, when added to estimates of heterozygosity in isozymes for other conifers, suggests that trees are among the most heterozygous of higher organisms analysed (Hamrick et al. 1980). Current reports on several species further suggest that most genetic variability is within geographic samples and a small proportion (about 7 percent) of total variation is between geographic samples.

THE PROCEDURES

Open-pollinated seed from individual parent trees germinates to the stage where the radicle just emerges beyond the seed coat and is then refrigerated until analysed.

As a minimum, six gametophytes from seed of each parent tree are individually macerated and electrophoresed. With this sample size, homozygotes are identified without error and true heterozygotes are expected to be misclassified in less than 3 percent of the samples. Electrophoresis and staining procedures are similar to those described by Fowler and Morris (1977), and Guries and Ledig (1978). Phenotypes of enzyme bands including migration distances and staining intensities are the basis for identifying allelic forms and inferring parent tree genotypes.

Seed materials came from a variety of sources (Table 1). Diploid tree genotypes were inferred for individual trees in the samples and allele frequencies for loci were assessed on the basis of two times the number of parent trees.

The techniques for obtaining enzyme bands is similar for all conifers, but the analysis of the species included in this study spans several years of research. Different numbers of loci per species resulted from improvements in laboratory procedures. Some bias toward more variable loci may be a feature of species with few numbers of loci, but all enzymes with clear bands are included in this survey.

Two measures of genetic variability are examined for each species. The average number of alleles per locus estimates the allelic differentiation within a species. A few loci in some species are monomorphic, but characteristically, from two to a maximum of nine different alleles are identified for each locus.

The second measure of variability estimates the average number of heterozygous loci per parent tree. Average hetereozygosity is calculated from species allele frequencies for each locus by subtracting the sum of the squared allele frequencies from 1.0. This heterozygosity value estimates the number of heterozygotes expected under Hardy-Weinberg conditions. For species in this study, the estimated numbers of heterozygotes approximates the actual count of heterozygotes. The choice of using computed rather than observed values is to make the data

Table 1. Species samples for evaluating the amount of allelic variation in isozyme loci.

Species and Location	Parent Trees 1/ in the Sample
Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco	
Natural population, Oregon	152
Loblolly pine, *Pinus taeda* L.	
Natural population, North Carolina	146
Superior trees	90
Sugar pine, *P. lambertiana* Dougl.	
California and Oregon	58
Jeffrey pine, *P. Jeffreyi* Grev. & Balf.	
Four natural stands, California	75
Lodgepole pine, *P. contorta* Dougl. ssp. *murrayana*	
Natural stand, California	40
Knobcone pine, *P. attenuata* Lemm.	
Ten geographic areas, Oregon and California	49

1/ Allele frequencies are 2 X these numbers.

compatible with other literature. The number of heterozygotes per locus tends to be large when allele frequencies have intermediate values and when alleles per locus are numerous.

RESULTS

Species differed in both measures of genetic variability (Table 2). Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and loblolly pine (*Pinus taeda* L.) averaged almost four alleles per enzyme locus. Sugar, pine (*P. lambertiana* Dougl., Jeffrey pine (*P. Jeffreyi* Grev. & Balf., and Lodgepole pine (*P. contorta* Dougl.) averaged slightly less than three alleles per locus, and knobcone pine (*P. attenuata* Lemm.) had only about two. Confidence intervals for the means indicate that the three groups

Table 2. Isozyme variability in several coniferous species.

Species	Loci Analysed	Alleles per Locus ($\bar{X} \pm$ S.D.)	Heterozygosity per Locus ($\bar{X} \pm$ S.D.)
Douglas-fir	19	4.00 ± .22	0.33 ± .03
Loblolly pine			
Natural stand	11	3.73 ± .32	.34 ± .04
Select trees	30	3.87 ± .13	.26 ± .01
Sugar pine	20	2.85 ± .18	.26 ± .03
Jeffrey pine	44	2.85 ± .33	.26 ± .05
Lodgepole pine	37	2.78 ± .08	.19 ± .01
Knobcone pine	22	2.09 ± .11	.14 ± .02

of species differ significantly from one another.

Mean heterozygosities per locus followed the trends set by the average number of alleles per locus. Douglas-fir and the natural stand of loblolly pine had trees that on the average were heterozygous at about one-third of the loci in the sample. The superior tree sample of loblolly pine had a lower estimate for heterozygosity than the natural stand (Table 2), but when the same genes are compared, the values are nearly equal. Additional loci analysed recently in our laboratory had several allelic variants per locus, but often had one allele in high frequency.

The select loblolly pines had the same values for heterozygosity per locus as did sugar and Jeffrey pines. These three species were heterozygous for about one-fourth of their loci. Lower values were observed for lodgepole pine (0.19) and knobcone pine (0.14).

DISCUSSION

Contrasts between conifer species for the amount of isozyme variation each possesses are heightened by the finding that red pine (*Pinus resinosa* Ait.) is estimated to have an average heterozygosity value of zero (Fowler and Morris 1977). Nine loci were studied for red

pine. In other conifers, several loci which were among those sampled in red pine, had many alleles and high estimates of heterozygosity. Because several of the red pine seed samples were from widely separated geographic locations, the lack of variation was not the result of restricted sampling. It is noteworthy that red pine has high self-compatibility and low levels of phenotypic variability in growth traits. The zero value for red pine heterozygosity is the low benchmark.

The values reported here for alleles per locus and heterozygosity per locus for Douglas-fir and loblolly pine are among the highest obtained for conifers. A similarly high value was reported for bristlecone pine (*P. aristata* Engelm.): 2.35 alleles per locus and heterozygosity of 0.36 (Hiebert 1977). The highest values for conifers, therefore, approach four alleles per locus and average heterozygosities in excess of one-third of the loci per individual.

CONCLUSIONS

Broad-scale comparisons of species and taxa are outdated as new data become available and should be interpreted cautiously because comparisons are rarely made on the basis of the same subset of loci. But inclusion of large samples of loci improves average values for species. Hamrick et al. (1979, 1980) summarize plant species information currently available. On the average, genetic variation in plants is roughly equivalent to that in invertebrates but significantly more than in vertebrate species. Trees average significantly more variation than herbaceous plants. Plant species, with the greatest number of alleles per locus and the highest values for heterozygosity, have large geographic distributions, high fecundities, outcrossing as the mode of reproduction, long generation times, and are wind-pollinated.

