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CLONAL FORESTRY:

IT'S IMPACT ON TREE IMPROVEMENT AND OUR FUTURE FORESTS

LA FORESTERIE CLONALE:

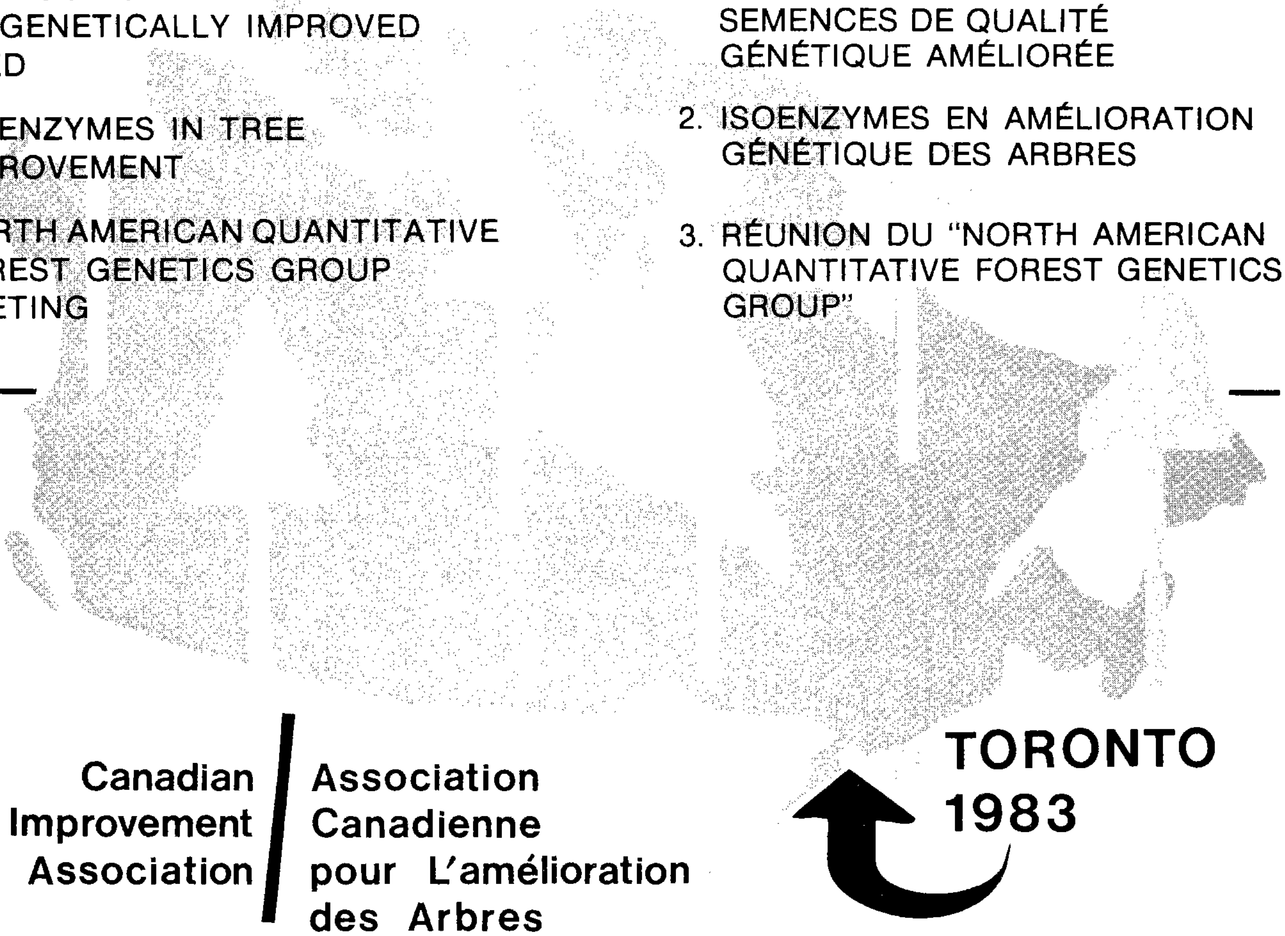
SON IMPACT SUR L'AMÉLIORATION GÉNÉTIQUE DES ARBRES ET NOTRE FORÊT FUTURE

WORKSHOPS

1. PRODUCTION AND UTILIZATION OF GENETICALLY IMPROVED SEED
2. ISOENZYMES IN TREE IMPROVEMENT
3. NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP MEETING

ATELIERS DE TRAVAIL

1. PRODUCTION ET UTILISATION DES SEMENCES DE QUALITÉ GÉNÉTIQUE AMÉLIORÉE
2. ISOENZYMES EN AMÉLIORATION GÉNÉTIQUE DES ARBRES
3. RÉUNION DU "NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP"



Canadian Tree Improvement Association / Association Canadienne pour L'amélioration des Arbres

TORONTO 1983

PROCEEDINGS

NINETEENTH MEETING
PART 2

COMPTE RENDUS

DIX-NEUVIÈME CONFÉRENCE
2e PARTIE

EDITORS / RÉDACTEURS
L. ZSUFFA
R.M. RAUTER
C.W. YEATMAN

PROCEEDINGS

OF THE NINETEENTH MEETING

OF THE

CANADIAN TREE IMPROVEMENT

ASSOCIATION

PART 2:

SYMPOSIUM ON

CLONAL FORESTRY: ITS IMPACT ON TREE IMPROVEMENT AND OUR FUTURE FORESTS

TORONTO, ONTARIO

AUGUST 22 - 26, 1983

EDITORS: L. ZSUFFA, R.M. RAUTER, C.W. YEATMAN

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Its Impact on Tree Improvement and Our Future Forests

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COMPTES RENDUS

DE LA

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DE

L'ASSOCIATION CANADIENNE POUR

L'AMÉLIORATION DES ARBRES

2^e PARTIE:

COLLOQUE SUR

LA FORESTERIE CLONALE: SON IMPACT SUR L'AMÉLIORATION GÉNÉTIQUE DES ARBRES ET NOTRE FORÊT FUTURE

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RÉDACTEURS: L. ZSUFFA, R.M. RAUTER, C.W. YEATMAN

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1985

PROCEEDINGS OF THE NINETEENTH MEETING OF THE CANADIAN TREE IMPROVEMENT ASSOCIATION

With the compliments of the Association

Enquiries may be addressed to the authors or to Mr. J.F. Coles, Executive Secretary, C.T.I.A./A.C.A.A., Room 147, Johnston Hall, University of Guelph, Guelph, Ontario, N1G 2W1.

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The Twentieth Meeting of the Association will be held in Quebec, Quebec, August 19-23, 1985. Speakers will be invited to address the topic of "Accelerated Genetic Gains through New Technologies". Canadian and foreign visitors are welcome. Further information concerning the 20th Meeting should be addressed to: Dr. Armand Corriveau, Centre de Recherches forestières du Laurentides, C.P. 3800, Saint-Foy, Québec. G1V 4C7.

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La vingtième conférence de l'association aura lieu à Québec (Québec) du 19 au 23 août 1985. Des orateurs seront invités à s'adresser au sujet de "l'accelération du développement de la génétique grâce à la nouvelle technologie". Tous sont les bienvenus. Pour d'autres renseignements concernant la vigtième conférence, s'adresser à: M. Armand Corriveau, Ph.D., Centre de recherches forestières des Laurentides, B.P. 3800, Sainte-Foy (Québec) G1V 4C7.

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ACKNOWLEDGEMENTS

On behalf of the Canadian Tree Improvement Association, I gratefully acknowledge the Faculty of Forestry, University of Toronto, and the Ontario Forest Industries Aassociation for their support towards the icebreaker, banquet and invited speakers at the Nineteenth Biennial Meeting.

We are grateful to the Ontario Ministry of Natural Resources for providing the competent assistance of Louis Zsuffa (program), Jim Hood (local arrangements) and support staff Brad Graham, Dan McKenney, and Celia Graham.

The Association is also indebted to Sam Foster of the North American Quantitative Forest Genetics Groups, to Bill Cheliak and George Buchert for their organization of the isozyme workshop, and to Ben Wang and Doug Skeates of the Tree Seed Working Group for enhancing the C.T.I.A. conference with their annual meeting and workshop, respectively.

R.M. Rauter, Chairman

REMERCIEMENTS

De la part de l'Association canadienne pour l'amélioration des arbres je suis très reconnaissant à la Faculté de Forestérie à l'Université de Toronto, et à la "Ontario Forest Industries Association" d'avoir participé au "bris-glace", au banquet et aux discours au dix-neuvième conférence biennal.

Nous témoignons de la gratitude pour l'excellent appui de Louis Zsuffa (programme), Jim Hood (arrangements locaux), et Brad Graham, Dan McKenney et Celila Graham (auxiliaires) du ministère des Ressources naturelles de l'Ontario.

L'association sait gré à Sam Foster des "North American Quantitative Forest Genetics Groups", à Bill Cheliak et George Buchert d'avoir organisé l'atelier sur les isoenzymes, et à Ben Wang et Doug Skeates du Groupe de travail sur les semences forestières d'avoir contribué au conférence de l'A.C.A.A. en tant que hôtes du conférence annuel et de l'atelier.

R.M. Rauter, Président

Dr. V. Nordin, Dean of the Faculty of Forestry, University of Toronto and Mr. W.T. Foster, Deputy Minister, Ontario Ministry of Natural Resources, presents Dr. C. Heimburger with a plaque in recognition of his outstanding contributions to the knowledge and advancement of forest genetics and tree breeding.

M. V. Nordin, doyen de la faculté de foresterie à l'Université de Toronto et M. W.T. Foster, adjoint ministre, ministère des Ressources naturelles de l'Ontario donnent une plaque à M. C. Heimburger en témoignage de reconnaissance de ses excellentes contributions aux connaissances sur l'amélioration génétique des arbres et donc au développement de celle-là.

APPRECIATION/RECONNAISSANCE

DR. CARL C. HEIMBURGER

The Canadian Tree Improvement Association, the Faculty of Forestry, University of Toronto, and the Ontario Ministry of Natural Resources were proud to extend a special honour to Dr. Carl C. Heimburger at its 19th Biennial Meeting. Dr. Heimburger was presented with a diploma certificate and a special plaque in recognition of his outstanding contributions to the knowledge and advancement of forest genetics and tree breeing.

It was through Dr. Heimburger's foresight and enthusiams that tree breeding got its foundation in Canada in the 1930's. Dr. Heimburger continued to make historical progress in tree breeding, conducting most of his work in Ontario at the former Southern Research Centre in Maple.

Dr. Heimburger's contributions are acknowledged in Canada and throughout the world. He is particularly noted for his work in poplar breeding and white pine hybridization.

M. CARL C. HEIMBURGER

L'Association canadienne pour l'amélioration des arbres, la Faculté de Foresterie à l'Université de Toronto, et le ministère des Ressources naturelles de l'Ontario ont eu le grand plaisir de rendre honneur à M. Carl C. Heimburger au dix-neuvième conférence biennial. On a donné à M. Heimburger un diplôme et une plaque spéciale pour témoigner de notre reconnaissance de ces contributions extraordinaires aux connaissances sur l'amélioration génétique des arbres, et à son développement.

C'était grâce à la prévoyance et au enthousiasme de M. Heimburger que les canadiens ont commencé à s'intéresser à l'amélioration des arbres dans les années 30. M. Heimburger a continué à réaliser des progrès historiques en ce qui concerne l'amélioration des arbres; il fait la plupart de son travail à Maple, en Ontario, à l'ancien Centre de recherche du Sud.

Les contributions de M. Heimburger sont reconnues au Canada et à travers le monde. Ses travaux sur l'amélioration des peupliers et sur l'hybridation des pins blancs sont les mieux connus.

C.T.I.A. EXECUTIVE AND GUEST SPEAKERS

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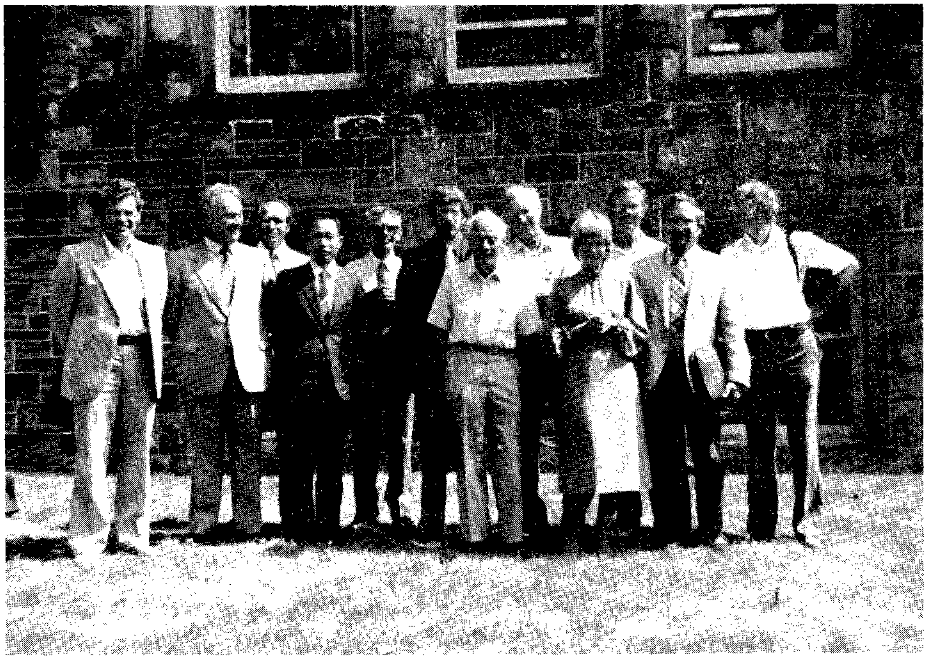

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KEYNOTE ADDRESS

WILLIAM J. LIBBY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA
U.S.A.

KEYNOTE ADDRESS

POTENTIAL OF CLONAL FORESTRY

W.J. Libby
Professor, Departments of Genetics
and of Forestry and Resources Management
University of California
Berkeley, California
U.S.A. 94720

Keywords: Deployment strategy, Diversity, Maturation, Selection, Silviculture

INTRODUCTION

To most people, the word "clone" is not a neutral word.

To some, the images are wonderful and even magical, full of the promises of modern molecular biology.

To others, the word is ominous, leading inexorably to visions of that worst of all monocultures: large, dreary, and biologically unstable monoclonal plantations.

RECENT HISTORY

In Canada, clonal forestry was pioneered during the 1940's by Carl Heimburger, largely with poplars. Here and in most other parts of the world, the idea of a clonal forest of conifers was rarely addressed until about a decade ago. Except for a few species, most of which were hardwoods, the biological problems kept clonal forestry from serious consideration. Among those biological problems, now largely solved or understood, are maturation state, cutting environment, cutting condition, problems associated with other ways of cloning, and survival and growth of propagules.

Maturation State

It was logical to select outstanding trees as cutting donors for clonal trials and for use in forest plantations. Trees of at least half rotation age seemed likely to provide better information for selection than younger trees; thus mature trees were generally used as sources of cuttings. It only took a few rooting trials to learn that cuttings from mature trees did not root very well. Furthermore, those cuttings that did root didn't grow very well. But few of those hopeful early cloners realized that the maturation state of their cuttings was the problem. Even fewer thought that maturation state could be controlled, or better yet, manipulated. It now seems clear that maturation can be slowed, perhaps arrested, and maybe even reversed.

Cutting Environment

We have learned much about the physical environment of detached cuttings, and we now can modify that environment so that cuttings are maintained in a healthy condition prior to their rooting. We know (with less certainty) that many elements of that environment, such as bottom and top temperature, also affect the rooting event.

Cutting Condition

We have learned much about the physiological states of cuttings, and we have particularly gained experience with various plant-growh regulators that promote or otherwise affect rooting.

Diseases

We are beginning to learn about various important pathosystems and their interactions in various rooting environments. The low rooting percentages common in many early experiments were not so much rooting failures as pathogen successes.

Other Ways of Cloning

Grafting was occasionally used in order to produce genetic uniformity for various experiments, and even for large-scale propagation. Cost, stock-scion incompatibility, and variable rootstock effects have largely ruled out these uses. Grafting is still appropriately and effectively used for seed-orchards, breeding-orchards, gene archives, and in some urban-forestry operations. Organ- and callus-culture techniques have progressed from exotic science to become increasingly reliable alternatives to the more classical rooting of cuttings. Encapsulated embryoids may soon be the method of choice.

Survival and Growth

Much of the early skepticism with regard to the practical usefulness of rooted cuttings (stecklings) was based on their poor survival and early growth in field conditions. We are gaining experience with the after-care of newly-independent stecklings. Increasingly, we can produce healthy stecklings that survive as well and grow as well as the best nursery-produced seedlings.

In short, while we still need to know the answers to a lot of biological questions to do clonal forestry well, these biological questions are no longer of a disqualifying nature, such that they block further consideration. We can now begin to think seriously about other kinds of questions. Questions concerning the economics of clonal forestry, and policy questions related to clonal forestry, are and will be important. However, in this paper, I am going to focus on opportunities and strategies.

DO WE NEED A WHOLE NEW DESIGN?

A favourite analogy has to do with the strategies followed by three major U.S. aircraft builders following World War II, with respect to the civilian passenger market[1]. In the 1930's, Lockheed was producing their two-engine "10" and "14"' series, and Boeing came out with their "247" in 1933. Beginning in 1936, Douglas had the DC-3, which for years was thought by many to be the best and most reliable airplane in service. The four-engine Boeing Stratoliner appeared in 1940, and then further civilian development paused for World War II. Near the end of that war, Allied air crews got their first looks at German jet aircraft.

Following the war, piston engines continued to be used on civilian airliners for more than a decade. Douglas' wartime four-engine DC-4 was soon replaced by its popular DC-6. Boeing upgraded its Stratoliner to a Stratocruiser. In the early and mid-1950's, Douglas followed the DC-6 with a series of DC-7's, culminating in the large, long-range DC-7C. Lockheed added two engines and a tail to evolve its 10A Lodestar to its Constellation, and then to its wonderful Superconstellation. These 1950's models were about as good as big piston-engine airliners ever got.

Responding to much pressure and direction from Eastern Air Lines' Captain Eddie Rickenbacker, in 1959 Lockheed produced the Electra, the first U.S. civilian airliner with jet engines. The Electra looked like a fat DC-7, with turbojets mounted where the piston engines used to be. (Perhaps that was comforting to those who were used to the DC-7, and thought it was wonderful.)

Meanwhile, Boeing had been gaining experience with a jet-powered military tanker, which was designed to take advantage of the jet and to meet its requirements. By 1959, they had modified it for civilian passenger use, and entered that market with the 707. A year later, Douglas brought out the DC-8. It looked a lot like the 707. Since 1960, no new major long-haul airliner has looked anything like the wonderful and successful DC-3 or DC-7, and most have looked rather like the 707.

I now suggest, perhaps inappropriately, that those forestry enterprises that attempt to use stecklings or culture-origin plantlets or embryoids in a forestry system designed for seedlings will be creating something about as successful as the Electra. Further, those that persist with the tried and true seedling as the propagule of choice will be about as competitive as a 1983 airliner salesman with a lot full of new DC-7's.

But, what are the probable design changes that will produce 707-like clonal forestry?

---

[1] The material on aircraft in this section, of which I know little, is from Wigton, D.C., 1963, From Jenny to Jet, Floyd Cramer, Los Angeles

BE CAUTIOUS WITH FANTASIES

I opened this talk speaking of promises of modern molecular biology and fears of unstable monocultures. I believe both are largely fantasies.

Molecular Biology Fantasies

As exciting as molecular biology is, there are several reasons that led me to believe that it will not soon provide much to forestry that is radically new. The first and most obvious reason is its expense. But clever people may solve the problems of expense.

A more serious problem is that plants produced by the more radical techniques of molecular biology will be totally new. And being totally new, they must be very carefully tested. Thus, the promise of faster entry of new clones to production forestry through molecular biology appears to me to be a fantasy, and perhaps a dangerous one. Because propagules for forest plantations must successfully occupy a variety of sites for very long periods, the tests of radically different trees should be conservative;i.e., they should be conducted on many sites for many years, exposing such trees to both the foreseen and unforeseen conditions that occur on operational plantation sites. It is the need for this conservative and long-term testing that will increase both the time and the expense of entering radically-engineered new clones.

In contrast, one may use clones whose histories include conventional selection from populations known to be adapted to plantation sites, followed by breeding, clonal testing, and further selection. Such clones are at least as reliable as seedlings from those same populations. They may be rapidly entered in large-scale use, with considerable assurance that most of them will not fail. This is not to say that radical new clones will never be used. It merely suggests that their use will (should) be neither soon nor cheap.

The safest technique and thus probably the most useful genetic-engineering technique for forestry, will be genetic surgery..... i.e., the insertion of single desirable genes (operons) into clones that are already outstanding in most other respects. At present, this technique is not operational with forest-tree species. Furthermore, few sufficiently outstanding recipient clones of forest trees are available..... perhaps the poplar clone I-214 is one such.

Large Monoclonal Fantasies

The fear of inexorable, large, unstable, monoclonal plantations is, I think, fear of a fantasy. Large monoclonal plantations are neither necessary nor likely components of clonal forestry. If they occur at all, they are best viewed as rather nasty management errors, and such errors may be avoided by education and/or regulation.

Well, none of the above leads obviously to our 707-like forestry.

SO WHAT IS TO BE REDESIGNED?

Production Options

With greater per-tree (or per-unit-area) productivity being acheived by genetic means, managers have the option of reducing the amount of land managed for harvest, or of increasing the total production on the previous land base. These options occur with any form of successful tree-improvement, but clonal forestry appears to offer the greatest genetic leverage.

Orchards

Classical tree-improvement has typically relied on seed-orchards to produce select stock at production levels. Seed-orchards also feature sexual recombination of the select genotypes, a mixed blessing at best. As clonal programs come on-line, seed-orchards will be replaced by much smaller and less demanding breeding-orchards, to satisfy the need for continued advanced breeding. It is appropriate to emphasize here that continued breeding will be as essential a component of clonal forestry as it is for classical tree-improvement. But for most clonal forestry, production-level multiplication of clones will be done in hedge-orchards, or by using young stecklings still in the nursery as donors, or by *in-vitro* propagation.

Returning to the airline analogy, piston-engine airliners still serve on local low-volume runs. Here the analogy breaks down. Seed-orchards are sensitive to economies of scale, and one can rarely justify a seed-orchard for local low volume demand. (In contrast, a relatively few hedges will satisfy such a special demand, and they can easily be included in a larger hedge-orchard.) Seed-orchards are site-demanding, can be labour-demanding when cones ripen, should be free of pollen contamination, and can have other annoying management problems. You may wish to take pictures of these wonderful old components of seedling-based forestry. If so, do it soon, for they will disappear soon after reliable clonal programs become available.

Nurseries

If clonal forestry employs plantlets or stecklings, nurseries will be substantially redesigned in their early operations and little affected in their later ones. Seed-handling equipment will be replaced by mechanized methods of sticking cuttings. If plantlets are employed, we will need new techniques and facilities to acclimatize the delicate plantlets from culture conditions to harsher field conditions. Compared to a seedling, a recently rooted steckling needs more attention to the early form of its roots, using either containers or root pruning for this purpose.

Once the stecklings or plantlets are well established, husbanding of their continued growth and their preparation for lifting will be very similar to what is (or should be) done for seedlings. If it becomes operationally possible to encapsulate cloned embryoids, then nurseries may handle these very much like they handle seeds today. However, in order to take full advantage of clonal forestry, clonal identities should be maintained at least until assignment of the propagules to a plantation site, and thus record-keeping at clonal nurseries will have greater detail.

Finally, when all clones in production use are of high genetic quality, culling percentages should be greatly reduced and the efficiency of nursery management will thereby be improved.

Reciprocal Effects

Much of what used to be known as "maternal effects" is turning out to be due to DNA contained in mitochondria and chloroplasts, exclusively (or largely) inherited from the mother. Much hard evidence is accumulating from molecular biology that this mitochondrial and chloroplast DNA is of importance and is variable. Softer evidence has been bothering us quantitative-genetic types for some time, in that open-pollinated families told us more about the female's performance as a mother than we thought they should.

For some time, I've been recommending single-pair matings as an efficient and cost-effective mating design for long-term (many-generation) breeding. And it is, at least for nuclear genes that are largely additive in their effects. But, if most or all mitochondrial and chloroplast DNA is inherited from the mother, as much as half of the variation in these genes will be lost in the first generation of single-pair matings, and more may be subsequently lost depending on the assignment of male or female roles to sibs. This requires some rethinking, no matter whether a clonal or seed-orchard option follows the breeding. But note that clones can capture this variability better than can an open-pollinated seed-orchard.

Silviculture

As with other tree-improvement options that increase the growth and value of plantations, clonal forestry will make various silvicultural manipulations more possible and more effective. Because clones will provide the greatest predictability, the timing and effectiveness of particular silvicultural treatments can be best optimized with known sets of clones. The nature of the treatments prescribed may also vary substantially, depending on the particular clones in a plantation.

Site Characterization

The clonal option allows the selection of sets of clones adapted to particular sites. Thus, with clonal forestry, there will be much greater attention to characterization of sites and subsites when planning plantations, prior to ordering the clonal mixtures for them.

Deployment Strategies

As we learn more about general theory and as we accumulate specific details about our best clones, the decisions we make about the numbers and arrangements of these clones may have large effects on both the appearance and the scheduling of our forest stands. The two deployment strategies being frequently debated are "widespread intimately mixed plantations" vs "mosaics of monoclonal stands".

Early in a program, it will be prudent to deploy large numbers of local clones to any given plantation site, and it will also be prudent to plant them in intimate mixture. Those that are well adapted will sort themselves out from those that are not, and, with close initial spacing, sites should thus remain fully occupied.

Later, both biological and management arguments may lead us to favour mosaics of small monoclonal plantings. For this option, we will probably use relatively few clones in each region. Ideally, these clones will be well known (i.e. thoroughly tested) and biologically dissimilar to each other.

When we learn even more, we may be able to program particular neighbour sequences during planting, such that adjacent clones make complementary demands on the site, or such that a thinning sequence is pre-programmed by clone and position. It may be that particular clones of two or more species can coexist in intimate mixture, even though random members of the same two or more species do not mix well.

THE TITLE OF THIS PAPER

I think that there are at least 16 major potential advantages to clonal forestry.

One advantage is the short-term ability to capture a greater proportion of the additive genetic variation, a greater proportion of the variation in chloroplast and mitochondrial genes, and a much greater proportion of the nonadditive genetic variation, than can be done using conventional seed-orchards. However, note that over several generations of breeding, additive nuclear alleles and favorable chloroplast and mitochondrial gene complexes will be accumulated, and the additional gain possible by cloning will be relatively reduced when compared to the total accumulated gain. Put another way, long-term genetic advances will depend on breeding; clonal techniques will add a gain increment in each generation, by locating and propagating those outstanding individuals that have unusually favourable combinations of genes.

A 2nd potential advantage is the ability to provide adapted (and probably selected) clones for unusual sites. Such sites are not well served by families selected for the more common sites, and these unusual sites do not require regeneration in numbers to justify their own conventional seed-orchards.

A 3rd is the elimination of all inbreds (including selfs) from production plantations. For most species. open-pollinated seed-orchards may produce an unacceptable percentages of selfs. Clones derived from pedigreed control-pollination crosses can be 100% non-inbred. This is particularly useful in species that exhibit serious inbreeding depression in plantations, but whose inbreds in good nursery conditions have high germination percentages, high viability, and early growth above cull levels.

A 4th is the mass production of valuable but expensive genotypes. These include various kinds of hybrids, the products of various genetic-engineering techniques, and pedigreed control-cross seedlings.

A 5th is the identification of broadly-adapted and/or highly interactive clones, with deployment of the latter then being restricted to those sites on which they do well.

A 6th is the ability to use maturation states other than juvenile. In at least some species, late juvenile or even early adolescent maturation states of their propagules at the time of planting confer substantial advantages with respect to subsequent stem-form and resistance to at least some important pests.

A 7th is the possibility of using "correlation breakers". Multiple-trait index gains attainable by sexual reproduction, on average, are constrained by genetic correlations between the indexed characteristics. However, these correlations are rarely 1.0, and unusual "correlation breakers" can be found by clonal testing.

An 8th allows us to shift biomass allocation in our plantations from cones and pollen to bole wood. While there is no convincing evidence that juvenile growth is negatively correlated to seed and pollen production, it makes sense that heavy commitments to reproductive ograns are probably at the expense of something else after reproductive maturity is achieved.

A 9th is the knowledge and experience that accumulates about outstanding successful clones. (We must avoid the mistake of using too high a proportion of too few such clones.) These well-known clones will probably remain more valuable than new clones or seedlings from the breeding program, which are genetically better but less known than the current clones. This may sound like a disadvantage with respect to gain per unit time, but the advantages of managing a well-understood set of clones are substantial.

A 10th is the control of genetic diversity in production plantations. Genetic diversity may be more precisely prescribed using sets of pedigreed clones than using the continuum of unique genotypes produced in the wild or in seed-orchards. Here, the goal is not merely plantations that are as safe as those using seedlings, but plantations that are significantly safer as a result of wise and informed deployment of clones.

An 11th, related to the 2nd and 10th, is the ability to improve and thus effectively use a greater number of species. Two elements of clonal forestry contribute to this. One, noted above, is the possibility of mixing compatible clones of species that do not typically mix well. The second is that, in a clonal program, the production of individual clones is not as sensitive to economies of scale as is classical seed-orchard production of improved seedlings. Thus, while it might be economically possible to genetically improve (and thus effectively use)only two or three species in a region by classical seed-orchard techniques, clonal selection and propagation may allow deployment of genetically superior clones from a much larger number of species.

A 12th is the greater simplicity of managing hedge-orchards (or other donor options) than of managing seed-orchards. In addition, the unit of management will be the individual clone, rather than the general seed-orchard. This removes several restrictions (such as putting clones that should not interbreed in the same hedge-orchard), and increases flexibility (each clone is individually deployable to different plantations).

This latter allows a 13th advantage, namely specific and probably optimal deployment of sets of clones from the available list of known clones to each plantation or even to each subsite of a plantation. It is likely that no two plantations will have exactly the same set of clones. In contrast, most seed-orchards produce the same general product for all their client areas.

A 14th, true in most species, is the shorter time between selection and production, compared to seed orchards.

A 15th, discussed above, is the ability to program planting sequences, such that productivity is increased by reducing negative competitive interactions, and/or such that thinning and other activites may be planned by clone and position.

The 16th concerns selection philosophy. In most seed-orchard programs, in an attempt to quickly use the very best trees from a large number of candidates, one usually selects a very few of them. These few selected individuals become the parents of extensive production plantations. In contrast, most clonal programs begin with many clones and selection is then incremental. As different characteristics develop at each round of evaluation during the tests, new increments of the poorer clones are identified and discontinued. The remaining clones contribute ever larger proportions to the clonal production plantations. This incrementally reduced proportion of clones in production is both increasingly better genetically and increasingly better known, and thus is increasingly reliable. This latter strategy seems much more in keeping with the conservative selection philosophy appropriate to a long-term crop such as forest trees.

THE BEGINNINGS OF CLONAL FORESTRY

One condition that is necessary is that the cloned ramets must develop into satisfactory trees. Given that, clonal forestry may be adopted when sufficient additional conditions are present. For example, clonal forestry has been pioneered with such species as cottonwoods, willow and tsugi, whose rooted cuttings cost little more (or even less) than seedlings.

Other sufficient conditions are that unit values are high and rotations short. The Christmas-tree industry is thus characterized, and it is beginning to replace seedlings with expensive but more reliable clones.

When tree values or maintenance costs are very high, very expensive clones (that produce unusually valued trees or that avoid costly problems) can be justified. The emerging field of urban forestry is rapidly adopting clonal propagation of select trees.

When trees are planted at wide spacings, as in agroforestry, clonal reliability is highly desirable. Thus, agroforestry will probably adopt the clonal option even though per-plant costs remain higher than those for seedlings, and values are not unusually high.

For more general wood-and-fiber forestry, two forces appear to be at work. On the one hand, stumpage values are expected to continue to outpace inflation; and land, labour, and transportation costs will make high unit-area productivity increasingly desirable. On the other hand, technical advances in producing clonal propagules are eliminating maturation-related problems, and are reducing unit costs. The joint effects of these forces will be to shift conventional forestry to clonal forestry, species by species and region by region, as values and costs become first favourable, and then compelling.

CONCLUSIONS

Forestry, compared to agriculture, has been very late in domesticating the organisms it depends on. Clonal forestry provides a major opportunity to close some of that gap. It seems inevitable that we will adopt a more aggressive management of the forest resource; the only thing likely to stop this intensification is the failure of civilization. But inevitable or not, it is exciting to be in on the development and expansion of clonal forestry. However, let me suggest that our excitement and enthusiasm should be tempered by the careful conservatism appropriate to husbanding a long-term resource, and that we should seek wisdom in instituting not only the techniques but the policies appropriate to clonal forestry.

Finally, I'm pleased to note that this first major North American conference featuring clonal forestry is being held in Ontario, where so much of the pioneering has been and is being done.

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SESSION I

MODERATOR:
DR. D.P. FOWLER
CANADIAN FORESTRY SERVICE
FREDERICTON, NEW BRUNSWICK

CONCEPTS AND EXPERIENCES IN CLONAL PLANTATIONS OF HARDWOODS

L. Zsuffa

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

ABSTRACT

Clonal silviculture has only evolved in poplars and willows. Therefore, consideration of the concepts and experiences in clonal plantations is restricted to these two genera.

Monoclonal planting is common in all forms of poplar and willow plantations because of ease of propagation by rooting of cuttings, advantages of growing and utilizing plantations of uniform trees, and limited availability of outstanding desired clones. Most of the plantings over large areas and in different countries are with very few clones.

Multiclonal plantations are a desirable alternative to existing monoclonal plantations in poplars and willows. The concept of "multiclonal varieties" in individual tree mixtures may be feasible for forest stand management. Mosaics of monoclonal blocks that are relatively small and similar in size in which clones are matched to soils, plantation systems and production objectives seem to be the only reasonable choice for intensively managed plantations.

Stock for multiclonal management has yet to be developed. However, active breeding projects in poplars and willows have the capacity for producing such stock. The prospects for similar development in other hardwood species are more distant.

RÉSUMÉ

La sylviculture des clones ne s'est développée que pour les peupliers et les saules. Par conséquent, il ne saurait être question que de la théorie et que des expériences touchant la plantation de clones de ces deux genres.

L'aménagement monoclonal est répandu dans les plantations de peupliers et de saules de toutes formes, en raison de la facilité de multiplication de ces espèces par racinage de boutures, des avatages de cultiver et d'utiliser des arbres uniformes et de la disponibilité limitée des clones exceptionnels voulus. La plupart des plantations sur de vastes territoires et dans différents pays sont établies avec très peu de clones.

Les plantations multiclonales représentent une solution de rechange souhaitable aux plantations monoclonales existantes de peupliers et de saules. L'idée d'avoir des "variétés multiclonales" dans des mélanges d'arbres individuels pourrait convenir à la gestion des peuplements forestiers. Des mosaïques de blocs monoclonaux de dimensions similaires, mais relativement petites, où les clones seraient choisis en fonction du sol, du système de plantation et des objectifs de production semblent la seule solution raisonnable pour les plantations en gestion intensive.

Le matériel nécessaire pour l'aménagement multiclonal n'est pas encore disponible. Toutefois, des travaux en cours dans le domaine de l'amélioration des peupliers et des saules pourraient produire ce matériel. Par ailleurs, les perspectives de réalisations similaires avec d'autres espèces feuillues sont plus éloignées.

INTRODUCTION

Clones of hardwood (broadleaved) tree species (Angiospermae Brongn.) have been used as ornamental and fruit producing varieties for many centuries. Hillier's manual of trees and shrubs (1974) lists more than 2,000 such clonal varieties, some dating back to the beginning of known human history. Some of these have been traditionally reproduced by grafting, while others have been reproduced by rooting of cuttings. Poplars (Populus L.) and willows (Salix L.), fall within this latter category. They are well-known for their ease of propagation by rooting of cuttings, and are, therefore, commonly used.

Ease of propagation by rooting of cuttings has significantly contributed to the distribution and use of hardwood clones. While varieties propagated by grafting remained restricted as high-value, individual trees for ornamental and fruit-producing purposes, other species which could be easily propagated by cuttings were inexpensively spread in large numbers. Such clonal material was also used in multi-tree, forest-type plantations for timber production. In addition to ease of rooting, the determining factors for such use were the utility of the wood, facility of plantation establishment, and adaptability to a variety of sites.

Poplars and willows conform well to these criteria and are valuable for timber production. Therefore, their clonal culture is the most widely spread of any hardwood species in a temperate climatic zone. Although some other hardwoods, such as Platanus L., have the potential for vegetative propagation, the concept of clonal silviculture evolved only in the case of poplars and willows as the result of a unique combination of economic demand, site requirements and ease of reproduction. Consideration of the concepts and experiences in clonal plantations of hardwoods in this paper is, therefore, restricted to these two genera.

HISTORICAL NOTES

Poplars are interspersed throughout all forests of the temperate and colder regions of the northern hemisphere. Willows have a wider natural distribution and also occur in the southern hemisphere. They grow in clusters and rarely expand into forests to a great extent. Many species of both genera are typical occupants of recent alluvial deposits.

In the International Poplar Commission's book on poplars and willows (FAO, 1979), the spread of cultivation of these species is described as follows: "when the forests of the plains of the Old World were cleared and converted to agriculture, many parts of the land were left aside because no worthwhile crops could be expected from them. However, it was there that poplars and willows were successful in establishing themselves. People came to look at these areas of natural growth to satisfy their needs for timber and fuelwood. Because of the value of the trees and the proven ease of propagating them by cuttings, people started planting them near their houses and around their fields to furnish wood and provide greenery and shade".

In Mid-Asia, the Near-East and around the edges of the Mediterranean, Salicaceae have been closely associated with agriculture since antiquity. For many centuries, pollard willows have provided fuel and withes, while poplars provided timber and fuel. As well, poplar leaves were used as forage and litter for animals. Even today, from Kashmir to the Atlantic in the Old World and throughout the New World, poplars and willows mark the neighbourhoods of human habitations.

The oldest type of poplar forest culture evolved with locally available trees; almost everywhere, this was with various forms of Populus nigra L. (Zsuffa, 1974). However, the introduction of P. deltoides Marsh from North America into Europe during the seventeenth century resulted in natural hybrids (P. deltoides x nigra = P. canadensis Moench, syn. P. x euramericana (Dode) Guinier) which in time revolutionized the ideas about poplar cultivation. Gradually, this led to the use of poplar in all modern wood industries.

The extremely rapid growth and ease of propagation by cuttings fascinated poplar growers. Nurseries were established and their output was in great demand. It was clearly appreciated that these poplars, although of the same origin, were not alike, and people wanted to know which clones were best suited to their needs. For planting, culture, and protection against pests, the empirical approach was the only answer.

During the first years of this century, poplar cultivation was already widely spread in Europe with the support of industry. Voices began to be raised on all sides deploring the general state of disorder, the ignorance of tree planters, and the ravages caused by insects and diseases. Scientists began to take interest in poplar; in France, Dode (1905); in England, Henry (1914), in Italy, Jacometti (1933); in Germany, Wettstein (1933); in the United States, Schreiner and Stout (1934); and in the Netherlands, Houtzagers (1937).

These initiatives were brought together in 1947 with the founding ofthe International Poplar Commission under the aegis of the Food and Agriculture Organization (FAO). The efforts of this Commission resulted in considerable progress in the knowledge of poplar types and their cultivation, and led to important agreements in nomenclature, registration of clones, varietal control, and the exchange of cuttings. The Commission, of which Canada is a member, is still very active, and is now also concerned with the cultivation and utilization of Salicaceae.

TYPES OF POPLAR AND WILLOW PLANTATIONS

The two main forms of plantations are in stands (blocks) and rows. A variety of each is practised in different countries depending on needs, tradition, tree varieties, sites, and economies. The examples which follow will illustrate these practises and also facilitate the discussion on clonal concepts and experiences.

In stands, poplar is grown most often in medium spacings (3.0 m to 4.0 m) and harvested in twenty to thirty year rotations. Rooted stock is used for planting, and some form of site preparation and initial tending is practised. The stands are at times pruned and thinned depending on growth vigour, tree variety, and the desired tree size and quality at harvesting. Such forms of plantations are found, for example, in Scandinavia and parts of mid- and eastern Europe with aspen (P. tremula L.) seedlings and its hybrid varieties (aspen does not propagate from cuttings), and throughout mid-Europe and in parts of North America with Euramerican (P. x euramericana) poplar and various balsam poplar hybrid clones.