Total genetic variation within a taxon can be partitioned to within and between subsamples. Recent studies of variation in conifers show that most of the genetic variation is within subsamples. Pitch pine (*P. rigida* Mill.), had 99 percent of total variation within populations (Guries and Ledig 1977) and only one percent between populations. Yeh (1980) reports similar large amounts of variation within populations of Douglas-fir, Sitka spruce, and lodgepole pine (ssp. *latifolia*) from British Columbia, Canada. From 92 to 97 percent of the total variation is within populations of these three species. Brown and Moran (1980) summarized 17 studies of forest trees and found that wind-pollinated species averaged 93 percent of total variation within subpopulations, 7 percent between populations.

These low levels of variation among subpopulations strengthen the inferences drawn from the species data reported in this study. Estimates of the amount of genetic variation of single populations are expected to compare favorably with samples representing species collections. Further work on forest trees is expected to assign tree species on a heterozygosity scale that ranges from near zero heterozygosity to the most heterozygous organisms studied to date.

It seems reasonable to assume that variation in enzymes is a direct measure of overall genetic variation. Isozymes have proved their usefulness for estimating relative amounts of genetic variability within taxonomic units and for comparing the degree of similarity between taxa. Allelic differences in enzymes, however, measure genetic variation in primary gene products. These primary gene products are many steps removed and represent only a small sample of the genes that contribute to the expression of a tree's phenotype. The challenge in research is to establish a relationship, if it exists, between enzyme data and phenotypically valuable traits in forest trees.

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DISCUSSION

Dr. E.K. Morgenstern. In the last slides you showed that a low amount of variation had been found in Norway spruce but a very large amount of variation in Monterey pine - two species which have range differences of the order of 1 000. Could you explain?

Dr. M.T. Conkle. Your observation should account for variation within the two species. The total isozyme variation for Norway spruce from Sweden was estimated to be three times larger than for California Monterey pine, 41 percent vs 15 percent. The illustration, which you referred to, divided total variation into two classes; among populations and within populations. Monterey pine had significant variation among populations (15 percent), while Norway spruce populations were less distinct (3 percent of total variation). The contrast between these two species suggests a relationship between variation in isozymes and the size and isolation of populations within species. Norway spruce is widely distributed with large population numbers and few restrictions to gene flow. Monterey pine has small populations separated by distances of 50-100 kilometres. The information for Norway spruce was from a 1979 Hereditas article by Kenneth Lundkvist. Data for California populations of Monterey pine were from a seminar by Gavin Moran.

Dr. E.K. Morgenstern. I guess in your Table 1 you have a low number of samples of Norway spruce. I wonder if you could explain this.

Dr. M.T. Conkle. Data for forest trees are just becoming available. I selected studies that reported ten or more loci to gain accuracy in the average estimates. Other studies of Norway spruce, however, give values similar to those in the illustration. Researchers, I might add, are working toward standardized isozyme analysis so results from different laboratories will be comparable.

Dr. D.P. Fowler. The question in Norway spruce is a bit bothersome. I suggest it makes very big differences if your three or four samples are from Poland, let us say, compared to a broader range-wide sample.

Dr. M.T. Conkle. Isozyme studies of various plants and animals commonly report large proportions of total variation within geographic samples and small proportions among samples. Thus, I expect variation within stands from Poland would be minor, similar to the findings of the Swedish study. Comparing Polish and Swedish samples, however, should

produce larger differences.

Forest geneticists are accustomed to significant differences among provenances in growth trials and these differences are often greater than 3 percent. But, the comparison of isozyme and growth results may not account for the compounding of growth differences over the life of a trial. Would growth differences within field trials average about 3 percent annual rates when discounted over several years? Francis Yeh's research addresses the comparison of isozyme and growth variability; we should watch his results as they become available.

Dr. R.B. Hall. This population versus within stand versus between stands selection seems to be very important and I am wondering how much extrapolation you can make between particular enzyme systems which may not be under strong selection pressures versus something like photoperiod response which would be under very strong selection pressures.

Dr. M.T. Conkle. People are actively researching your question. Some isozyme variation probably represents noise (random variation) while other isozyme variation results from selection. Isozyme alleles differ in heat deactivation and temperature optima, and the biochemical function of most enzymes is known. Some isozyme allele frequencies may reflect selection as strongly as photoperiod response.

Dr. F.C. Yeh. I would like to add a little bit to the analysis. We have further subdivided the analysis into subpopulation aspects. Like we looked at within populations. This is confounded because it included family variation within populations. And that component comes to about one-third of the variation. Therefore, family differences within populations account for one-third of the differences that you see.

CELL AND TISSUE CULTURE FOR TREE IMPROVEMENT OF CONIFEROUS SPECIES

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ABSTRACT

In recent years, cell and tissue culture technology has been oriented increasingly toward tree improvement programs, largely to overcome many of the constraints embodied in existing practice and to meet industry's needs for trees. The trees are needed in large numbers, with high quality, hardiness, and rapid growth, especially on the poorer sites. For Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco)and loblolly pine (*Pinus taeda* L.), tissue culture technology, involving materials from juvenile tissue or from improved seed has increased by a hundred-fold the ability to clone high quality material for container planting programs. The variability and field performance of this type of approach has not yet been evaluated. Substantial progress has been made in another approach using cell suspensions from more mature and proven trees. For Douglas-fir and loblolly pine, the mass production of embryo-like structures from somatic cells (1 000 per 10 ml) has been obtained only recently in the authors' laboratory. There is every reason to believe that the same advancement may be obtained with some boreal species such as white spruce (*Picea glauca* (Moench) Voss) and jack pine (*P. banksiana* Lamb.). The opportunities that this progress promises, are outlined to show, first, how cell and tissue culture methodology could contribute to domestication and mass cloning and, second, how existing barriers to crossability might be removed using protoplast and genetic engineering technology.

RÉSUMÉ

Ces dernières années la technologie de la culture des cellules et des tissus a été appliquée davantage aux programmes d'amélioration des arbres, essentiellement afin de surmonter un certain nombre de contraintes particulières aux méthodes existantes et de répondre aux besoins en arbres de l'industrie. Il faut que les arbres soient nombreux, de haute qualité, et qu'ils aient une croissance rapide, surtout sur les terrains pauvres. Dans de cas du sapin Douglas (*Pseudotsuga menziesii* (Mirb.) Franco et du pin à l'encens (*Pinus taeda* L.), la technologie de la culture des cellules et des tissus basée sur des matières provenant de tissus juvéniles ou de semences perfectionnées a permis de multiplier par cent la production de

clones de haute qualité appliqués à des programmes de plantation en container. On n'a pas encore évalué la variabilité ni les résultats sur le terrain de ce genre d'approche. D'importants progrès ont été réalisés dans la cadre d'une autre méthode utilisant les suspensions cellulaires sur des arbres plus mûrs et plus éprouvés. La production massive de structures de type embryonnaire à partir de cellules somatiques (1 000/10 ml) ne vient d'être obtenue que récemment dans le laboratoire de l'auteur. Nous avons de connes raisons de croire que les mêmes progrès peuvent être réalisés avec certaines espèces boréales comme l'épinette blanche (*Picea glauca* (Moench) Voss) et le pin de Banks (*Pinus banksiana* Lamb.). Les possibilités que ces progrès peuvent apporter montrent d'abord comment la méthodologie de cellule et de tissu pourrait contribuer à la domestication et au clonage de masse et aussi comment l'on pourrait surmonter les obstacles actuels à l'hybridabilité en utilisant la technologie du protoplasme et de la génétique.

INTRODUCTION

Problems associated with the improvement and domestication of coniferous species impose constraints on the yields of forest products. Attempts to widen the range of multiple uses of trees in response to the current energy and materials crisis impose additional demands on our dwindling forest resources. In the USA, the opportunities are clear for southern pines. Hall (1979) has estimated that on managed plantations tree improvement over several generations, when coupled with a program of asexual propagation, could increase the forest productivity by a factor of 3.7 over a minimal base line of 7 to 11 m^3/ha/yr. In the boreal forest, factors such as available nitrogen on site classes, genetic variability, slow growth, and tolerance of severe climatic conditions often determine whether land should be invested in tree growth or put to alternative uses. Nevertheless, the problem remains that should large numbers of trees be planted, what would be the source and quality of these trees?

Should a desirable source or specimen be found, asexual or vegetative propagation can be used to multiply individuals with specific genetic gains. The gains would include the following properties: rapid growth rate, resistance to insects and disease, desirable wood quality, good shape and form, responsiveness to silvicultural practices and reliable cone and seed production (Durzan and Campbell 1974). In cloned individuals these traits must be matched with performance under specific soil conditions and at typical sites preferably close to the mill.

This report will deal with a review of recent advances in cell and tissue culture technology aimed at the development of methods for the mass propagation of conifer species. In the short term, this alternate technology in vegetative propagation is believed to have its merit as a complementary system to existing proven methods of propagation. Because of the long and complex life cycle of trees, the value of cell and tissue culture methods remains to be properly evaluated and field-tested. Over the long term, the greatest benefit of cell and tissue culture lies in

the convenient manipulation of the genetics of individual cells. Through the enzymic removal of the cell wall to form protoplasts, new opportunities in reducing the barriers to crossability and hybridization emerge through protoplast fusion and genetic engineering.

VEGETATIVE PROPAGATION SYSTEM

The use of cell and tissue culture falls into a wider vegetative propagation scheme (Durzan 1979). Figure 1 contains a sequence of decisions and alternatives that deremines whether or not propagation involves tissues or cells. The key steps are:

i. Determination of client needs and the genetic gains to be realized. This includes identifying heritable traits that are essential and non-essential, balancing or counterbalancing, and desirable or undesirable. The specific genetic trait must be evaluated with a knowledge of its history. Screening and testing methods for the trait should be developed if not already available. The economic gain and options in the systems should also be evaluated and known.

ii. Introduction of precultural factors unique to the species to facilitate propagation, particularly from more mature individual trees. Barriers to propagation that must be removed need to be identified, and steps should be taken to overcome these factors (e.g. Boulay 1976). This could include removal of apical dominance in shoots of mature trees and pretreatments that convert mature tissues to a more juvenile phase. Choice of location of explant, choice of culture system, and screening of the explant for totipotency are other factors. A feasibility study should reveal unknown factors and identify the permissive or inductive situations allowing morphogenesis.

iii. Cell, tissue, and organ (meristem) culture alternatives. The systems of choice will involve partially organized explants or the fully unorganized tissue route. The options will be determined by the number of required propagants, success in generating callus, cell suspensions, numbers of clones, and by the fidelity required in the product. The partially organized route is labor-intensive and requires a developmental integration of plant organs that are sequentially induced. The genetic stability of gymnosperm cells in vitro makes the unorganized route attractive for many of industry's needs (Fig. 2).

iv. Once plantlets have been regenerated, quality control and packaging of the product is essential. Embryos can be pelleted by special coatings to form artificial seeds that can be easily handled for mass planting (e.g. Murashige 1978). Alternatively, plants can be distributed in containers for field planting.

v. For woody species, physiological preconditioning in the field may be essential. Control over nursery diseases is important. This