Another common form of poplar plantations uses widely spaced trees (5.0 to 8.0 m) and harvested in ten to fifteen year rotations. These are clonal plantations of Euramerican poplar, cottonwoods, and similar easy-to-propagate varieties. Large-size rooted stock is usually planted on well-prepared sites, and the plantations are well tended. An example of this is the well-known Italian poplar culture. It has spread, together with clones developed in Italy, throughout the world. Such poplar plantations, when established on productive agricultural soils, are associated with annual farm crops (corn, potatoes, etc.) for 2 to 4 years until the crops become shaded by fast growing poplars. In other cases, forage, such as alfalfa, is grown and grazed by cattle in widely spaced poplar plantations.

A traditional form of poplar cultivation is practised in Asia, the Mid East and North Africa. Fastigiate (pyramidal) poplar clones (usually of P. nigra and P. alba L. origin) are grown in very dense spacings (1.0 m) in ten to twenty year rotations. The trees are an important source of timber for rural construction, and fuel, and leafy branches are used for animal feed.

Another form of poplar plantation has been developed in North America. Unrooted cuttings of easy-to-propagate clones are used for medium density plantations, and the production goal is usually industrial fibre. The planting sites are prepared by ploughing, and the plantations are tended by cultivation and weed control. The rotation age is approximately ten years, and the trees are occasionally regenerated from coppices.

Biomass plantations are a new concept in poplar and willow cultivation. These are very dense plantings (0.5 m to 1.5 m) of unrooted cuttings, harvested frequently (every 1 to 5 years), and subsequently regenerated by coppice growth. The blocks are monoclonal, and the trees only grow well on fertile sites and when properly tended. The whole above-ground portion of the trees, sometimes even the foliage, is harvested and used as a source of fibre, energy and food. A traditional form of willow plantations with a concept similar to biomass plantations are the osiers.

Willow stands are in many cases similar to poplar stands. However, dense plantings (3.0 m) are more commonly practiced, and tending is usually less frequent and extensive. Willow cultivation is widely spread in Europe along the Danube River, and in Argentina in the valley of the Parana River.

In row plantings, there is a variety of cultivation forms of poplars and willows with different cultivars. A common form of row plantation uses Euramerican poplar clones along ditches, roads, and farm boundaries. The spacing is usually wide (4.0 m to 10.0 m) and large, rooted stock is planted in single rows, or occasionally, in double rows. The trees are cut at twenty to forty years-of-age and replaced by new plantings. This form of row planting is very common throughout Europe. In some countries, such as parts of Holland, France and Italy, approximately 40 trees grow in such plantings on every hectare of farmland.

In the Mid East and North Africa, fastigiate poplars are often grown in single or multiple rows in dense (1.0 m to 2.0 m) arrangements. In the Soviet Union and Canada, poplars are an important component of windbreaks. In New Zealand, row plantations of poplar check soil erosion as a result of sheep and cattle grazing on hilly land.

A traditional concept of row plantations, practiced in many Old World countries, are the pollard willows. The crowns of these willows are cut back periodically to the trunk and the branches are used as fuel, withes and stakes.

CONCEPTS IN CLONAL PLANTATIONS

Monoclonal planting is common in all forms of poplar and willow plantations. This concept was applied almost automatically as the most reasonable and practical method of poplar and willow cultivation.

The reasons for, and practicality of, monoclonal poplar and willow culture are: ease of propagation by rooting of cuttings; the advantages of growing and utilizing plantations of uniform trees; large variation within populations and families; and the occurrence of unusual, desired individual types. The ease of vegetative propagation alone, if dealing with relatively uniform populations and sibs containing many desired types, could have lead to multiclonal cultures. However, poplar species in general, and families of interspecific hybrid poplars in particular, are well-noted for large tree-to-tree variation. In fact P. nigra clonal selections of fastigiate forms, such as cvs. italica, thevestina and plantierensis, which have been propagated for centuries, represent unique or rare occurrences. Old selections of Euramerican poplars, such as cvs. serotina, regenerata and robusta, are also distinguished by particular features of form, growth and site adaptation. For this reason, and because of the relatively late start of planned poplar and willow breeding programs, the number of clones used in monoclonal plantations throughout the world has remained relatively small.

The International Poplar Commission has registered fifty-two clones and has eight more clones which are candidates for registration (FAO, 1983). Most of these clones are Euramerican poplar hybrids. The Commission's textbook on poplars and willows (FAO, 1979) describes five clones of P. nigra, twenty-two clones of P. deltoides (in use since 1970), forty-four clones of Euramerican poplar (about half of them in use since 1970), and twenty clones of balsam poplars and their hybrids (eight of which have been in use since 1970). Thus, the total number of poplar clones registered and in commercial use is less than one hundred.

The situation with willow clones is similar in that the total number of clones in commercial use does not exceed one hundred. However, many of these are osier willows (shrubs); few clones are actual trees.

While osiers and biomass (energy) plantations are always monoclonal, tree plantations of willows, especially of Salix alba L. along the Danube River, are occasionally multiclonal. This is because special selections were not made earlier within this species which is native to the flood plain of the river, and the plantations established on similar sites are extensively managed as forest stands.

Only a portion of registered poplar and willow clones are widely used in plantations. According to the reports of the National Poplar Commissions submitted to the 16th Session of the International Poplar Commission, the number of clones planted on a large scale is very small in most of the countries (Chardenon, 1980). In Hungary, three clones (P. x euramericana cvs. Robusta, I-214, and Marilandica) represent 81.0% of the plantations, in the Netherlands, Euramerican poplar clones Robusta and Zealand account for 60.0%, and in France, Robusta and I-214 account for 70.0% of the plantings. In the Republic of Korea, Euramerican poplar clones I-214 and I-476 are planted almost entirely on the plains, while seedlings of a single hybrid aspen family (P. alba x glandulosa) are planted in the hills and lower mountain ranges on a total of 428,000 ha. A few of the clones, such as I-214 and Robusta, have been very successful and are, therefore, widely planted in many countries. Fewer than ten clones represent well over 50.0% of all poplar plantations, the extent of which can be estimated at more than 1,500,000 ha worldwide.

The situation in willow plantations with regard to the number of clones registered and planted over large areas is similar to poplar. In England, a single clone (S. alba v. coerulea) has been planted and used for the production of cricket bats. In osiers, as well as in tree plantings, a handful of clones are favoured and used throughout the world.

Multiclonal concepts were introduced in poplar and willow culture in the 1960's in conjunction with the dawn of new ideas in plantation technology, spreading of plantations to different sites, and well-planned breeding programs. The ideas on integrated use of trees (the biomass concept), and new needs and possibilities in biomass utilization (such as for composite boards, energy, food) led to studies of matching clonal characteristics to production and utilization technology. These studies showed significant clonal variation and resulted in new clonal selections and diversification.

After the Second World War, poplar plantations spread to new and different sites, such as forest sites, upland sites, and different countries and climatic zones. Occasionally, traditional clones did not perform satisfactorily in such conditions and a search for new selections was initiated. At the same time, some of the well-established breeding programs, such as in Belgium, (Steenackers, 1970), Germany (Mohrdiek et al., 1979) and Holland (Koster, 1976), have come to fruition. In addition, new breeding programs were initiated by geneticists and specialists.

All this resulted in a variety of new breeding stock in the arboreta with new combinations of hybrids, and a large number of new clonal selections. However, although the need for diversification of clonal stock was realized and breeding efforts for this diversification were made, multiclonal concepts were only considered and developed in a few cases.

The two main concepts promoted for clonal mixtures were: (i) the use of mosaics of relatively small monoclonal blocks which were similar in size, and (b) the use of row-to-row or tree-to-tree type clonal mixtures.

The concept of mosaics of monoclonal blocks in poplar plantations was developed and implemented in Ontario, Canada (OMNR, 83). According to this concept, clones are carefully matched to site, plantation system and production goal. Sites are planted with several, rather than one or two, different clones. Monoclonal blocks are no larger than 5.0 ha in size. Each clone in production is continuously being tested for growth and pest resistance. As better clones become available from on-going breeding work, the poorer clones are removed from nursery propagation and planting. The number of clones in propagation and planting is more than fifty at any one time, and 5.0 to 10.0% of the clones in production are changed annually by phasing-out and new introductions.

The poplar plantation program in Ontario, as well as the breeding work in support of this program, has intensified since the late 1960's. The existing clones did not, in many cases, satisfy the needs, and studies demonstrated the significant potential for new combinations of hybrids and clones (Zsuffa, 1976, 1979). Team-work, and cooperation between research, technology development and management led to the development and successful application of this multiclonal concept in Ontario.

The use of row-to-row and tree-to-tree type clonal mixtures was attempted in Holland and Germany. Kolster (1978) investigated the possibility of decreasing monoclonal risks by mixing two clones by rows in plantations established with five hundred to six hundred and twenty-five trees/ha. The results indicated that this method did not offer practical possibilities. The growth of one clone was suppressed by the other. As well, the loss in wood production by one clone was not compensated by the increased growth of the trees of the other clone. For this reason, the mixing of rows of different clones was discouraged. The same result was expected when individual trees of different clones were mixed.

The concept of such mixtures of individual trees of several clones, or "multiclonal varieties", was furthered by Weisgerber (1979) in WestGermany. With the spreading of poplar culture to forest land in that country, attention turned to aspen and balsam poplars which placed less demand on climate and soil than cottonwoods and black poplars (section Aigeiros Duby). While aspen stock is rarely clonal due to difficulties in rooting of cuttings, balsam poplar stock often occurs as clones. In 1972, three balsam poplar clones comprised 54.0% of the market, and the proportion of balsam poplars to all poplar stock produced grew rapidly (to 70.0% by 1977). With the introduction of Populus trichocarpa Torr. and Gray provenances, efforts were made to enrich the gene pool and enable new selections. The development of multiclonal varieties is envisaged from such selections for growth in pure or mixed stands (with other deciduous and coniferous species). Balsam poplars are considered to be better suited than Euramerican hybrids to this forest stand concept.

Multiclonal varieties may succeed better in forest stands with species mixtures, thinnings, and less intensive tending practices than in intensively managed plantations. In forest stands, the suppression and loss of individual trees has less effect on yield than in intensively managed plantations. In addition, balsam poplars are more tolerant than Euramerican poplars and more adapted to growth in clonal stands.

Multiclonal varieties will create problems of maintenance and control. It will be difficult to maintain a certain predetermined clonal mixture in the stock because of problems in clonal identification and differences in the rate of rooting of cuttings of various clones. In addition, it will be difficult to judge the performance of individual clones in the mixture and adjust and improve the multiclonal variety accordingly. Consequently, the concept of clonal mixtures in poplars and willows can only be conceived as a mixture of uniform monoclonal plantations of sizes dictated by site and other needs. In his study of models of clonal plantations, Libby (1980) came to a similar conclusion that monoclonal plantations are frequently the best strategy, while mixtures of 2 or 3 clones are frequently the worst and rarely, or never, the best. Mixtures of a large number of clones in either multiclonal or monoclonal plantings are as safe as seedling plantations.

EXPERIENCES IN CLONAL PLANTATIONS

Experiences in clonal plantations have resulted from monoclonal plantations of poplars and willows, with a few clones planted on large areas, various sites, over a relatively long period of time, and in a variety of plantings. The successes and failures of these monoclonal plantations with regard to performance of clones, changes of clonal characteristics in time, and pest problems will be discussed.

Exceptional clonal performance was experienced with only a few clones. Up until the 1960's, virtually millions of ortets and clones were screened within the framework of many breeding programs and only several hundred clones were retained for propagation. Mohrdiek et al (1979) reported that in West Germany from 1948 to 1967, five hundred and fifty-six crosses were made and approximately 1.0% of the seedlings were retained for selecting promising clones. Very few of the several hundred tested clones equalled or surpassed the performance of standard clones. Thus, new clones of the quality of *I-214*, *Robusta* and *Androscoggin* were difficult to obtain. It is not surprising that these outstanding clones were so widely distributed.

Some of the exceptional clones adapted to a large variety of sites and growing conditions. A good example is *I-214*, which outperformed other clones (including local ones) in various countries of Europe, Asia, and the New World. Naturally, its ecological niche has limitations; in the north, frost limits its growth, while in southern latitudes, it is outperformed by clones adapted to shorter days. This is the situation in the southern United States where local selections of *P. deltoides* outperform most Euramerican poplars, including clone *I-214*. A change in photoperiod, as well as sites and growing conditions, limits the performance of clones. On forest sites and in forest stands, clones of balsam poplar origin, which are less demanding and more tolerant than black poplars, will outperform many clones from the latter group. In such conditions, *Androscoggin* is preferred to *I-214*. Thus, the outstanding clones performed well, but only within the range of characteristics determined by species and parent trees from which they originated.

The same clones used over a long period of time have not aged and their characteristics have not changed. *P.* x *euramericana* cl. *serotina* Hartig is an early selection described in 1775. More than two hundred years later, its stock is still in use, and its clonal characteristics and growth pattern appear to be unchanged. The same applies to very widely used clones, such as *Robusta* (selected in 1904) and *I-214* (selected in 1940). This phenomenon can be explained by the well-known ability of poplars and willows to rejuvenate when grafted onto young stock or rooted from cuttings. Unfortunately, this is not the case with many other tree species, where ageing, associated with symptoms of cyclophysis, may present a problem.

Clonal pest resistance has not changed over a long period of time unless new strains of pests appeared. Poplar clones, selected in western Europe for their resistance to leaf rust (*Melampsora* spp.), have remained virtually immune for many years (Steenackers, 1972). However, some of the same clones became susceptible when moved to North America and exposed to different species and strains of leaf rust. Clones in cultivation in Lombardy (Italy) in the 1920's succumbed to a newly spreading disease (*Venturia populina* (Vuill) Fabr) causing spring defoliation; however, clones subsequently selected for resistance to the same disease remained resistance for over fifty years. *Marssonina brunea* (Ell. and Ev.) Magn, a fungus causing browning of leaves and green shoots first described in North America and noted in Europe in 1958, caused serious problems in monoclonal plantations in Europe in the 1960's. More than one-half of the clones and plantations suffered serious set-backs from the disease. Since then, new clonal selections resistant to the disease were found and are replacing the susceptible clones. It is obvious that pests can move from surrounding natural vegetation to monocultures, new strains of pests can develop, and new pests can be imported from other countries and continents. The problems created are easier to surpass in short-rotation than in long-rotation monocultures, especially if the former are supported by active breeding programs. However, multiclonal plantations combined with continuous phasing-out and introduction of clones can lower the risk even further and represent a more efficient method of pest management.

Monoclonal plantations of poplars and willows have thrived for more than a century. Despite periodic pest problems, they have been successful. Monoclonal plantations are easier to manage than multiclonal plantations; fewer clones cause less problems in stock production, handling and varietal control. The determination of clonal mixtures and matching of clones to sites can be a difficult and complicated task. Therefore, is it worth doing all the additional work associated with multiclonal plantations? What other benefits, in addition to better pest management, can be derived from such plantations?

The advantages of multiclonal plantations, in addition to lowering the risks of pest problems, are in utilizing significant variations in clonal performances by matching these to sites, plantation systems and production goals.

Clone-site trials in Ontario (OMNR, 1983) demonstrated that the quality of the soil of the planting sites significantly changed over a small area; systematic analysis of the planting sites was necessary to map these changes. The performance of the clones at four and five years-of-age

Clones can be chosen effectively for different plantation systems. For example, fastigiate and shade tolerant clones are better adapted to dense plantings, and clones demonstrating rapid juvenile growth are better adapted to biomass plantations. The ranking of clones in trials in Ontario changed for a period of five years. Clones which initially grew well were surpassed by other clones when spacing allowed for such competition (Zsuffa, 1975). The errors in selection for height growth alone would have amounted to almost 50.0%.

Clonal biomass qualities vary significantly and large gains can be realized by the proper matching of clones to production goals. Clonal variations in poplar biomass qualities were demonstrated in Ontario by Anderson and Zsuffa (1975, 1976, 1982, 1983). Very significant clonal differences (sometimes more than 50.0%) were observed in many qualities of wood, bark and foliage (e.g. specific gravity, fibre qualities, gross heat of combustion, nutrient content, protein concentration and composition). The feasibility of simultaneous selection for several characteristics (such as growth rate and specific gravity, high biomass productivity and low nutrient content, etc.) was also demonstrated.

Until recently, poplar and willow breeding programs were unable to produce a sufficient number of clones of known and desired qualities for specific sites, plantation systems and production goals. Breeding programs currently underway, especially in poplar, renewed interest, and new considerations given to genetics and improvement of poplars and willows have provided hope for the realization of such goals. The genetic base for breeding has been significantly broadened, large collections of native and exotic species have been assembled and genetic variation studies are underway. As well, studies of genetic-environment interactions and parameters for many traits are yielding results. New and better planned crosses are producing rich pools of hybrids for selection and clonal development. Internationally coordinated programs, such as those within the frame of the International Energy Agency (IEA), the International Union of Forest Research Organizations (IUFRO) and the International Poplar Commission (IPC) provide good exchange of information and faster results.

A broadened genetic base, new knowledge, and good coordination can yield the desired stock for proper multiclonal management of poplars and willows. It is doubtful whether this can be achieved within the foreseeable future with other hardwood species where breeding programs do not exist or are just being initiated.

Pure clonal stands are easier for the silviculturist, the nursery man, the designated authority for control of planting stock, and the industrial user. However, if the clonal balancing is left to the user, there is the danger that one or two clones will be favoured and thus constitute the majority of plantings. In order to prevent this, each single clone should have a high and specific usability. Monoclonal plantations tailored to sites, plantations systems and production goals will be advantageous for intensive, industrial production. On the other hand, mixtures of clones, or multiclonal varieties, may be advantageous in forest stands when components, site, or their interactions are unknown (Heybrook, 1978).

CONCLUSIONS

Multiclonal plantations are a desired alternative to existing monoclonal plantations in poplars and willows. The concept of "multiclonal varieties" in individual tree mixtures may be feasible for forest stand management. The concept of mosaics of monoclonal blocks which are relatively small and similar in size in which clones are matched to soils, plantation systems and production objectives appears to be the only reasonable choice for intensively managed plantations.

Stock for multiclonal management has yet to be developed; however, active breeding projects in poplars and willows have the capacity for producing such stock. The prospects for the same development in other hardwood species are more distant.

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CONCEPTS AND EXPERIENCES IN CLONAL PLANTATIONS OF CONIFERS

J. Kleinschmit

Lower Saxony Forest Research Institute
3513 Escherode, Federal Republic of Germany

ABSTRACT

The general background of the concepts of clonal propagation and clonal plantations of conifers is discussed in the first part of the paper.

The main factors which influence production risk are reduction of genetic variation, pure plantations, little knowledge of plantations or tests, longer rotations, heterogeneity of environment, extent of plantation, exotic species, lack of knowledge about juvenile-mature correlations, and lack of basic genetic knowledge.

In the second part of the paper, results of a systematic breeding and selection program, including cutting propagation with Norway spruce, are discussed. Early testing, genotype x site interaction, and ageing with repeated repropagation are also discussed.

Clonal propagation may not only improve breeding programs, but it may also improve the transfer of the bred material to application.

RÉSUMÉ

La base théorique générale de la multiplication par clones et des plantations de clones de conifères est examinée dans la première partie de l'article.

Les principaux facteurs de risques pour la production sont la réduction de la variation génétique, les plantations pures, le peu de connaissances sur les plantations ou sur les tests, les révolutions plus longues, l'hétérogénéité de l'environnement, l'importance de la plantation, les espèces exotiques, le manque de connaissances sur les corrélations état juvénile-état mature et l'insuffisance des connaissances de base en génétique.

Dans la deuxième partie de l'article, les résultats d'un programme systématique d'amélioration génétique et de sélection, comprenant la multiplication par boutures de l'épinette de Norvège, sont examinée. On traite également des contrôles précoces, de l'interaction

génotype x emplacement, et du vieillissement accompagnant le reclonage répéte.

La multiplication par clones peut non seulement être profitable aux programmes en génétique, mais également améliorer la mise en application du matériel produit.