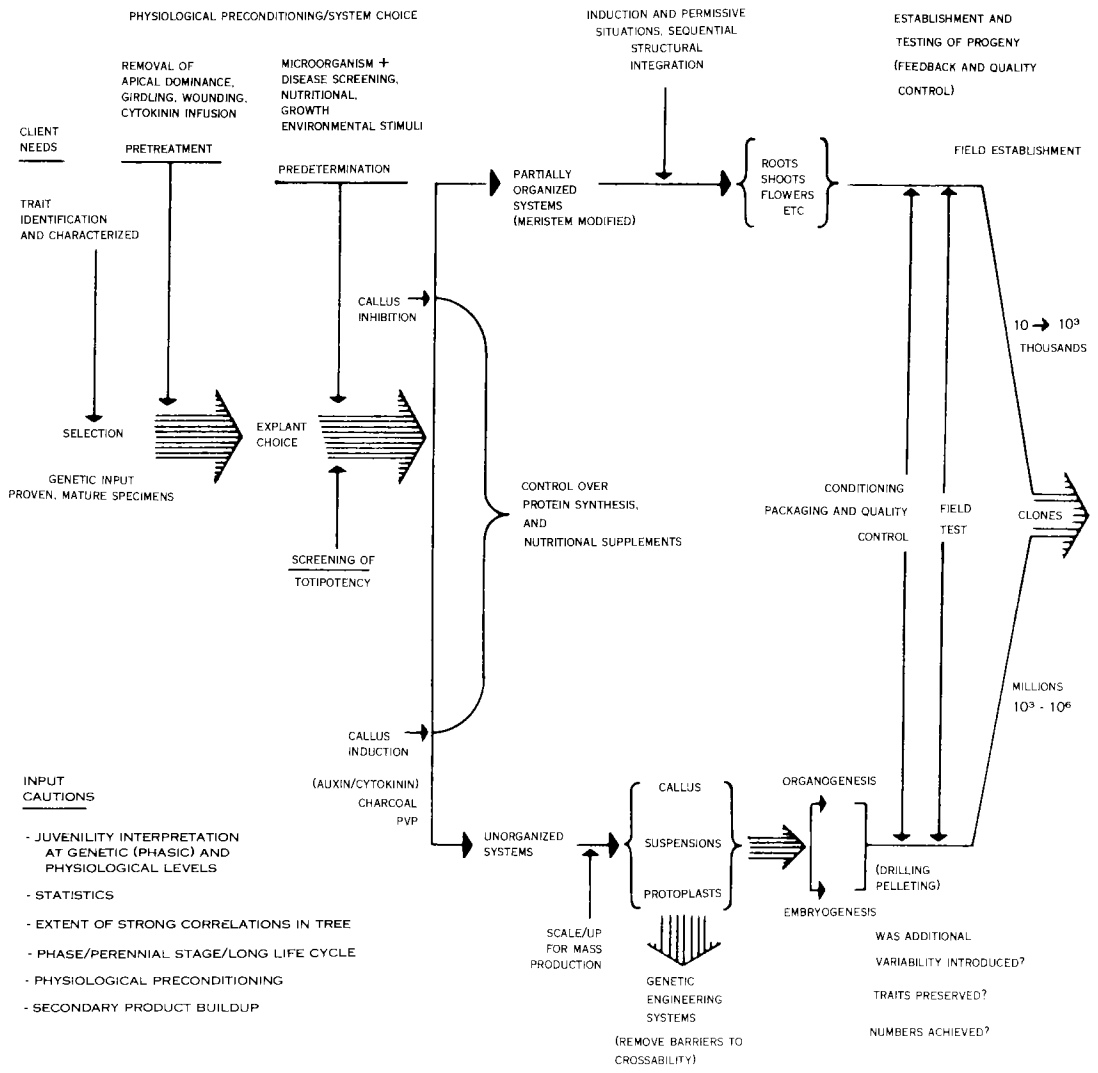

Fig. 1. Scheme for alternatives in the vegetative propagation of coniferous species. The various stages in the sequence are identified at the top of the diagram from the upper left to the right.

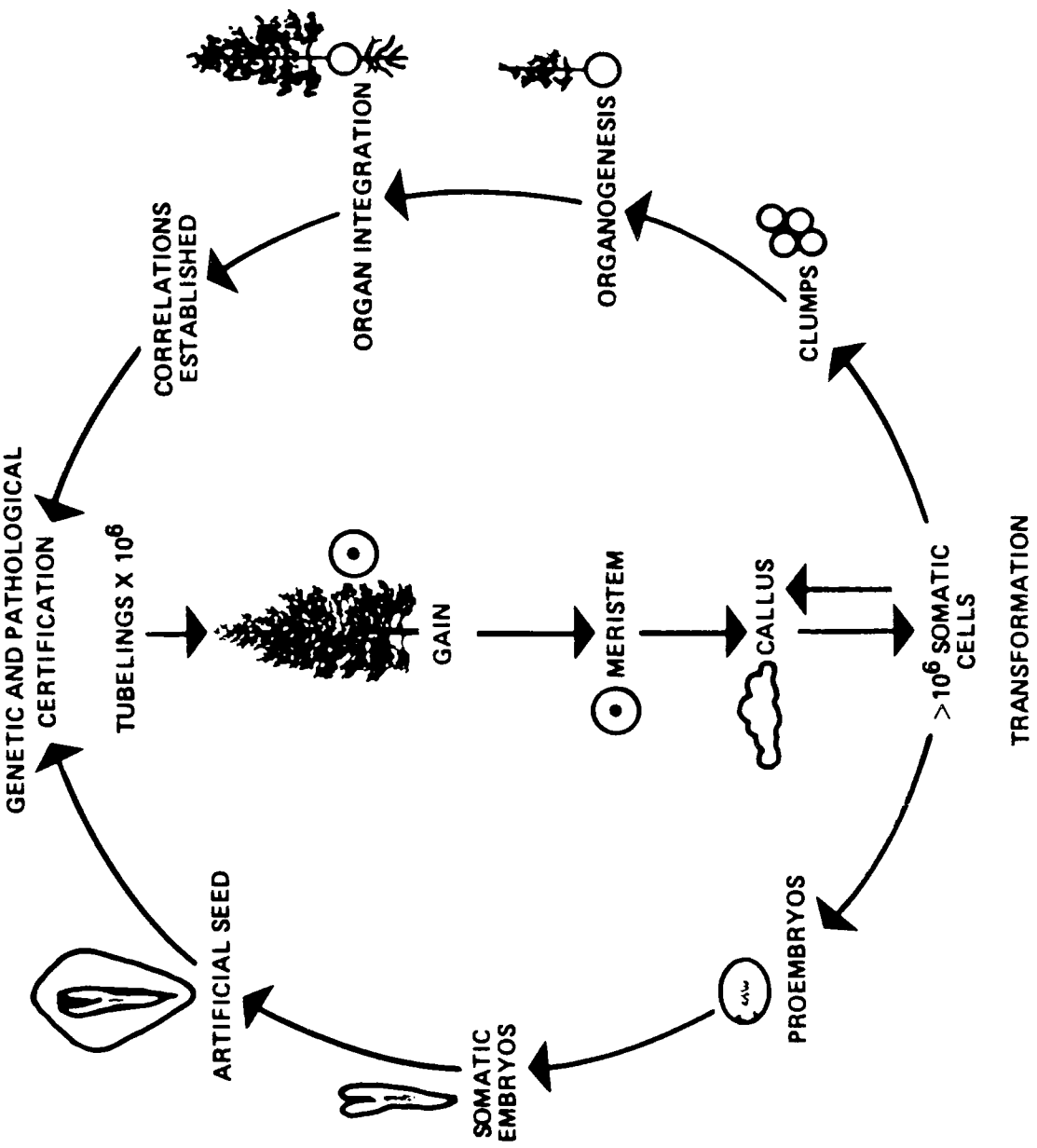

Fig. 2. Cloning cycle for conifers using cell suspension cultures. Embryogenesis occurs along the pathway to the left whereas organogenesis proceeds to the right.