INTRODUCTION

Clonal plantations of coniferous species are more restricted in area than broadleafed trees. Due to the difficulties in rooting many coniferous species, most of the plantations are not very advanced in age. In Japan, only easy-to-root species, such as *Cryptomeria japonica* and *Chamaecyparis obtusa*, have been primarily planted in monoclonal plantations for a long period of time. However, vegetative propagation has progressed rapidly and has reached the point where operational clonal programs with coniferous species have been, or are being, initiated. Early clonal selection programs have been started in New Zealand with *Pinus radiata* (Thulin and Faulds, 1968), in California with *Pinus radiata* and *Sequoia sempervirens* (Libby and Conkle, 1966), and in Canada with spruce (Rauter, 1971). In addition, a great deal of research has been devoted to Douglas fir in Canada and the United States.

More than one million cuttings of Norway spruce have been produced annually for many years in Germany. Even more cuttings have been produced in Sweden in recent years and it is anticipated that two million cuttings will be sold next year. It appears that a re-evaluation of the concepts and experiences is necessary.

The most important literature on vegetative propagation is:

(1) IUFRO meeting on vegetative propagation in Rotorua, New Zealand, 1973. Published in New Zealand Journal of Forestry Science (4) 1974.

(2) Symposium on juvenility in Wood Perennials, College Park/Berlin 1975, Acta Horticulturae (56) 1976.

(3) Meeting on vegetative propagation of forest trees - physiology and practice, Uppsala, Sweden, 1977. Published by the Institute of Forest Improvement and the Dept. of Forest Genetics, Swedish University of Agricultural Sciences, 1977.

(4) Symposium on clonal forestry, Uppsala, Sweden, 1981. Published by the Institute of Forest Genetics, Dept. of Forest Genetics, Swedish University of Agricultural Sciences, research Notes No. 32, 1981.

(5) IUFRO meeting on *in vitro* propagation of forest tree species, Fontainebleau, France, 1981. Published by Associacion Foret Cellulose, Nangis, France, 1982.

(6) IUFRO meeting on breeding strategies including multiclonal varieties, Sensenstein, Federal Republic of Germany, 1982. Published by the Dept. of Forest Tree Breeding, Lower Saxony Forest Research Institute, 1982.

Within the proceedings of the last meeting mentioned above, the papers by Rauter on "Recent advances in vegetative propagation including biological and economic considerations and future potential" and by Burdon on "The roles and optimal place of vegetative propagation in tree breeding strategies" are worth reading.

In the first part of my paper, I will concentrate on the general aspects for the use of clonal plantations in breeding programs and for commercial use. In the second part, I will summarize our own experiences with clonal selection and plantations of Norway spruce.

GENERAL BACKGROUND

Vegetative propagules in conifers are generally more expensive to produce than seedling plants. Therefore, production is worthwhile only if the material is superior to seedling material, or if sufficient seedlings of similar genetic constitution cannot be produced by conventional methods.

Clonal Plantations in Breeding Programs

Vegetative propagation can be quite useful within breeding programs (e.g. for seed orchards, clonal archives, testing of material, special physiological research projects and studies of population architecture) (Burdon, 1982). However, I will only deal with plantations of clonal material which are also established for wood production.

Vegetative reproduction is only one means for propagation. However, it should never be used alone in a breeding program as this would limit progress. For long-term breeding (Kang, 1982), generative propagation must be used as the main line of a program and vegetative propagation should be used as a supplement.

In production populations, clonal propagation can be a powerful tool for utilizing, or even improving, genetic gain. Clonal selection and testing can provide considerable progress compared to bulk propagation of bred material (e.g. seed orchard progenies, controlled-pollinated families). These clones can be used at the same time for future breeding work.

The main restriction of coniferous species is the ageing process. It not only influences rootability, but also topophysis effects, growth vigour, physiological behaviour and root intensity (Roulund, 1979; Olesen, 1978). Up to now, only limited progress has been made in the field of rejuvenation of conifers (Franclet, 1981). Hedging of ortets and serial propagation are two common ways to delay ageing in conifers. Hedging, which means repeated pruning, has been successfully applied by

Libby *et al.* (1972) and Libby and Hood (1976) in *Pinus radiata*. Repeated repropagation of Norway spruce has been used in our program for fifteen years with limited or doubtful success. Another approach, used by Rauter (1982), is the propagation of very young seedlings. However, this is not clonal selection in the first stage, but mass propagation of superior and rare seedling material; a very interesting tool for practical forestry and forest tree breeding.

Concepts and Experiences for Production Populations

For most foresters, it is important to have clear and well-founded concepts for the utilization of clones in silviculture. The criteria on which their decisions are based are high production with minimal risk, and reasonable economic return.

The productivity of the clones must be guaranteed by the breeder. The extent to which he is able to do this is the basis for economic return. Risk depends on a number of factors which are influenced by the species as well as by the silvicultural concept. Production risk increases with reduction of genetic variation, pure plantations, little knowledge of plantations or tests, longer rotations, heterogeneity of environment, extent of plantations, exotic species, lack of knowledge about juvenile-mature correlations, and lack of basic genetic knowledge.

Reduction of Genetic Variation

Most clonal plantations have less genetic variation than seedling populations of natural stands. The most extreme example would be a monoclonal plantation. Many variations, from monoclonal plantations to clonal mixtures of several thousand clones, which was the method used in our early selection stages of Norway spruce, are possible and have been proposed. Libby (1981) suggested that there may be a small, optimal number of clones on the basis of theoretical assumptions. This may be true in theory, however, in practical programs, there are many arguments for keeping the number of clones high. Very limited sound information exists on this question. Hühn (1983) initiated a study from a theoretical point of view and some experimental data will be presented in this paper.

Finally, the objective of a clonal program could be to simply reproduce a seedling population. In this case, there would not be any difference between generatively and vegetatively propagated material. Seedling populations can also have a narrow genetic base, such as full-sib families. Arguments for their utilization parallel the arguments for clonal plantations of the same material.

From a risk point of view, the mixture of good clones with seedling plants of the same species, or with other species, in a systematic pattern may be advantageous. Such a system enables expensive and rare clonal material to be used without increasing production risk or reducing economic return. Such systems have been used with timber species, such as *Sequoiadendron giganteum*, and with Norway spruce clones selected for frost hardiness in Germany.

Knowledge of Clonal Performance

The knowledge of clonal performance of most coniferous species, especially those produced by repeated repropagation, is very limited. On the other hand, there is a large amount of seedling material available from breeding programs which cannot be reproduced sufficiently by generative means.

The differences between seedlings and cuttings have been described for those species which have been vegetatively reproduced for many years. There are species with a pronounced topophysis effect in cuttings. *Araucaria heterophylla* is one extreme example where cuttings from branches of second order remain branches of second order, branches of first order which are rooted remain branches of first order, and only terminal buds ofthe leader, or the leader itself, develops into a complete tree. Most *Abies* species show a strong topophysis influence. Cuttings of mature trees may maintain the branch habit for more than thirty years while others change to orthotropic growth after a number of years, often starting from an adventitious bud. However, severe trunk deformations can result.

Although not as common in *Abies* species, plagiotropism in Douglas fir is a limiting factor of a clonal propagation program. Cuttings from young ortets change to orthotropic growth within a few years and after seven years, it is difficult to see the differences between a seedling and a cutting plant. If the age of the ortet exceeds ten years, the change to orthotropic growth may take so long that permanent trunk deformation results (Vieitez *et al.*, 1977).

The differences are less obvious in the genera *Larix*, *Pinus* and *Picea*, however, minor differences between seedling plants and rooted cuttings have been described for these species. Generally, the differences increase with the age of the ortets and topophysis effects partially account for the differences (Fielding, 1970; Roulund, 1979; Kleinschmit, 1978; Kleinschmit and Svolba, 1980). In *Pinus radiata*, the stem diameter of cuttings was lower than that of seedlings of comparable height. The diameter of cuttings of Norway spruce was greater. In a recent study, we found that *Larix* had a better root system in seedlings than cuttings. Norway spruce seedlings and cuttings had comparable root percentage, however, considerable clonal variation existed in root percentage. There are differences between seedlings and cuttings in root collar diameter and in a number of branches of first and second order.

These few examples may be sufficient to stress the importance of the species influence on clonal performance. Before starting an intensive clonal propagation program, sufficient knowledge of clonal performance compared to seedling plants should be obtained. As a rule, differences between seedlings and cuttings will be less with very young ortets and risk is likely to increase with ortets of advanced age.

Rotation Time

Limited knowledge of clonal performance and a limited testing period are serious problems for species grown in long rotations. For Christmas tree growing or short-rotation forestry, such as pulp wood production, a shorter testing period is acceptable whereas valuable timber production requires a rotation period of one hundred years or more. The period of testing should be realistically related to rotation time. It is difficult to establish general rules since some characteristics, such as flushing and bud set, may be judged at a very early age. If the aim of the breeding program is to improve these characteristics, early selection from locally-adapted populations are sufficient. Other characteristics, such as wood quality, snow-break resistance, or straightness of stem require a much longer period of observation and, therefore, early selection can be quite misleading. With respect to growth, it is necessary to follow the clonal performance at least until competition counterbalances the differences in vigour.

Heterogeneity of Environment

Clonal testing can only be performed on a limited range of sites. The more uniform the growing conditions, the easier it is to select clones which will reproduce their growth at other locations. For example, it is more difficult to select clones for Norway or Switzerland than for the pine region in the lowland of Northern Germany.

Genotype x site interactions are more strongly expressed on a clonal level than within families. These interactions account for approximately 50.0% of the total variation in Norway spruce clones. Genetic variation, as a buffer to environmental heterogeneity, does not exist on a clonal level. On the other hand, different clones show pronounced differences in their ecological stability (e.g. ecovalence). This may be partially due to differences in the degree of heterozygosity. This supports the concept of combined selection for vigour and stability. The risk of failure can be counterbalanced by a mixture of clones with different ecological adaptations.

Extent of Plantations

There is a difference if a selected clone is planted in a home garden or if it covers 10.0% of the woodland area of a state. Risk increases with the extent of the area of the plantation. In our clonal tests with Norway spruce, obvious clonal differences in susceptibility to diseases were found and only appeared if a clone occurred in high frequency. Pathogene populations can easily build on a homogeneous substrate. The strategies for resistance breeding, including clonal propagation, have recently been discussed by Thielges (1982). Either a strategy of slowly increasing the plantation area while increasing knowledge about a certain set of clones, or a long period of intensive testing which may cause ageing problems, is necessary if large areas are to be planted. A limitation of the maximum number of ramets per clone is one method of controlling the extent of the plantations.

Considering both risk and the logic of a breeding program, it would be wise to use flexible systems for conifers. This may include shifting clonal composition and different clone numbers depending on the progress of testing time and knowledge.

Exotic Species

The risk of failure of clonal plantations increases with those species where little is known about their performance under plantation conditions. The history of growing exotic species in Europe provides examples of originally promising results ending in disaster, even with seedling populations. Adaptation to a certain environment is important not only in conventional breeding but even more so in clonal forestry. Recent observations in Sweden indicate that on a provenance level, adaptation to local climate only appears at an age of more than twenty years (Karlsson, 1983). We found that in Norway spruce, early selection from provenances which are not autochthonous is much less effective than from indigenous material. This is probably the case with hybrid material which can be regarded as exotic in so far as the specific combination of genomes has never been tested under the ecological conditions of the planting site. The probability of failure of hybrids increases if the ecological conditions of the parent species, especially photoperiod and length of vegetation time, deviates significantly from those of the planting site (Dormling *et al.*, 1974).

Lack of Knowlege about Juvenile-Mature Correlations and Other Genetic Parameters

For species which have been in a breeding program for many years, basic information regarding juvenile-mature correlations and other genetic parameters is available. For these species, early selection for a clonal program is more soundly based. It must be stressed, however, that correlations calculated from populations cannot be used in clones since individuals may react differently than a population (Kleinschmit *et al.*, 1981). On the other hand, this is a possible advantage for a clonal selection program (e.g. to select correlation breakers).

Administrative Regulation Concepts

The above considerations led to the first administrative regulations for the use of clones of coniferous species in Sweden (Hedström and Krutzsch, 1982). These regulations are well-founded and consider bothdifferent and important aspects, and are a good base for similar regulations being developed for different European countries. These rules are as follows:

(a) Clone-identified material may only be used in mixtures of clones

(b) Minimum numbers of clones and maximum numbers of ramets per clone depend on the duration of the clonal tests (Table 1)

(c) Bulk propagation of seedling material is allowed for propagation of approved forest reproductive material where the seed supply is poor

(d) It is not necessary to maintain clonal identity

(e) Bulk propagation of previously clone-identified material is permitted under certain conditions. In bulk propagated material, less than one hundred ramets per seedling are permitted. The reproductive material must contain at least twenty full sib families with different parents or fifteen half sib families mixed in equal proportions

Table 1. Swedish regulations for clone-identified material

Testing				Application	
Test Level	Duration of Test	Minimum Number of Test Sites	Minimum Number of Replications	Minimum Number of Clones/ Mixture	Maximum Number of Ramets/ Clone
1	6	2	5	120	250,000
2	9	4	7	60	750,000
3	12	6	7	30	1,500,000

The difficulty with such a system is effective control; the concept, however, seems to be well-balanced.

EXPERIENCES WITH NORWAY SPRUCE CUTTING PROPAGATION

Background

The first Norway spruce cuttings were rooted as early as 1830 by Pfifferling (1830). Clonal propagation of Norway spruce was first initated in Escherode in 1948 by my father, Richard Kleinschmit. It was also started in other places in Germany and Europe at approximately the same time. Summaries of the experiences with spruce cuttings are given by Girouard (1974) and Kleinschmit *et al*. (1973).

Norway spruce is the most important forest tree species in Germany and central Europe and covers more than 40.0% of the total woodland area. One problem encountered in breeding Norway spruce was that it only started to flower after twenty to twenty-five years, even in grafted seed orchards. As a result of this delay, rapid propagation is more desirable. We expect to receive a better return from provenance selection combined with clonal selection than from seed orchard establishment.

Program

Concept

The general concept is presented in Figure 1. The left side shows the traditional line of a breeding program using provenance testing and selection, plus-tree selection, testing, and seed orchard establishment. The first clonal selection and testing can be done at the provenance level, thus improving the best provenances by early selection in the nursery. The best individuals are selected at an early age (usually at age four) from open-pollinated families, controlled-pollinated families, or species hybrids. Each individual is vegetatively reproduced and included in clonal tests.

Our hybridization work with spruce has increased considerably since 1974. To date, we have attempted more than two hundred hybrid combinations out of a possible 1260 theoretical combinations. Our aim is to include up to 10.0% hybrids in clonal mixtures. If the hybrids subsequently fail, there is no economic loss; if they succeed, they can form up to 100.0% of the final crop.

The scheme for clonal selection and testing is presented in Figure 2. It shows the close integration of a general breeding program, clonal testing, and practical application. A set of clones, selected in one year (e.g. 1974) is propagated and early-tested in the nursery. After three years, the best clones are then planted in field tests which are established at three sites in single tree plots with seven replications per clone. Ramets of these clones are already being used for practical mass propagation. For this purpose, a number of propagation units have been developed in state forest nurseries with a total capacity of approximately one million cuttings annually. The selected clones are repropagated and new clones enter from the breeding program. The information collected from the field tests is used for the reselection of the clones in the nursery three years later and the first set of clones is replaced by the next in application. The best clones from the clonal test may be subsequently used for advanced breeding material in controlled crosses.

This system guarantees the quick transfer of breeding results to application without greatly narrowing the genetic variation at an early stage. If ageing becomes a severe problem in advanced propagation cycles, the older clones can be stopped in propagation and a shift in clonal composition subsequently results. This is advantageous in maintaining genetic variation, however, it limits possible genetic gain.

Application

This system has been applied since 1968, and in 1980, we reached

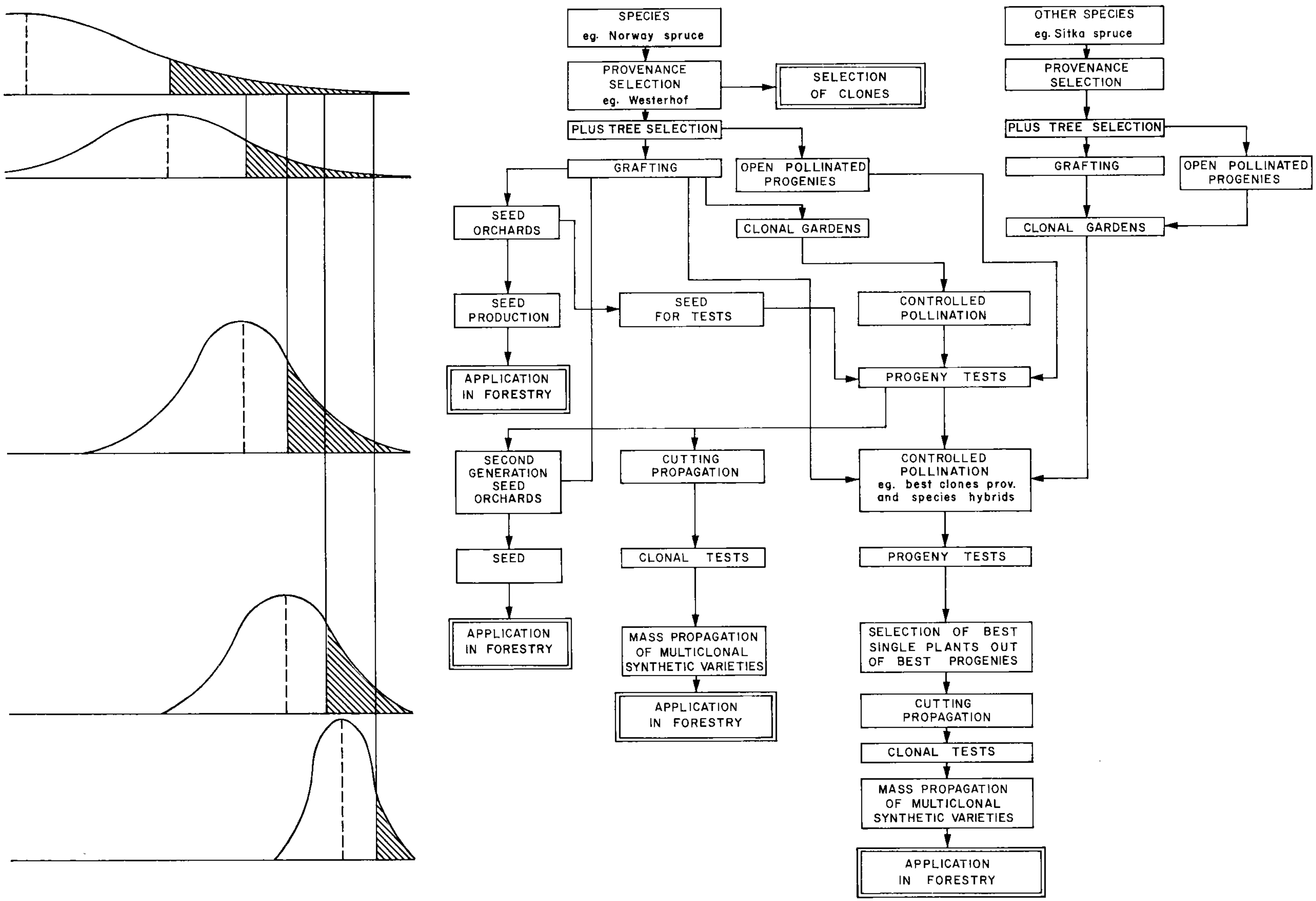

Figure 1. General concept of a breeding program

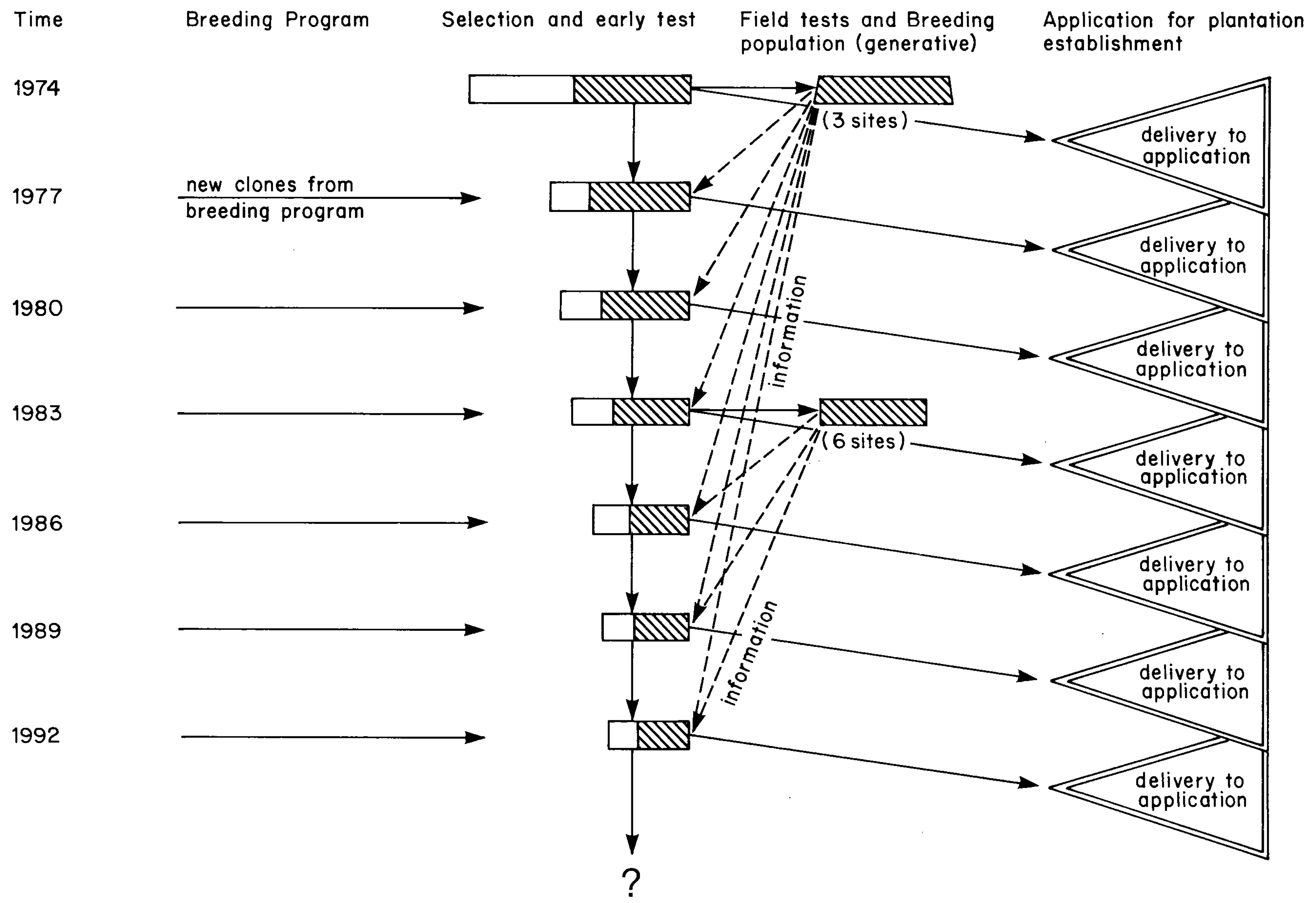

Figure 2. Scheme for clonal selection and testing

the fifth propagation cycle. To date, approximately 55,000 clones have been selected, one-third of which are still under propagation.

More than one hundred and fifty hectares of clonal tests, with single tree plots, have been established and the number of hybrids in our tests is increasing. The annual plantation area of clonal mixtures is approximately four hundred hectares. Clonal mixtures contain one thousand to five thousand clones, depending on the progress of selection. They are planted in pure stands with 2.0 x 2.0 m spacing, which is approximately ten times the number of the final crop and 20.0% less than in seedling plantations. Clonal identity is only maintained in our breeding and selection program.

The method used for application is bulk propagation of the clones which we developed. We have established seven propagation units; only three other units exist in Germany and belong to other state forest tree breeding institutes. Most of the clones originate from selections of tested provenances. However, the number of clones selected from half sib or full sib families is increasing. On the practical side, to date there have been only limited difficulties as we had good control of the propagation units and material.

The main problems of this type of program are early testing, genotype x site interaction, and ageing. We have collected a great deal of information on these problems and some of our results are presented in the following sections.

Early Selection and Testing

In 1968, we initiated an early selection program using material from Langlet's international provenance experiment. We selected thirty four-year-old clones from ten different provenances. Each provenance was represented by ten clones of inferior plants, ten clones of mean plants, and ten clones of superior plants.

We followed the growth of the ortets and two sets of ramets taken in 1970 at age six, and 1971 at age seven. The results are presented in Figures 3, 4 and 5. These results show that significant progress can be made at an early age by intensive selection, however, this is not without exception. Provenances from climatically-different regions, such as Kolonowskie, Poland, may behave quite contradictorily which can only be explained by different adaptational behaviour.

As growth continued, changes in rank took place. The amount of change was due to differences in the test and nursery environments. If these environments are similar, rank changes are comparatively small, however, they can be quite drastic. The correlations calculated for a series of field experiments are presented in Table 2 and Figure 6. Similar results are described for Norway spruce by Dietrichson and Kierulf (1982). However, as these figures are based on clonal means and use ranking, they exaggerate the real changes.

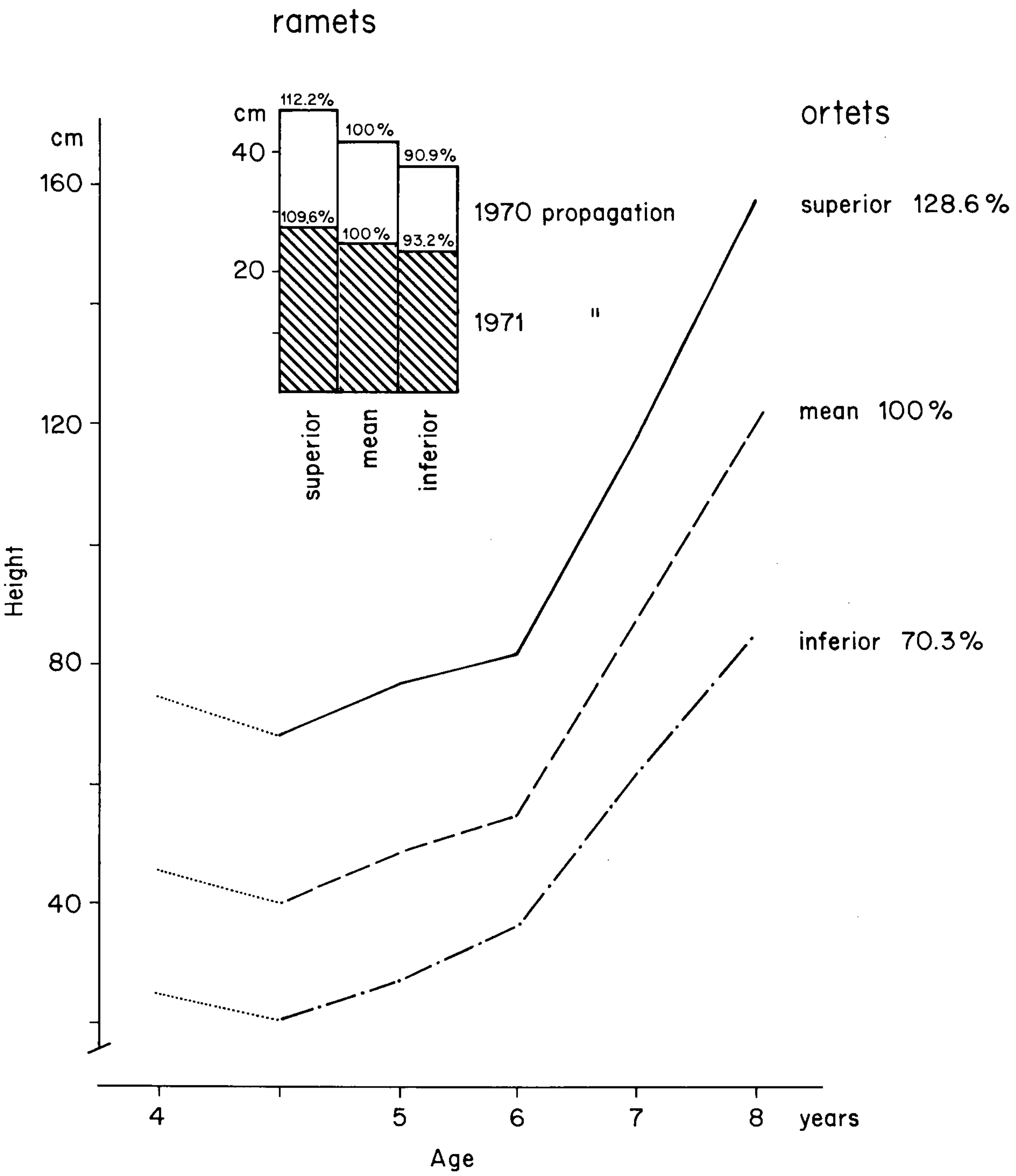

Figure 3. Early selection of Norway spruce
Langlet overall-mean
(10 provenances with 30 clones each)

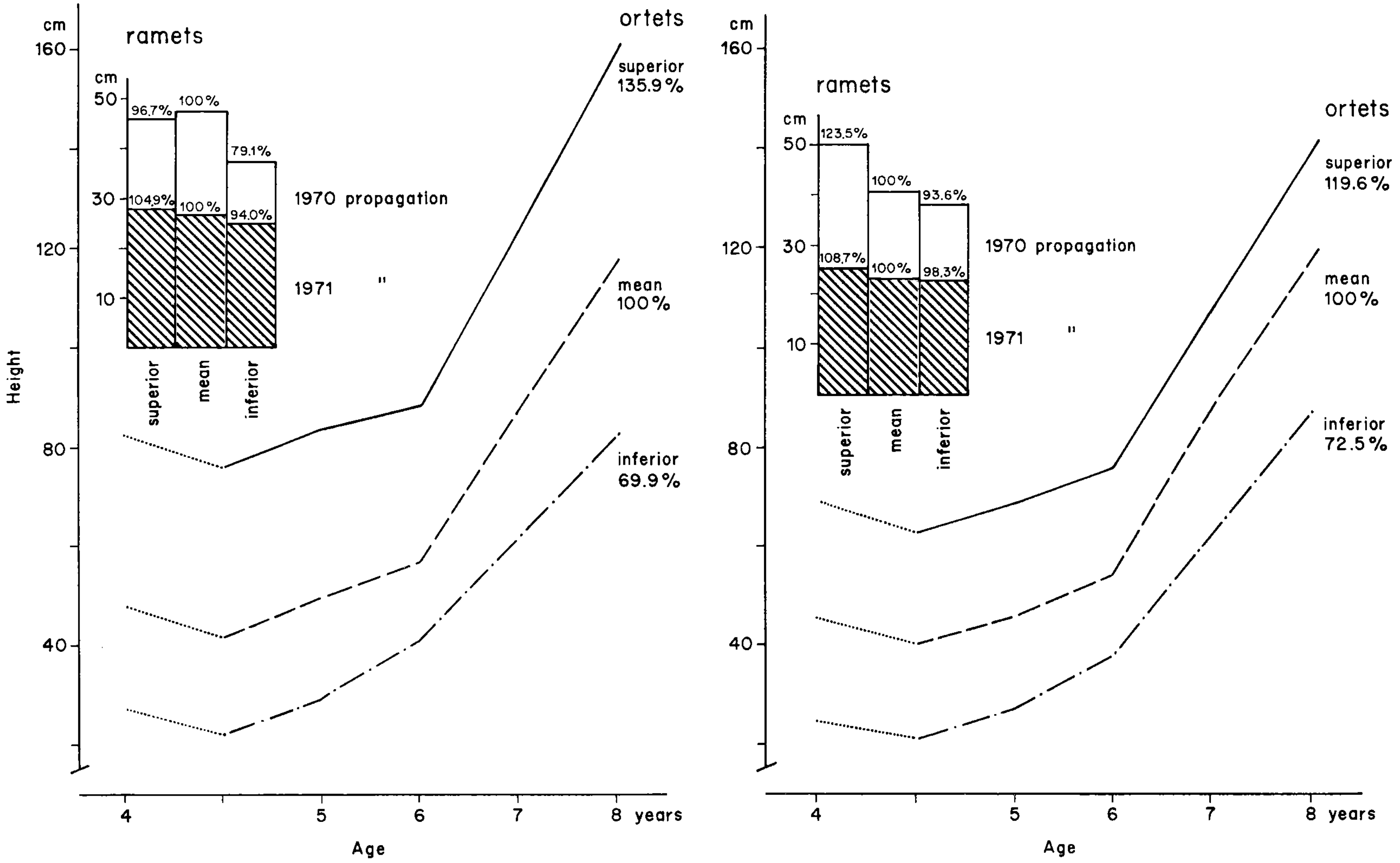

Figure 4. Early selection of Norway spruce
Provenances from Poland and Czechoslovakia

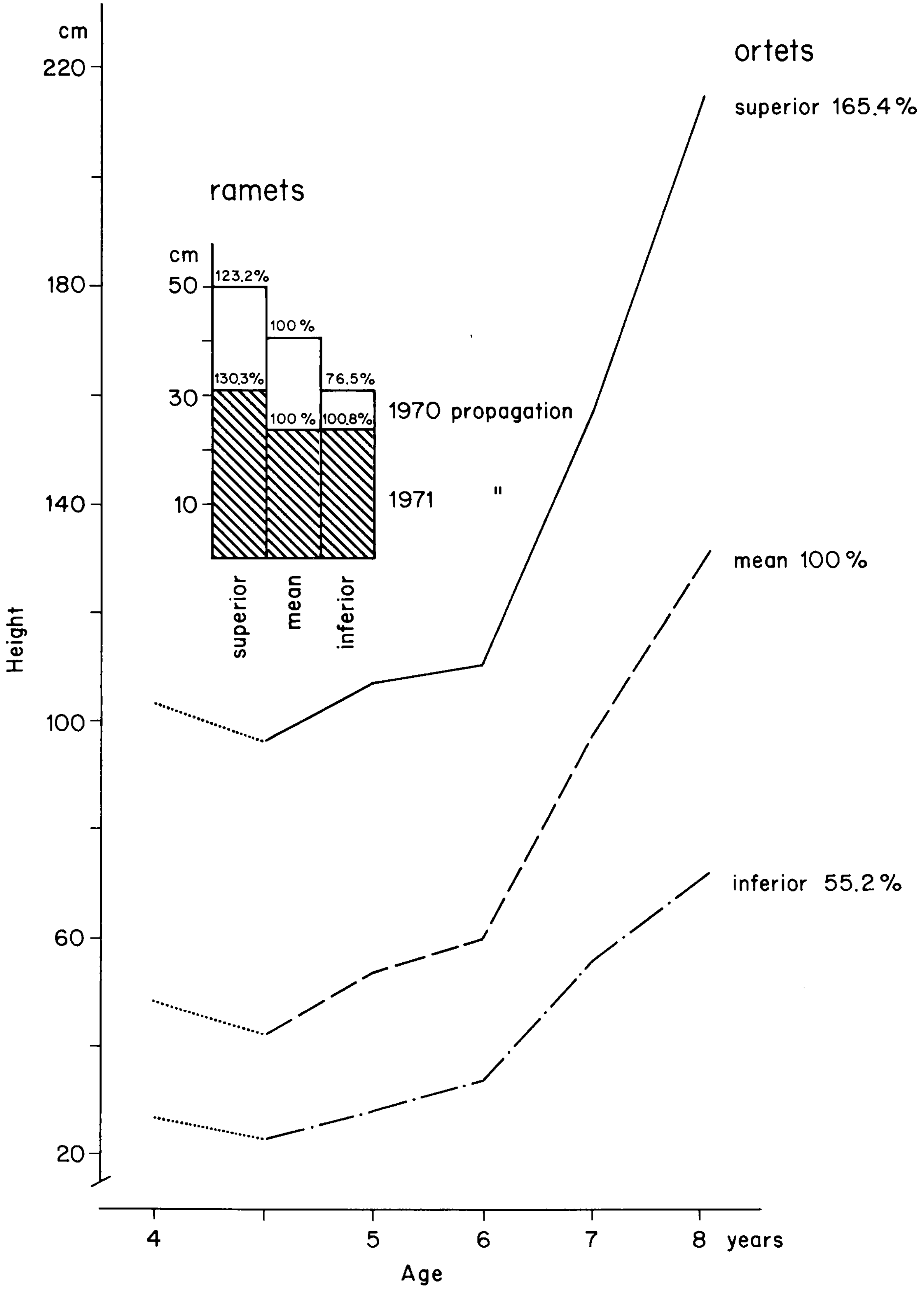

Figure 5. Early selection of Norway spruce
Provenance Westerhof

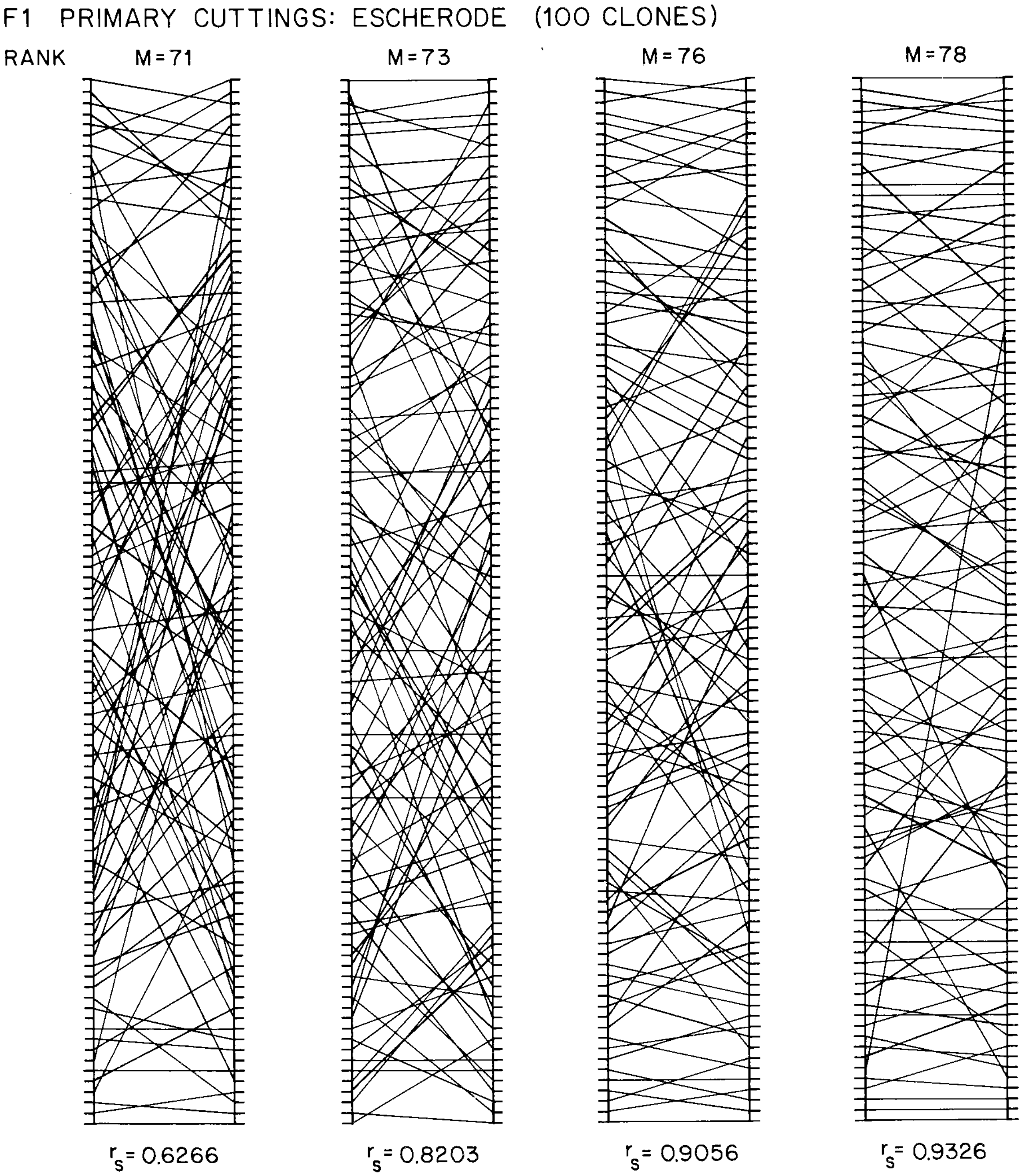

Figure 6. Norway spruce clones - Changes of ranking with age

Table 2. Correlations for clonal height development on twenty field experiments from age 3, 6, 8 (N = 61, 36, 21)

	3 - 6	6 - 8	3 - 8
secondary	0.69	0.90	0.52
	0.82	0.80	0.57
	0.82	0.80	0.69
	0.52	0.84	0.30
	0.70	0.84	0.44
tertiary	0.84	0.50	0.20
	0.62	0.85	0.55
	0.76	0.70	0.36
	0.65	0.64	0.28
	0.83	0.48	0.13
	0.84	0.53	0.16
	0.81	0.56	0.23
	0.63	0.44	0.30
	0.81	0.70	0.35
	0.84	0.70	0.54
quatenary	0.69		
	0.86		
	0.85		
	0.76		
	0.80		
	0.84		
	0.81		
	0.81		

Details of rank changes are not very clear. They may fluctuate or go in one direction. Some clones maintain their positions while others change drastically. This may be due to changes of annual weather conditions (frost damage, drought, etc.), developmental differences, or genotype x site interactions.