may be achieved by placing fungicides in the pellet around the somatic embryo or propagule. Disease-free certification of the product by a pathologist is a requirement.

vi. Field testing and establishment of the clones require the input of population geneticists who can decide upon the ideal genetic blends for specific sites and end uses. The overall system presents several end-uses. These include domestication, trees for energy plantations, production of pathogen-free trees, improved and efficient breeding systems and progeny testing. Viable cell suspension cultures with demonstrated potential for totipotency provide excellent experimental system for an understanding of biological and physiological processes underlying growth and development of conifers.

SOME ADVANTAGES AND LANDMARKS

In tissue culture practice, gymnosperms offer an advantage not always shared by angiosperms. In the developing seed cone the multicellular female gametophyte is derived from a single haploid cell and is thus genetically homogeneous. The gametophyte passes through a "free-nuclear" stage, i.e., one where the cell contains numerous free nuclei that are not separated by cell walls. This means that we may be able to use the free-nuclear stage of the gametophytes to induce homozygous diploid and tetraploid tissues which could be organized into plants. The haploid state also provides a means of testing for structural linkage among genetic loci responsible for the production of certain enzymes (Guries et al. 1978). Trees were recalcitrant for showing classical genetic segregation and assortment until a tight genetic linkage was shown for haploid tissue from pitch pine (*P. rigida* Mill.). This work represents a start in the mapping of the conifer genome.

In fact some of the pioneering studies on in vitro systems of haploid tissues were done on gymnosperms. Gymnosperm callus, derived from pollen was obtained five to ten years before this approach was successfully applied to angiosperms (Sommer and Brown 1979). Larue obtained callus from pollen of *Taxus brevifolia* Nutt. (cf. Tulecke 1959), and Tulecke (1957) derived a green and colourless haploid callus from pollen of *Ginkgo biloba* L. Plantlet formation did not occur in either of these cases. Perhaps it was not attempted. Larue (1954) reported that the large haploid female gametophytes of genera *Zamia* and *Cycas* were able to grow in culture and regenerate new plants. *Zamia* and *Cycas* belong to the class *Cycadopsida*, and are evolutionarily closely related to the gymnosperms and ferns. Thus, plantlets were induced from haploid tissue in plants belonging to a class which is evolutionarily intermediate between the angiosperms and gymnospemrs. Bonga (1977) and Steinhauer and Huhtinen (1978) claimed to have shoot regeneration from haploid megagametophyte cultures of *Picea abies* (L.) Karst. Others have successfully cultured female gametophyte tissue from conifers. At the Institute of Paper Chemistry we have established cell suspensions of haploid cells from Douglas-fir and loblolly pine (unpublished). It would be extremely

Fig. 3A. Somatic cell embryogenesis in conifers. The shoot tip of loblolly pine may serve as donor tissue.

B. After production of callus on a chemically defined nutrient medium with growth regulators, the callus is fragmented in rotating flasks to form cell suspension cultures. Illustrated is a Douglas-fir cell ca. 60 microns long.

C. After removal of the stimulus for callus growth and dedifferentiation in darkness, the cell suspension is transformed after one month into white clusters of proembryolike cells.

D. The polyembryonic clusters are attached to each other by cells that may function as a suspensor.

E. After two months cells in globular structures continue to grow in a regular pattern and green-up in darkness.

F. After another month, polarity emerges with one end forming nodules where the seed leaves would normally appear.

G. A cross section of cells comprising the globular stage of somatic embryos.

H. A cross section of the larger (ca. 0.5 mm) somatic embryos when axial elongation starts.

I. An elongated somatic embryo (ca. 1 mm in length) of Douglas-fir with multiple primordia at the top where the seed leaves should appear.