Early, final decisions cannot be made due to rank changes which occur in the first years in the field. Rank changes between clones depend primarily on the age of the plantation. They are quite drastic in the first years after establishment and more or less disappear after ten to twelve years-of-age after which, changes only occur in one direction, from up to down, due to competition in the plantation.

We were interested to see how correlations change if groups of clones as in a clonal mixture were considered. We formed groups of different sizes beginning with one clone and increasing the number of clones stepwise. As expected, the genetic variation slowly increased within the groups. However, the expected gain between groups as well as the genotype x site interactions decreased. Ranking was done according to the first measurement of the field tests and all subsequent grouping followed this early ranking. For this purpose, we evaluated our older Norway spruce clonal tests. The general results (Figure 7) show that even if one begins with very poor correlations, such as 0.3 with one clone, these improve rapidly if the groups increase; with ten clones, correlations reached 0.8; with twenty clones, 0.9; with fifty clones, 0.95; and with sixty-five to one hundred clones, 0.99 to 1.0, depending on the site. At the same time, differences in height between the groups mean decreased and gain decreased. However, gain can only be realized at an early stage if correlations are good. By increasing the group size from one to five clones, the difference between groups decrease from more than 100.0% to 15.0to 20.0%. A 15.0% gain can be achieved at the end of the nursery stage whereas a 100.0% gain requires an additional nine years. This implies all the problems connected with ageing. An additional selection of 50.0% of the best clones in the nursery would have produced an 8.0% gain; 20.0% of the best clones would have produced a 15.0% gain; and 10.0% of the best clones, an 18.0% gain. Considering the cost of field testing, this information can aid in considerably improving the selection procedure. A selection program which takes these results into consideration is presented in Figure 8.

Genotype x Site Interactions

Significant rank changes can be observed between clonal means on different sites. Genotype x site interaction is significant in all series and accounts for 8.0 to 50.0% of total variation.

The ecological stability of the clones can be quite different. We calculated different stability parameters (Wricke, 1964; Hühn, 1979) and found differences of 1:10 to 1:20 for the clones. Therefore, extreme differences are expected in adaptational behaviour of different clones (Figure 9).

All this seems quite pessimistic for the use of clones in a breeding program. The practical success, however, can be measured in the superiority of the clones as compared to seedling standards. We have as a standard in our experiments, a tested stand, provenance Westerhof, which is our best indigenous source.

As a mean figure from all primary tests, the seedling standard ranks between 75.0 to 95.0% depending on the age. The top clones reach 50.0 to 120.0% superiority as compared to the overall clonal mean. In all secondary propagated material, we reached a mean figure of the seedling standard of 85.9% and 84.9% respectively, with the best clones ranging from 31.0% to 66.0% superiority as compared to the overall clonal mean. As a mean figure of the tertiary propagated material, the seedling standard had 84.0% with the best clones showing 40% superiority as compared to the overall mean.

The quatenary cuttings only reached six years of age at the last measurement. As a result, comparisons are not very valid at this time as this material had a poor start in comparison to the seedlings which

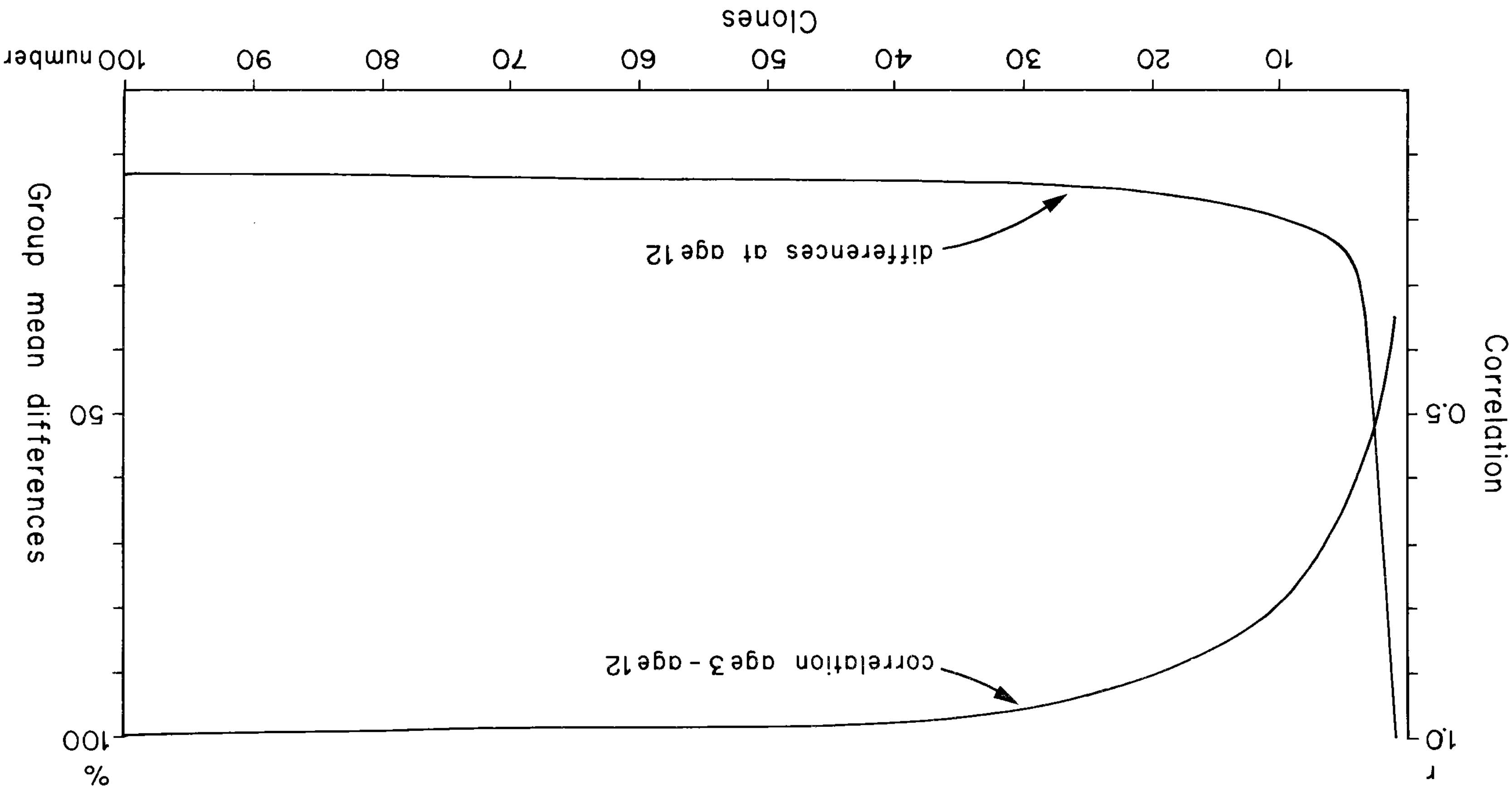

Figure 7. Changes of correlations and differences between group means for height with grop size for Norway spruce clones

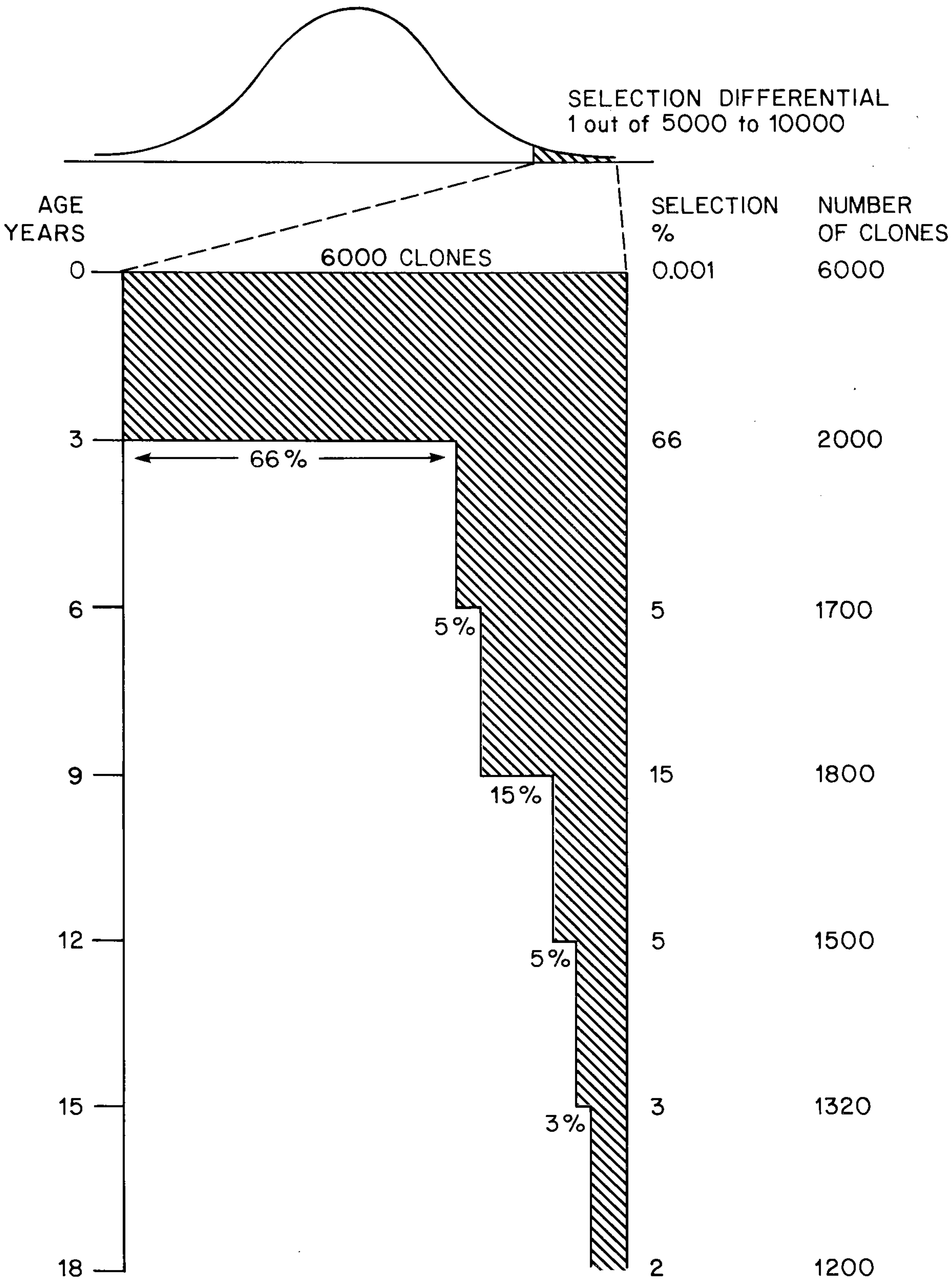

Figure 8. Proposed selection program for Norway spruce clones
3 years propagation cycle

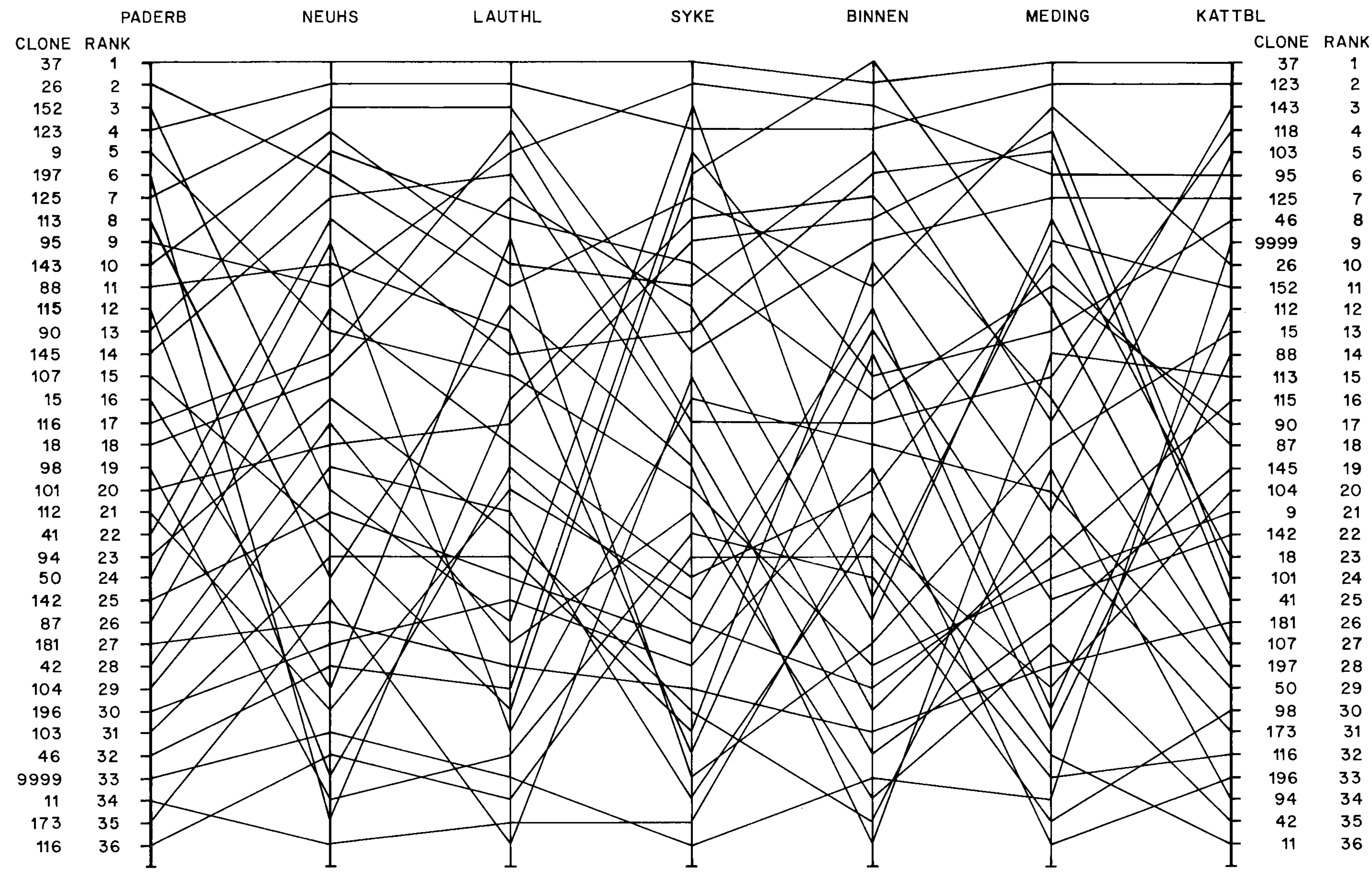

Figure 9. Norway spruce cuttings - tertiary propagation
7 field tests
Rank changes of clonal means due to location

originated from a different nursery bed and had an initial superiority which subsequently decreased.

Selection of single clones from one location appears to be quite ineffective. Therefore, we increased the groups of clones using the same method we used for different age measurements.

The following illustrates the improvment of the correlations for a set of 425 clones tested on two very contrasting sites (age 8).

Number of clones	Correlation coefficient	Group differences (%)
1	0.39	250.0
10	0.84	41.0
50	0.93	33.0
75	1.00	19.0

At the same time, differences between groups decreased. Conclusions can be drawn from rank changes due to age influences and genotype x site interactions. The gain became more stable with mixtures of clones than with single clones, however, the amount of gain decreased.

Ageing

Ageing is the most important problem in clonal programs with conifers and can have severe implications.

In the autumn of 1979, we had a large amount of material of primary to quatenary cuttings which had been aged in the nursery for three years. The different stages were represented as follows: primary, 2000 clones; secondary, 2197 clones; tertiary, 8417 clones; and quartenary, 5387 clones.

Figure 10 presents the rooting percentage and height growth in the nursery. There is an obvious increase in rooting potential from secondary to quatenary propagation. There is also an obvious superiority in heightin the first propagation cycle which may result from having a good selection base for primary material. The difference between height growth from the second to fourth propagation cycle was not significant

We took a representative sample of twenty clones from each propagation cycle, which had been replicated four times, for further analysis. The results are presented in Figures 11 and 12. There is no obvious disadvantage between the fourth propagation cycle and the second or third. The general shape, tropismus and habitus are even better. The only difference is a shift from branches of first order to branches of second order.

In 1982, we had collected material from the first to the fifth propagation cycles in our nursery. The results of height measurement are presented in Figure 13. This material is, more or less, a random sample of different propagations, only biased by the different, original basis for selection. The fifth selection, however, includes all remaining

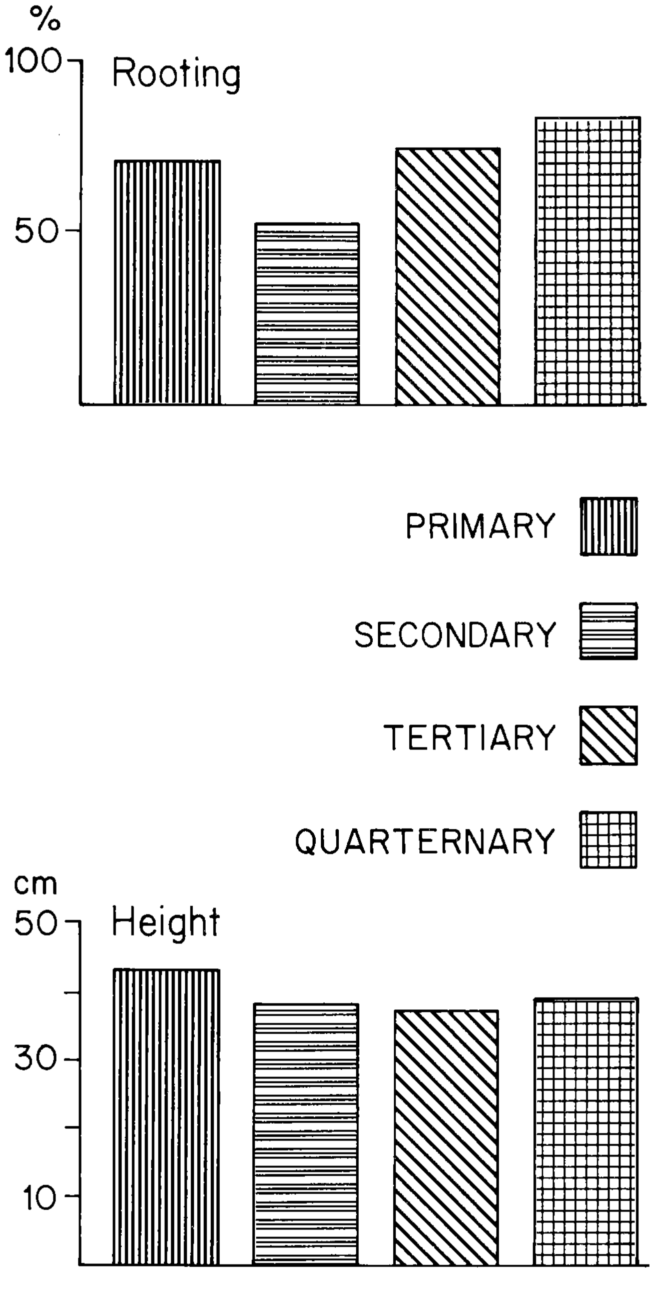

Figure 10. Rooting and height growth of Norway spruce cuttings in different propagation cycles

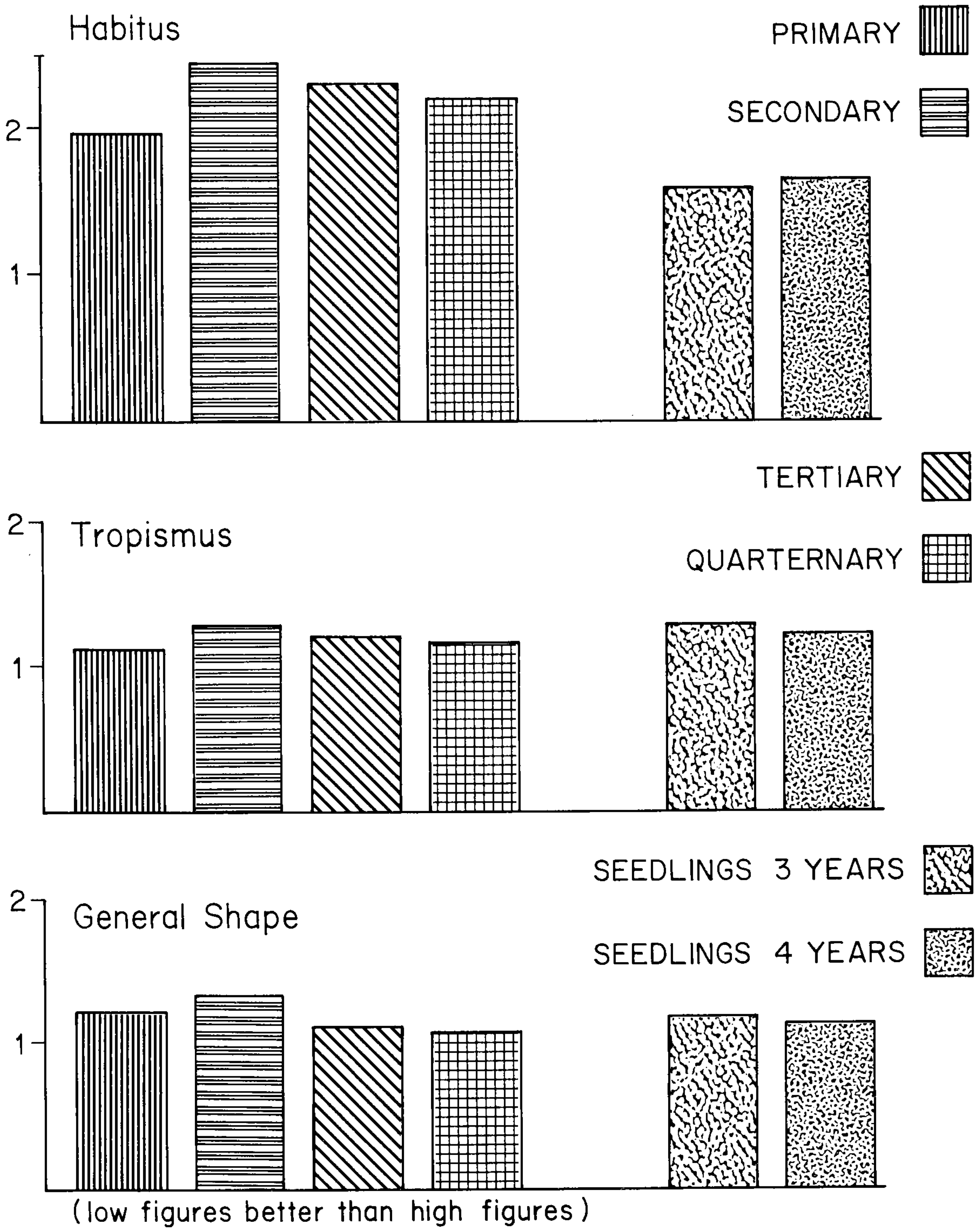

Figure 11. Structure of cuttings (3 years) from different propagation cycles and from seedlings

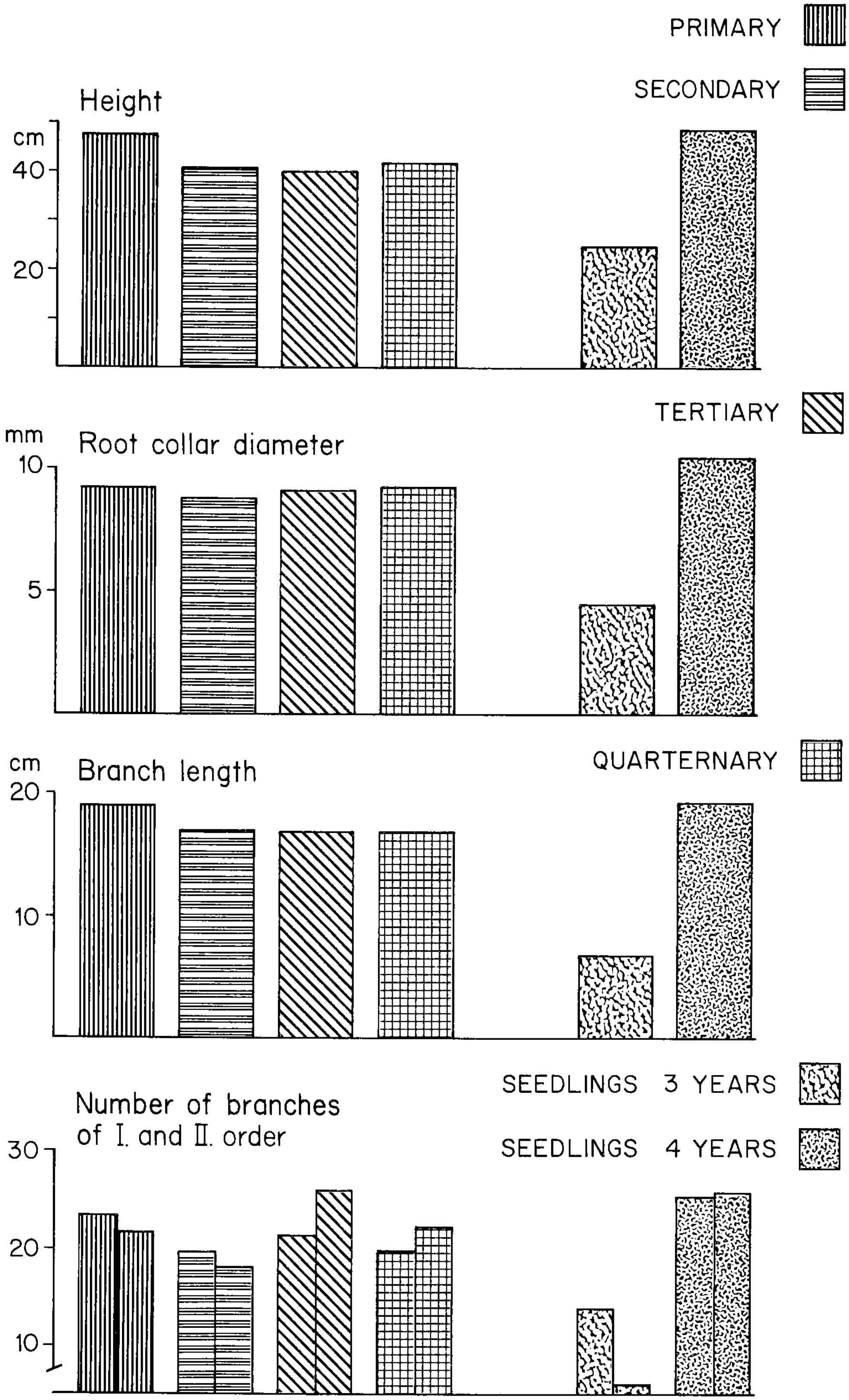

Figure 12. Structure of cuttings (3 years) from different propagation cycles and from seedlings

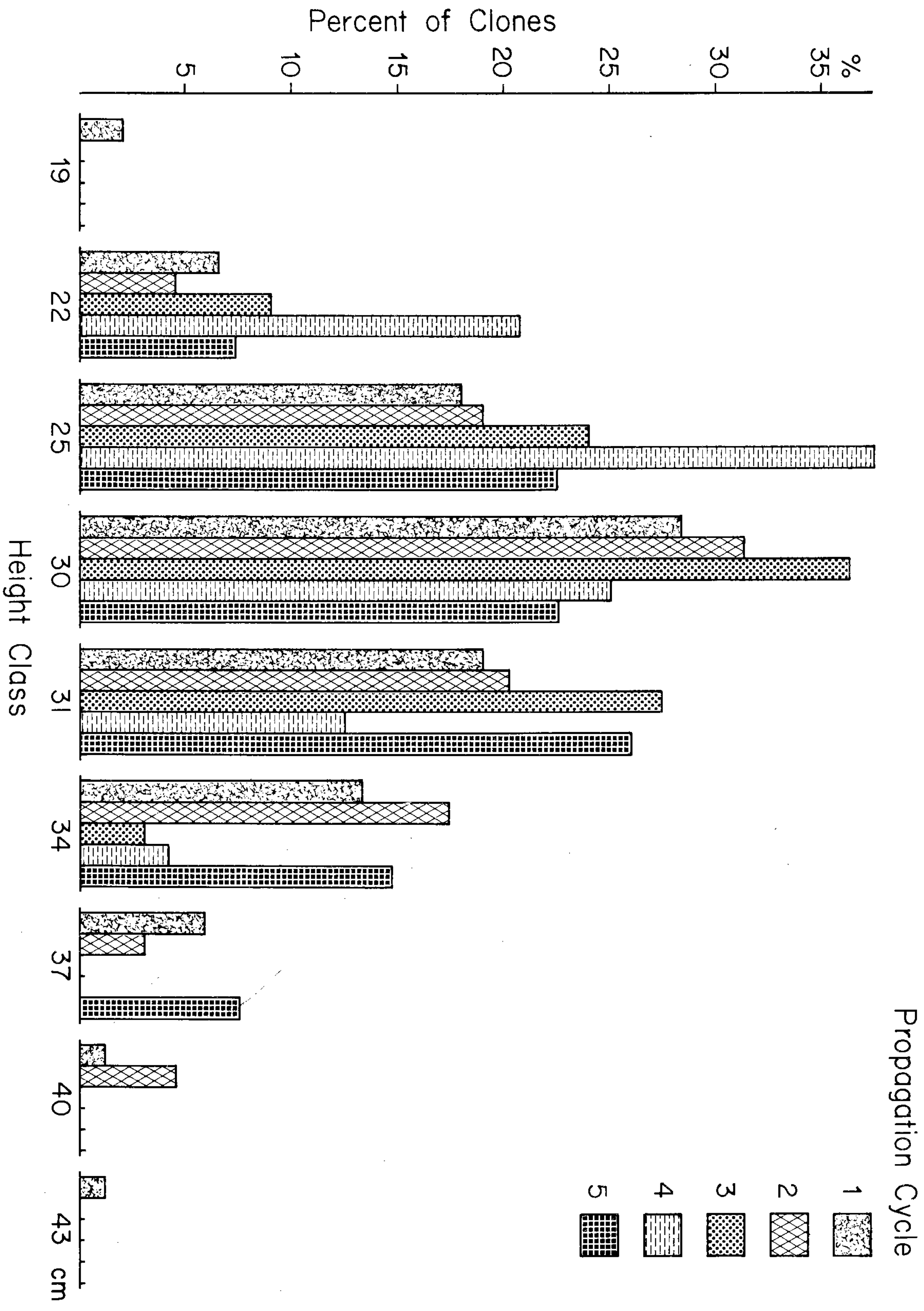

Figure 13. Height of clones of different propagation cycles
(ramdom samples)

clones. The respective mean values from three replications are: first propagation, 31.1 cm; second propagation 30.4 cm; third propagation, 28.3 cm; fourth propagation 26.8 cm; and fifth propagation 30.0 cm. There appears to be a slow decrease from the first to the fourth propagation cycle. The fifth, however, is better in height than the third and fourth. The effect of higher selection intensity in this material cannot be excluded.

The above is an evaluation of developent in a young stage. Physiological activity and re-establishment of inherent growth vigour may take longer in higher propagation cycles. These small differences, however, may not be too important from the point of view of production.

Finally, we correlated the growth of the second, third and fourth propagation cycle of the same clones tested on six (secondary), seven(tertiary) and seven (quatenary) sites, with seven replications on each site. There are still significant correlations between the second and third propagation cycles for the same clones, however, there are almost none between the third and fourth.

The same is true at a more advanced age. The analysis of variance calculated with clones, propagation cycles and replications within propagation cycles generally provided low values for clones, accounting for 10.0 to 20.0% of the total variation. Propagation cycles account for 30.0 to 80.0% of total variation, and interaction clone x propagation cycle accounts for 13.0 to 64.0% of total variation, depending on the respective set of experiments and propagation cycles.

The lowest repeatability of the clones was generally found in the third and fourth propagation cycle with correlation coefficients decreasing as far as zero or -0.017. As the quatenary material experienced frost heaving in the nursery, these figures are not very important, and further development of this and other experiments including quartenary propagation should be anticipated.

If groups of clones are increased as was done for different ages and plantations sites, correlations improve with groups of five clones to 0.5, and to 0.9 with groups of ten clones. Since the total number of clones which passed the fourth propagation cycle was limited, it was not possible to experimentally evaluate at which clone number the correlations reached 0.99 to 1.0. However, group differences are much smaller with different propagation cycles than with height and location, and they did not surpass 20.0% of the overall mean. This evaluation, however, has one more weak point which must be mentioned to avoid a pessimistic view of repeated repropagation: all clones that had reached the fourth propagation cycle has passed four selection cycles too. Therefore, total variation had been reduced considerably and the comparison was made between the top clones of the species. The selection intensity at this stage was 1 out of 40,000 to 1 out of 80,000 original seedling plants (see Figure 8).

This means that rank changes are quite possibly due to small differences between clones, or low correlations could be an indication for successful selection as well as for differences in the ageing progress of the clones. There are indications for both versions.

More information must be collected before the question of ageing of clones in repeated repropagation cycles can finally be answered.

CONCLUSIONS

Concepts of clonal selection programs in conifers are generally more difficult than in broadleaved species due to the ageing process. They require precautions that guarantee a genetic gain even if ageing is taking place.

The easiest and most secure concept is that of bulk propagation of tested superior material. However, this limits the possible gain and is not a clonal selection and testing program.

The next possibility, which improved bulk propagation material to a certain extent, is a subsequent screening at the end of the nursery phase which guarantees an additional gain anywhere between 5.0 to 25.0% depending on selection intensity. Again, this is possible without a clonal selection and testing program. An intensive clonal selection from good provenance or tested material with subsequent testing of the clones can provide considerable gain as long as the genetic base is not drastically reduced and if ageing does not counterbalance the gain. Due to errors as a result of early selection, genotype x site interactions, and genotype x propagation cycle interactions, the success of selection depends not only on selection intensity, but also on the size of the selected population of clones. It is not necessary to keep the clones separate from the applied part of the production. However, they must be treated separately in the selection and testing part of the program. A selection program should be kept open to introduce new clones if ageing becomes a severe problem. The situation may be quite different from one species to another.

Even if superior clones are detected so late that optimal propagation is impossible, they are not lost from the breeding program. They can be used in the advanced breeding population and rejuvenated by generative means. This possibility has been neglected up to now in many programs.

Production of outstanding hybrid families, and bulk propagation of these families or the best single plants selected out of these is another option. To prevent risks, these may be planted with conventional seedling material (e.g. in a percentage which corresponds to the trees of the final crop).

Finally, since tissue culture is most probably a tool for influencing a clone without disturbing the correlating systems in the plant, it may hopefully be a possibility not only for mass propagation, but also for future rejuvenation with conifers.

There is reason for optimism with clonal programs in conifers, and clonal propagation, selection, and testing are all good methods of improving the genetic gain and flexibility of tree breeding in conifers.

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SESSION II

MODERATOR:
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CURRENT STATUS OF MACROPROPAGATION

R. Marie Rauter

Forest Resources Branch
Ontario Ministry of Natural Resources
Toronto, Ontario, Canada

ABSTRACT

Rooting of cuttings from forest trees, particularly conifers, has reached a level of success in recent years that encourages further development towards fully operational clonal programs. Optimal conditions for rooting involve the physical conditions of both the plant material and the rooting environment. Maturation state, type of cutting, crown position, physiological condition of the cutting, and season of collection all affect the rootability and speed of rooting. Though conditions may vary with species, a general prescription would be to use stem cuttings from the lower crown, collected in early spring from young trees in a good nutritional state. Rooting media, air and soil temperature, humidity, light intensity and chemical treatments are all external factors that may affect the rooting of cuttings. Although the technology to root cuttings has progressed rapidly, there remains a number of biological constraints impeding the rapid implementation of clonal forestry. Control of maturation state and methods of early testing are crucial problems to be solved. Questions of genetic diversity, deployment strategies and growth habit of cuttings must also be further investigated. In light of recent advances in both practice and theory of the above issues, the clonal option is rapidly assuming a larger role in operational forestry.

RÉSUMÉ

Ces dernières années, on est parvenu à induire le développement des racines des boutures d'arbres forestiers, notamment de conifères, au point de croire à la possiblité de programmes de clonage tout à fait opérationnels. Parmi les conditions optimales du développement des racines, il y a les conditions physiques dans lesquelles baignent le matériel biologique et le milieu d'enracinement. La maturation, le type de bouture, son origine dans la cime, l'état physiologique de la bouture et la saison du prélèvement influent tous sur les possibilités et la vitesse d'enracinement. Même si les conditions peuvent varier selon l'espèce, la règle générale serait de prélever les boutures de la tige, au début du printemps, dans la partie inférieure de la cime de jeunes arbres en bon équilibre nutritif. Le milieu d'enracinement, la température de l'air et du sol, l'humidité, l'intensité de l'éclairage et les traitements chimiques sont tous des facteurs externes qui peuvent influer sur le développement des racines. Même si la technique favorisant le développement des racines des boutures a rapidement évolué, il subsiste un certain nombre de contraintes biologiques qui empêchent l'utilisation rapide des clones en foresterie. La maîtrise de la maturation et les méthodes permettant des tests précoces sont capitales. Il fait aussi examiner davantage les questions de diversité génétique, de stratégies de mise en place et de port des boutures. Compte tenu des progrès récents dans la pratique et la théorie des questions susmentionnées, les clones prennent rapidement de plus en plus d'importance en foresterie opérationnelle.

INTRODUCTION

Rooting of cuttings for the majority of tree species (particularly conifers) has been deemed sufficiently successfuly only within the last ten to fifteen years, and is only now being seriously considered in artifical regeneration and breeding programs. Some genera, such as willow (Salix L.), poplar (Populus L.), and cryptomeria (Cryptomeria japonica (L.F.) Don) have been routinely rooted for many years (Chemlar, 1974; Toda, 1974; Zsuffa, 1976). The successes of programs using these genera have provided much of the encouragement and impetus to solve the problems of difficult-to-root species and genera.

The following sections will (i) summarize the optimal conditions for successful rooting, with examples showing the variability in tolerance amongst species, (ii) outline some biological concerns and (iii) suggest areas where developmental work is needed to make rooted cuttings more economically competitive with seedlings.

OPTIMAL CONDITIONS FOR SUCCESSFUL ROOTING

Rooting success is dependent upon optimizing many endogenous and exogenous factors. The endogenous variables include maturation state of the cutting donor, type of cutting, physiological condition of the cutting, preconditioning of the cutting or cutting donor, and the season of collection. The exogenous variables include rooting medium, ambient temperature and humidity, photoperiod and light intensity, and hormone and chemical treatments.

The degree of control required for each of these variables is directly dependent upon whether the individual species is an easy or difficult rooter. Many hard pines require specific preconditioning treatments (Hare, 1978) or well-controlled environmental conditions (Hare, 1978) to obtain any degree of rooting success, while many poplar and willow species root easily, and even unrooted cuttings can be planted directly onto a plantation site (Zsuffa et al., 1977); Densmore and Zasada, 1978).

Endogenous Factors

Maturation State of the Cutting Donor

The less mature (i.e. the more juvenile) the cutting donor, the greater the success in rooting. As the state of maturation increases, percent rooting decreases. For example, juvenile cuttings of black spruce (Picea mariana (Mill.) B.S.P.) taken from twelve-week-old seedlings rooted more than 99.0% (Armson et al., 1980), whereas cuttings from four-year-old seedlings of the same species averaged 62.0% rooting with clonal variation in rooting ability ranging from 0% to 95.0% (Rauter, 1971). As the donor plant matures, not only does rooting decrease, but the length of time to develop roots increases (Isikawa, 1968) and the quality of the root system changes (Libby and Hood, 1976). Similarly, the subsequent rate of height growth decreases (Kleinschmit and Schmidt, 1977; Rauter, unpubl. data) and the effects of topophysis are more pronounced and take longer to overcome (Kleinschmit, 1961).