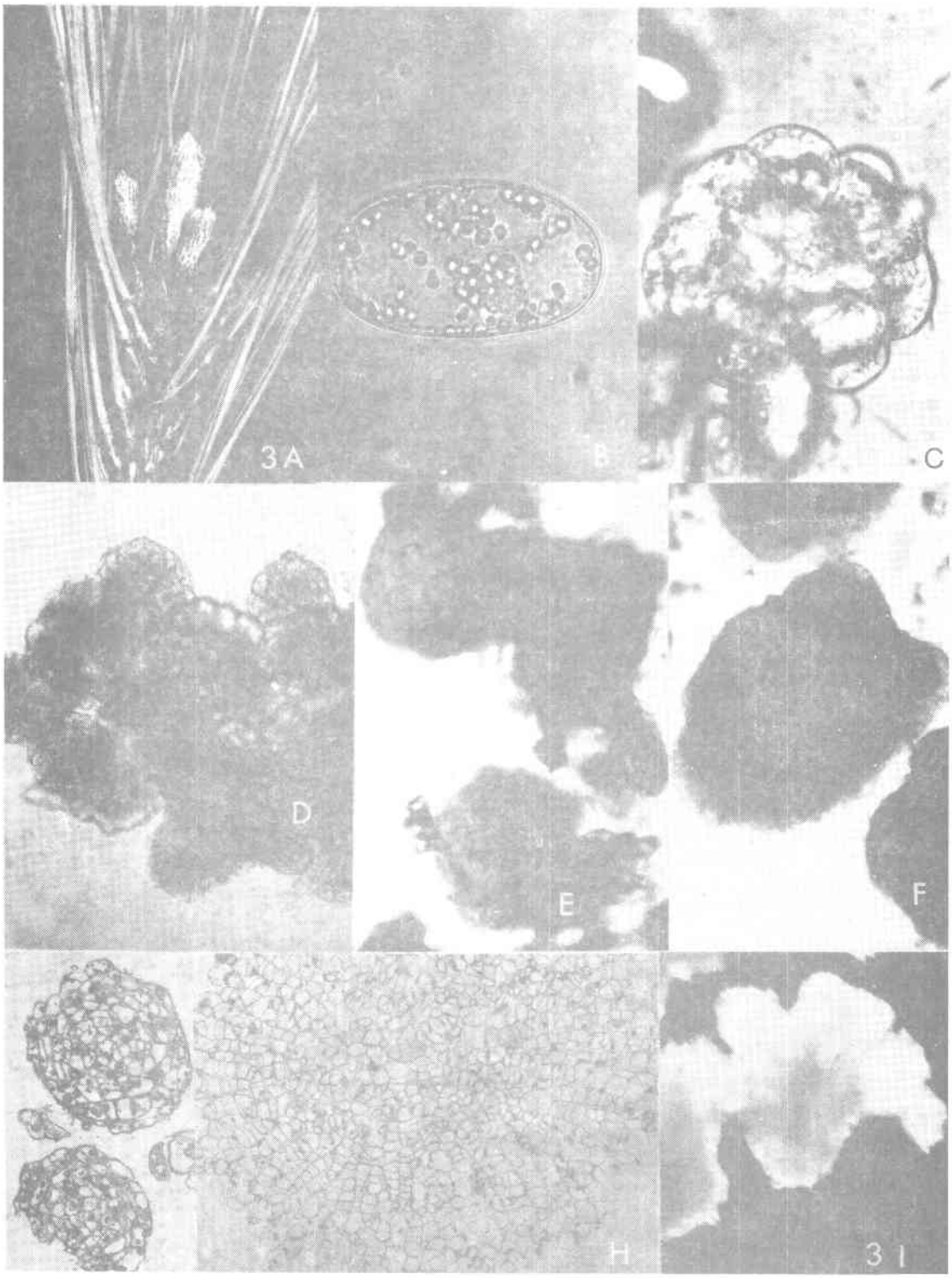

Fig. 3. Somatic cell embryogenesis in suspension cultures of Douglas-fir and loblolly pine.

advantageous if hundreds of thousands of the haploid plantlets and the recombinant products of meiosis be generated at one time through cell and tissue culture.

Diploid homozygous plants might be regenerated from haploid tissues. Doubling of the chromosome number by fusion, colchicine or other agents to produce homozygous individuals for breeding purposes would provide the tree breeder with opportunities not attainable by any other technique currently available.

Landmarks in the development of trees from diploid cells in tissue culture have been encouraging (Durzan and Campbell 1974; Sommer and Brown 1979). Cell suspension cultures from conifers were established in 1966 by Durzan and Steward (1968). The American elm (*Ulmus americana* L.) was the first tree produced from cell suspension cultures (Durzan and Lopushanski 1975). More recently, the following species have been cloned from partially organized tissues; *Sequoia* (Ball 1978), Douglas-fir (Wochok and Abo-El-Nil 1978, Cheng 1979), white spruce (Campbell and Durzan 1976) and numerous pines. The first conifer produced from tissue cultures was longleaf pine (*P. palustris* Mill.) (Sommer et al. 1975) followed by white spruce (Campbell and Durzan 1976). In the latter, induction of organs was in tissues devoid of apical meristems, and the events were shown to be related strictly to auxin-cytokinin ratios rather than the complex media changes of Sommer et al. (1975).

Since 1977, over 95 species of gymnosperms from 25 genera and 9 families have been placed in sterile tissue culture. Of these 95 species, 87 are members of the *Coniferae* and almost half belong to the genus *Pinus* (Brown and Sommer 1977). So far, propagation in commercial forests via tissue culture involves organized tissues, e.g., Douglas-fir (Wochok and Abo-El-Nil 1978). While no examples of successful generation of a conifer seedling are available from the culture of unorganized cells, these systems are currently under intensive development. Recently, at the Institute of Paper Chemistry, somatic cell embryogenesis has been induced in suspension cultures derived from explants taken from mature trees of Douglas-fir and 2-year-old saplings of Douglas-fir and loblolly pine (Fig. 3).

CLONING OF EMBRYONIC BUDS vs. MORPHOGENESIS IN CELL SUSPENSIONS

Many investigators have been successful in initiating single and multiple buds on the cotyledonary explants of conifers grown in sterile culture. The buds are then developed into fully organized plantlets under the influence of growth regulators (Blackman 1979). This technique is now employed on a pilot scale by Weyerhaeuser and the International Paper Company. Although it shows some promise as a means of vegetative propagation, it does have some shortcomings. (1) If only a limited number of buds are induced per seedling, it will be impossible to achieve the production of large numbers of plants because the separation and recult-uring of buds is labor-intensive. (2) The rooting of the shoots is often found difficult. (3) The desirability of plants established by this

procedure cannot be ascertained until the plants themselves are grown because the genetic potential of the seed giving rise to the cotyledonary buds is unknown. It is clear that only cell cultures can provide the required numbers of propagants and that the cells should be taken from proven, mature, and elite trees. It must be emphasized, however, that both systems have great value in the study of the genetic control of tree development under laboratory conditions.

The advantages of mass propagation from suspension cultures are clear. Once the genetic gain has been secured and reduced to callus and cell suspensions, the multiplication of totipotent cells is feasible on a more economical and rapid time scale. Millions of cells can be generated within a matter of a few days and used to reconstruct the tendency to recapitulate ontogeny.

One alternative to the fate of cells, discussed in more detail below, is that shown in Fig. 2, i.e., somatic cell embryogenesis. Two other alternatives are possible. If given the appropriate stimuli and auxin-cytokinin levels, organogenesis from cell suspensions can proceed. Unpublished data in Fig. 4 show that roots have been obtained from clumps with cambial-like cellular arrays in white spruce and jack pine suspension cultures, that were reported by Durzan et al. (1973). If the levels of growth regulators are excessive, the cells will revert to the continued production of callus until the nutrients in the medium become limiting.

Whenever a satisfactory cloning procedure becomes available for coniferous trees, it will be necessary to include many strains or ecotypes suited to particular environmental conditions. This requirement resists the temptation to plant large areas with a single or a few clones. With proper attention to the dangers inherent in genetic uniformity, calamities such as those that affect monocultures of herbaceous crops can be avoided (Day 1977).

EMBRYOGENESIS IN CELL SUSPENSIONS

In this approach the ultimate goal is to obtain trees from cell suspensions (Manson 1977). While the American elm has been propagated from cells (Durzan and Lopushanski 1975) there are as yet no reports for conifers although considerable evidence now indicates that it is only a matter of time before plants are obtained.

Evidence for the induction of somatic cell embryogenesis in suspension cultures has been obtained for Douglas-fir and loblolly pine (Fig. 3). Proembryolike clusters developed from clumps of cells between 60 and 120 μ in diameter. The clusters were characteristic of the growth patterns found during the natural early embryogenesis of conifers. Developmental patterns continued through several stages of growth and yielded individual embryo-like somatic structures with cohesive and polarized growth patterns. The developmental fate of these structures could be modified by auxins and cytokinins to produce precocious vascular elements and multiple shoot primordia. If structures were left unperturbed by

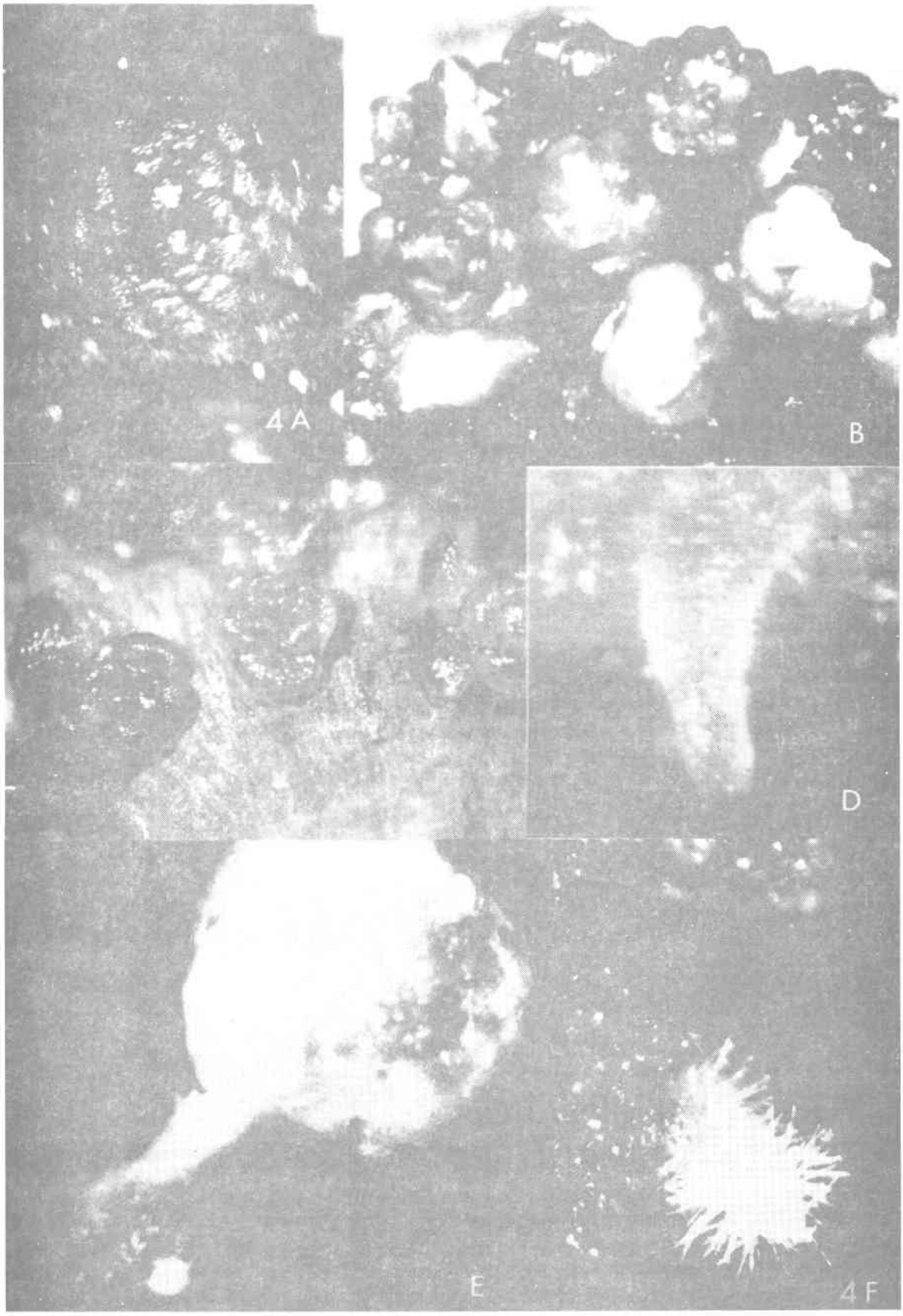

Fig. 4. Organogenesis in cell clumps derived from cell suspension cultures.

Fig. 4 A. Induction of a bud on a clump of white spruce from cell suspensions. The clumps were exposed to benzylaminopurine (10^{-5}M).

B. Induction of multiple buds each (0.5 mm diameter) by the same treatment in A.

C. Induction of multiple buds on a white spruce hypocotyl segment to contrast the difference in organized structures formed on partially organized tissue and from unorganized cells in suspension culture.

D. Induction of root-like protrusions (2 mm) on a clump of jack pine cells with naphthalene acetic acid in the presence of benzylaminopurine.

E. The formation of a root (6 mm long) without a shoot on a white spruce cell clump.

F. Development of root hairs from a root-like protrusion on a clump of white spruce cells.

auxins or cytokinins further embryonic development proceeded slowly until nutrients ran out. Current work is focussed on the physical and chemical conditions required to ensure continued development of the somatic structures.

This work involved some new ingredients. First, embryos were obtained from tissues of trees not less than two-years old and as old as 50 years. Before this, nearly all experiments were done with embryonic or very juvenile structures, e.g., cotyledons (Sommer and Brown 1979). Second, in most cases, the regeneration of plants was through tissues with a pre-existing built-in meristem. In our case, tissues were completely reduced to cells, and meristems had to be reconstructed. Third, at all stages of the technology, a completely chemically-defined medium was used. The medium formulations were based on the specific requirements of the tissue under consideration. Fourth, in a few cases, somatic cell embryogenesis was obtained from spherical cells resembling protoplasts, i.e., certain cells had only partially complete cell walls as viewed under the scanning electron microscope. Fifth, the yield of embryos approximated 1 000 per 10 ml.

The future steps will involve the fine tuning of the process and the pre-conditioning and further differentiation of embryos into plants for the autotrophic environment, i.e, one in which the embryos can be placed in an artificial seed or planted directly in soil. This stage will require the input of geneticists and pathologists so that a quality control can be imposed on the products of somatic cell embryogenesis.

GENETIC ENGINEERING

A wide range of promising genes cannot be introduced by sexual crossing because of natural crossability barriers. Recent evidence suggests that some barriers may be overcome by genetic engineering (e.g, Melchers 1980). For our purposes, genetic engineering is defined as any non-conventional method of genetic manipulation dealing with transfer of genes between trees and from other organisms to trees. This prospect becomes significant with the approach of controlled morphogenesis from cell suspensions and with recent developments in protoplast technology described in an International Workshop (Sala et al. 1980).

SUMMARY

Through domestication, tree improvement, and the promise of cell and tissue culture technology, industry can now anticipate that large numbers of superior trees can be cloned with quality gains and time savings in two areas. First, through more uniform rapid growth of cloned material in man-made forests the rotation age can be shortened and the juvenile phase promoted. Second, through vegetative propagation the effect of the long developmental period and unpredictability of seed years can be avoided or minimized.

It is postulated that the best approach to propagation of conifers starts at the cellular level. Haploid tissues are readily available and cell suspension cultures of mature tissues on defined media are becoming routine. Recently, a limited control over organogenesis and the steps of somatic cell embryogenesis has been observed. There is no longer any doubt that, while conifers show similar tendencies of totipotency as other plants, the mechanisms and nutrient media required to evoke responses are quite different. Furthermore, protoplasts are now easily prepared from the cell suspensions. Through protoplast fusion and genetic engineering new opportunities emerge for the removal of crossability barriers. The creation of new species perhaps more suitable for manufacturing needs of the forest industry, can be contemplated using the new biological systems that centre around protoplast technology.

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DISCUSSION

Dr. J.L. Farrar. It is very exciting. What are your achievements in the use of mature trees as a source of original culture? You did not say much about that. Just what tissue did you use?

Dr. D.J. Durzan. We have used the lateral branches from Douglas-fir and loblolly pine up to age of approximately four-years-old. We have been able to induce globular structures from the cells from trees that old. Our first success came from potted saplings that were growing in the greenhouse. These were approximately two to four years old. Samples were taken of terminal buds of lateral branches. We thought we might be able to push the age a little further and that was with the Douglas-fir tree I showed you which was 45 to 48 years old. We have been able to get somatic embryos from the older trees. We are now exploring how widely applicable this is. I think this is a significant departure from the material that is being used by other investigators. No doubt, we can establish cell-suspension cultures from mature specimens. I think by reducing the older tissue to cells and by removing the restraints of neighbouring cells as to revitalization we have a way to revitalize or evoke the ability to regenerate plants for propagation purposes.

Dr. M.T. Conkle. Your 9 o'clock breakthrough requires repackaging and embryo development. Have you given any thought to implanting de-programmed cells in unpollinated conelets hoping to get clonally propagated diploids?

Dr. D.J. Durzan. That is an interesting possibility. No, we have not gone to that stage. We have done all our work up to a few months ago on completely defined media and we very much wanted to see how far we can go on this media. Further development of embryo-like structures

is more complicated. We have gone to the use of extracts from the gametophytes of a number of seed sources and the responses are coming along nicely. It may be that we should try the approach that you have suggested. We are now at a point that if we are going to push the material all the way to completion, we have to use the more complicated nutrient supplements and culture systems.

Mr. A. Filauro. I just want to ask Don how long it would take before you make a complete circle?

Dr. D.J. Durzan. I would think maybe 10 years to work out the required technology.

Mr. A. Filauro. And how long did it take to get to the stage that you are at now?

Dr. D.J. Durzan. We started from scratch 25 months ago and we have gone three-quarters of the way around one cloning cycle. We can foresee that the problems are getting a little more complicated. Initially we try to predetermine the events in the flask but you can predetermine only a few of the variables. As the work progresses you lose control of the predetermined variables and what we have to do now is to move to different culture systems that control the reactions in the flask. Right now we do not have any control over pH or redox potential of the colloidal properties of the solution or even the nutrient fluxes that are taking place. I think that when we come to the central stage we can look at the factors such as pH and supplemental nutrients and then we will be better off to answer your earlier questions. And, I might say while it may take us 10 years to complete the details for one revolution of the cloning cycle, it may also occur in two years, if we are lucky.

CLOSING REMARKS

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Lively discussions, well-prepared presentations, an informative field trip, and the presence of geneticists and tree improvers from many locations have all combined to make this meeting highly successful. Many people mentioned during the course of the meeting that they were attracted to Newfoundland by their curiosity about the forests of easternmost North America. Camera shutters snapped at every stop, twig samples were stuffed into jacket pockets, and berries were jammed into mouths by the handfuls. I don't think Don Fowler knew the laxative power of these little berries at the time but he seems to have respect for them now. Indeed, we all have gained new respect for the forests, the research staff, and forest managers of this unique area.

Successful meetings do not plan themselves, and weeks, if not months, were involved in preparing for this meeting. We, who have enjoyed being here, wish to recognize and thank all who have worked hard to make these meetings an important event. Special thanks and highest regards go to members of the Newfoundland Forest Research Centre. We appreciate the time and thoughtfulness that all speakers gave in preparing and presenting their talks. And those of you, who keep alive the rich cultural heritage of Newfoundland, we thank you for sharing the language, the songs, and the stories. There is a certain sadness in leaving this meeting but we look forward to reunions in the near future.

Plans are underway for the next Canadian meetings to be held in Victoria, British Columbia. There are two other meetings of interest. The North American Forest Biology Group will meet in Edmonton, Alberta, and the Western Forest Genetics Association will meet in Coeur d'Alene, Idaho, during late summer, 1980.

PROCEEDINGS PART 1 - MINUTES AND MEMBERS' REPORTS
COMPTES RENDUS PARTIE 1 - PROCES-VERBAUX ET RAPPORTS DES MEMBRES

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