In Ontario, we experienced quick (in less than 5 weeks) and very efficient (better than 99.0%) rooting of very juvenile cuttings. This changed our approach in starting from more promising, mature trees-ortets and lower rooting percentages to propagating young seedlings from selected full-sib families with fast, reliable rooting. The poorer performing clones can be dropped from this program if the juvenile state can be maintained. The remaining clones can be propagated on a large scale. The quality of the resultant clonal plantations will be substantially improved over those produced from seed orchard seed.

There are several promising methods for retaining juvenility including hedging (Libby and Hood, 1976; Hartney, 1980; van den Driessche, 1983) and serial propagation (Kleinschmit, 1977; Morgan et al., 1980). However, it appears that these methods are successful with only some species. For example, serial propagation rooting percentages over a three year period decreasedless when cuttings were continously taken from black spruce donors, and more when taken from white spruce (P. glauca (Moench) Voss) donors (Phillion, unpubl. data). Also, black spruce exhibited less topophysis than white spruce.

Type of Cutting

Many recent studies confirm the results of earlier work on selecting the best type of cutting. For some species, root cuttings and brachyblast cuttings (made of shoots originating from fascicular buds) are the more successful types. Since most aspen species are difficult to root from stem cuttings, reasonable success can be achieved by taking root cuttings from the parent donor, producing suckers, and then rooting the resultant suckers (Zufa, 1971; Schier and Campbell, 1976). This is similar to the work done by Muhle Larsen in the 1940's (in Nienstaedt et al., 1958). Brachyblast cuttings from nine to twelve-year-old trees of Pinus densiflora Sieb. and Zucc., P. thunbergiana Franco, and P. rigida Mill. root significantly better than stem cuttings taken from the same trees and treated in the same manner (Hong, 1974). Brachyblast cuttings have also been successfully used in Ontario for jack pine (P. banksiana Lamb.) and scots pine (P. sylvestris L.) (Phillion, pers. comm.).

Stem cuttings are the most common form of propagules and usually perform the best. The length of the stem cutting can affect the success of rooting. In jack pine, cuttings less than 2.5 cm in length did not root as well as those 2.5 to 7.5 cm (Zsuffa, 1974). The growth of the roots was directly proportional to the length of the basswood (Tilia americana L.) cuttings (Morsink and Smith, 1974).

The Influence of Crown Position

The crown position from which the cutting is obtained is important and becomes more crucial as the maturation state of the donor increases. Spruce cuttings taken from the lowermost portion of the crown root better than those from the uppermost portion, and laterals root better than terminals (Girouard, 1971). Roulund (1979) found that Norway spruce (Picea abies (L.) Karst.) cuttings from the lowermost portion of the crown exhibited less plagiotropism than those from the top. In redwood (Sequoia sempervirens (D. Don) Endl.), cuttings from the lower crown rooted better than those from the upper crown and the duration and severity of plagiotropism was greater in the lower crown than the upper crown (Libby, pers. comm.). Most of the evidence is consistent with the hypothesis that upper crown cuttings are in a more advanced state of maturation than lower crown cuttings.

Physiological Condition of the Cutting

Several factors determine the physiological state of the cuttings; unfortunately, only a few of these are understood. Although it is known that sufficient carbohydrate reserves in combination with a high C/N ratio favour rooting (Haissig, 1974b), the relationship between carbohydrate metabolism, root primordium development, and enzyme activity is not understood. Haissig (1982) attempted to identify enzymes and enzyme systems, and found that these were positively related to primordium development and to the metabolism of reducing sugars. He assumed that these enzyme activities had a physiological impact and influenced the speed and amount of primordium development in the cuttings.

Cuttings must have sufficient nutrient reserves to survive the rooting period until proper growth occurs (Land Jr., 1977). Without sufficient reserves, cuttings are unable to photosynthesize properly and die soon after insertion (John, 1979). According to Davis and Potter (in Haissig, 1982), photosynthesis may contribute sugar that is translocated to the base of the cutting. This increase in sugar availability at the base of the cutting also enchances root primordium development.

In some difficult-to-root species, the presence of leaves or needles for photosynthesis appears essential for rooting. In sycamore (Platanus occidentalis L.), dormant cuttings leafed out after being struck, probably because of stored food reserves. The cuttings then dropped their leaves within a short period of time and died (Land Jr., 1977; Foster and Thor, 1977). In conifers, active shoot growth and foliage extension often occur simultaneously with rooting. Thus, there is competition for the available carbohydrates. Cameron and Rook (1974) showed that 75.0% of the photosynthates supplied to the roots of the cutting came from the old foliage. In Eucalyptus camaldulensis Dehn., the addition of auxins, sucrose, and nitrogenous compounds was not a sufficient substitute for the presence of leaves (Hartney, 1980).

Even though nutrient levels may be favourable, other physiological factors, such as the presence of root inhibitors, may depress rooting (Haissig, 1974b). Endogenous levels of root inhibitors increase with the maturation state of the donor (Davidson, 1974; Hartney, 1980). This increase in the concentration of the root inhibitor is directly correlated with a decrease in the ability of the cuttings to root (Paton et al., 1981).

Preconditioning of the Cutting or Donor Plant

Preconditioning can increase the rooting performance of the cutting by altering its physiological state at the time of striking. The storage of poplar cuttings in a refrigerator (2°C) induced callus formation and reduced the time between striking and rooting (Zsuffa, pers. comm.). Normally, cuttings from mature Sitka spruce (P. sitchensis (Bong.) Carr.) do not root; however, when they were placed in cool storage for up to three months, rooting success of 15.0 to 20.0% was achieved (McMullen, pers. comm.). Van den Driessche (1983) found that dormant Sitka spruce required ten weeks chilling at 2°C and Fraser fir (Abies fraseri (Porsh) Poir.) required eight weeks at 4°C to obtain the most rapid and complete rooting. In hybrid larch (Larix eurolepis (Henry)), cool storage (2°C) of dormant cuttings for six to nine weeks dramatically increased rooting, whereas warm storage (25°C) of more than three weeks resulted in good callus development, but significantly reduced subsequent rooting (John, 1979).

Ring-barking (girdling the stem prior to taking cuttings) contributes to rooting success. This technique is successful with some difficult-to-root species and with donors that have a more advanced state of maturation. In slash pine (P. elliottii Engelm.), up to 34.0% of the cuttings from girdled branches rooted, whereas a maximum of 3.0% rooted from ungirdled branches (Hare, 1978). Similarly, in sycamore, 100.0% of the treated branches rooted, but only 34.0% of the untreated ones rooted (Hare, 1976). This technique has made operational rooting of mature radiata pine feasible for seed orchard establishment.

It is important to minimize moisture stress at the time of striking cuttings. Most water is taken by cuttings through the cut base of the shoot and through the foliage in contact with moist soil. In Chamaecyparis obtusa (Sieb. a Zucc.) Endl. cuttings, the rate of water absorption decreased with time from when the cut was made (Toyuoka, 1977). A fresh cut at the base of the cutting at the time of striking resulted in a temporary increase in water absorption and in significantly higher rooting percentages. Substantially lower moisture deficits in the cuttings can be maintained if the foliage is sprayed with water (Cameron and Rook, 1974).

Removal of water soluble root inhibitors may be possible by soaking cuttings prior to striking. Good (in Bonga, 1977) showed that an inhibitor resembling abscisic acid could be removed from the foliage of Sitka spruce and European white birch (*Betula pendula* Roth.) by this method.

Season of Collection

The optimal collection times vary by species and conditions, but generally are best in early spring, just prior to the start of the growing season, and in late summer when growth extension is complete and lignification is just starting (Hare, 1978; John, 1979, Roulund, 1981). Under controlled growing conditions, however, collection periods can be extended. For example, when black spruce is grown under greenhouse conditions, cuttings can be taken throughout the year and successfully rooted, provided that supplemental lighting is available during the short winter days.

Exogenous Factors

Rooting Medium

The following table provides some examples of different media which can be successfully used.

Table 1. Examples of different media which can be successfully used

Rooting Medium	Species	Reference
Coarse gravel	Norway spruce	Kleinschmit *et al.* (1973)
Vermiculite and perlite	slash pine	Hare (1978)
Peat and sand	hybrid larch	John (1979)
Sphagnum peat and vermiculite	black spruce	Armson *et al.* (1980)
Sphagnum moss and coarse sand	several hardwoods	Tervonnen (1981)

The type of medium used influences the type of root system that develops. In Douglas fir (*Pseudotuga menziesii* (Mirb.) Franco), differences in root thickness, flexibility, and branching were observed among cuttings grown in different rooting media (Copes, 1977). When higher proportions of sphagnum peat were used, the root systems were finer and more branched than when smaller proportions were used. As the proportion of sand increased, the root systems that developed were longer, relatively unbranched, and less flexible. Similar results occurred in radiata pine (*P. radiata* D. Don) (Hill and Libby, 1969) and in white and black spruce (Rauter, unpubl. data). Spruce cuttings grown in 100.0% sand not only had long, unbranched roots, but the roots also had a tendency to break at the base of the cutting when disturbed. If these cuttings were left through the winter dormant season and lifted in the spring prior to the onset of growth, their roots became flexible and easily manipulated (Rauter, unpubl. data).

The success of rooting and growth of roots is dependent upon the proper balance of aeration and moisture within the medium. Copes (1977) found that a medium with a high content of sphagnum peat was easily saturated, whereas one with pure perlite or perlite-sand required frequent watering and media with either too little or too much water produced poor rooting results. Rauter (unpubl. data) found that when the soil was saturated, sufficient aeration was not provided and the roots grew upwards through the rooting medium and into the air.

Ambient Temperature and Humidity

The few studies done on the success of rooting at varying temperatures have shown that cuttings can generally tolerate wide fluctuations. Rauter (1971) noted that even though spruce cuttings were exposed to temperatures ranging from 10°C to 32°C, there was no apparent detrimental effects on rooting. Werner (in Roulund, 1981) observed no damage to spruce cuttings exposed for short time periods to temperatures of 35°C to 40°C.

Ambient air temperatures can influence the length of time from striking to rooting. Ideal temperatures appear to be in the range of 20°C to 22°C for many species. As the temperature decreases, the length of the time required to root cuttings increases. Juvenile cuttings of black spruce that would normally have rooted within five to seven weeks remained green yet unrooted for four months due to low temperatures (Miller, pers. comm.).

Ideally, cuttings root well in cool moist air surrounding the tops and warm, solid conditions around the base. This temperature gradient allows greater activity in the base, while minimizing respiration and moisture stress on the top of the cutting. However, the benefit of heating cables is greatly reduced once the ambient temperature is 22°C or higher.

High humidity in the air surrounding the cuttings can reduce the stress caused by transpiration and thus promote rooting. However, high humidity when combined with high temperatures can promote the development of diseases such as *Botrytis*. It is also important to be able to maintain high humidity without saturating the rooting medium, particularly if the medium contains a high proportion of sphagnum peat. High humidity can be maintained by covering the cuttings with plastic sheets and shade cloth for greenhouse conditions (Armson *et al.*, 1980) or aluminum-painted hoop-houses for nursery conditions.

Several types of misting equipment and control devices have been developed over the years to maintain humidity and humidity control. Many have been tried in the Ontario program. The most reliable to date is one that can be set on a timer and adjusted for varying weather conditions.

Photoperiod and Light Intensity

During the spring and summer months, natural light intensity is sufficient for rooting. If cuttings are rooted during the short days of winter, it may be necessary to supplement the natural light. Supplemental lighting can also be used to induce bud formation and subsequent branch development in order to increase the number of available cuttings on donor plants. Phillion (pers. comm.) uses high-pressure sodium-vapour lamps to intensify the level of brightness to 500, 1000 and 2000 foot candles. The higher the light intensity, the greater the number of cuttings that are produced on juvenile scots pine donors.

Hormone and Chemical Application

Results of hormone and chemical application for improved rooting are contradictory. This can be partially attributed to the different experimental conditions, the different physiological states of the cuttings, and to our lack of understanding of some of the chemical and physiological processes occurring within the plant.

Although many recent studies have shown the beneficial effects of auxin application (Bhatnagar and Joshi, 1972; Hare, 1974; Morsink and Smith, 1974; Tervonnen, 1981), there has been little attempt to explain the nature of its effect. The enhancement of rooting may not be a result of the actual chemical applied, but perhaps a transformation of that chemical when absorbed by the cutting (Haissig, 1974a) or a physiological response of the cutting to the chemical (Haissig, 1982).

Both natural and synthetic auxins when applied to cuttings, usually increase the development of already existing root primordia (Haissig, 1974a). Thus, cuttings with initiated primordia would show better rooting percentages than those without preformed primordia. As an exception, recent work in jack pine has shown that a new synthetic chemical, an aryl ester of indole-3-butyric acid, has the ability to initiate root primordia (Haissig, 1979).

Chemicals have also been used to try to induce bud formation on donor plants. For example, a benzo-adenine purine spray applied to white spruce donors increased bud development significantly, but unfortunately, also created some toxic effects on the plant (Phillion, pers. comm.). Modification of concentrations and carriers used may reduce the toxic effect yet still be effective in promoting bud development.

Several of the above factors are closely interrelated. The alteration of one factor often necessitates the adjustment of others to retain rooting success. The large number of factors that influence rooting success also make it difficult to evaluate the effect of any one factor. Although modifications to improve rooting techniques are likely for years to come, standards have been established and these are being successfully used to reproduce selected clones (Kleinschmit et al., 1973; Rauter, 1979).

BIOLOGICAL CONCERNS

Important problem areas are juvenility and maturation, early testing, genetic diversity, deployment strategies and clonal comparability. The following will highlight the current status of these problems as related to macropropagation.

Undue concern over biological problems has deterred the rapid development of clonal forestry, particularly with coniferous genera. To reiterate a statement of Libby (1984), 'we still need to know the answers to a lot of biological questions to do clonal forestry well, but they are no longer the sort of disqualifying questions that block further consideration.'. Many of these biological problems have been used by the traditionalists to oppose clonal forestry, rather than as a challenge to find solutions to the problems. Many satisfactory solutions to the various biological problems do exist, although they may not yet be the optimal solutions.

Juvenility and Maturation

One reason why vegetative propagation by rooting is not more widely used is our inability to sufficiently manipulate both ontogenetical and physiological ageing. We must be able to rejuvenate material that has an advanced state of maturation, otherwise selections for rooting programs must be made on young trees and we must accept the risks associated with early selection. These risks result from changes in growth rhythm and morphological characteristics as a tree shifts from the juvenile to the mature phase. This is of particular concern with species characterized by a large amount of free growth and only a moderate amount of predetermined growth, because selections may show a height advantage in the early years, but may not retain this advantage in later years (Eriksson, 1980). On the other hand, early selection can be effective for such traits as stem form and branch angle as there is little change in the expression of these over time.

Several ways have been developed to at least partially retard maturation and occasionally induce rejuvenation. Maturation can be retarded either by hedging the parent donor and not allowing upward growth (Libby and Hood, 1976; Hartney, 1980), by serial propagation of rooted cuttings (Kleinschmit, 1977; Morgan et al., 1980), or by using basal epicormic shoots which develop in many hardwood species (Hartney, 1980) and sprouting conifers (Libby, pers. comm.). In one instance, Dr. Ball of California produced some redwood propagules from tissue culture material derived from mature trees, and these propagules appear to be more juvenile than seedlings. Rejuvenation also seems to occur when scions from older trees are grafted and regrafted several times onto younger root stock (Franclet, 1981). All of these methods should improve the physiological state of the plant by shortening the internal transport system and improving the supply of water and nutrients to the periphery of the hedge, cutting or graft (Fortanier and Jonkers, 1976). It appears that juvenility in plant tissue is dependent upon the distance of the tissue from the root system. As distances from the peripheries of the plant are shortened, there is also a reduction in the amount of root inhibitor substance present (Paton et al., 1981).

Fortanier and Jonkers (1976) found that hedging induces the formation of buds and tissues less mature than those being removed, thus inducing semi-ontogenetical rejuvenation. They emphasize that theoretically such rejuvenation cannot be repeated indefinitely because each pruning activates the meristems and thus stimulates ontogenetical ageing.

Increased maturation of the donor plant will continue to be a problem until more information is available on the physiological and biochemical processes of ageing. In the rooting of cuttings, not only is rooting percentage affected by the maturation state of the donor, but the speed and quality of rooting, subsequent growth rate, form and wood properties (Hood and Libby, 1978) and within-clone variation (Kleinschmit, 1977) are also affected.

Early Testing

Associated with the problem of maturation is that of early testing. Since much of the material can only be propagated in the juvenile state, test material must be evaluated early enough so that the donors can still be propagated with a degree of success and good future development. However, the earlier the evaluations are made, the greater the risk that these selections will not maintain their superiority at the end of their rotation. Kleinschmit (1977) states that this change in performance can be partially explained by differential gene activity over time. To minimize the risk of early evaluations and early selections, he suggests using adapted populations, maintaining a high genetic variation combined with repeated selection, selecting under typical field conditions and using close spacing in plantation establishment. The risk can also be reduced by testing full-sib families whose parents were superior performers or whose sibs already exist in older tests. Seed from the best families can be germinated and the resulting material used for clonal tests and mass propagation. If reliable relationships can be developed between growth potential and physiological and biochemical processes, such as photosynthetic efficiency, then the risks encountered through early selection may be overcome.

Genetic Diversity

Sufficient variation within any production program must be maintained to accomodate the wide variety of environmental conditions encountered by all commercially important species, even within a single seed zone or planting region. Thus, it is necesary to have a large number of clones in the clone bank, even though only a relatively small number may be in use in the production program at any given time. As some are eliminated from the production program, others must be added. Eventually, these new additions will come from advanced generation breeding selections. There must also be close control on the number of propagules per clone that are produced for any production program. It is human nature to select only a few of the very best clones for production, however, this can lead to severe field losses. Vegetative propagation, as a production tool, could come into disfavour (Toda, 1974). Sweden and other countries have, or are contemplating, the establishment of rules controlling the number of clones and ramets per clone to ensure genetic diversity (Hedstrom and Krutzsch, 1983).

Deployment Strategies

When discussing deployment strategies, it is important to consider the size of the area under discussion. Large monoclonal plantations can be disasterous. In Japan, a single clone of cryptomeria, when planted on a small scale, did not show susceptibility to stem canker and foliage mites, but when planted over extensive areas, severe losses were incurred due to these pests (Toda, 1974). Monoclonal plantations may be lessserious with short-rotation species such as poplar. With short rotations, the pests do not have the same opportunity for a population build-up and there is less likelihood of abnormal weather conditions occurring. Even if serious losses do occur and the stand has to be removed, not as much time and money is lost when compared to a long-rotation species.

Small monoclonal plantations can be beneficial. Libby (1981) and Zobel (1982) recommended a mosaic of monoclonal stands rather than an intimate mixture of clones for a management system. When a small area (a few hectares or less) of single clones are planted, the variation within a plot is less than when a mixture of clones are planted. If individual clones fail, the problem can be readily recognized in the mosaic planting and these clones removed and replaced. This management method is more expensive because the identity of the clones must be maintained from collection through planting, yet greater gains can be made through better site utilization. If a program is sufficiently well-developed, then specific clones can be placed on specific sites (Zsüffa, pers. comm.). If there is a significant microsite variation, then a clonal mix similar to that proposed by Kleinschmit (1977) may be more realistic.

The number of clones to be used in a multiclonal mix is still under discussion. Initially, Japan favoured a large number of clones, expecting a larger gain than from seed orchard seed of the same parentage (Toda, 1974). However, when the performance of the clonal plantations was compared to these seedling plantations, the anticipated gain could not be confirmed due to the many silvicultural thinnings through the rotation. Libby (1981) recommends seven to thirty clones as a safe number in any single production plantation. The National Forestry Board in Sweden stipulated that a minimum of thirty to one hundred and twenty clones must be used depending upon the degree of testing for these clones (Twetman, pers. comm.). The number that should be used is affected by such variables as acceptable risk level, intensity of plantation management, spacing, plantation size and rotation age.

Since the production and establishment of many identified clones are more expensive than the production of a multiclonal mix, the extra costs have to be measured against the potential gains, potential risks and the most efficient use of the planting site.

Comparability of Clones

Before embarking on a large-scale clonal forestry program, there must be some assurance that material will maintain its superiority from the time of establishment until the time of harvest. For cuttings, Libby (1981) referred to this as the "comparability assumption". Shelbourne and Thulin (1974) found that the initial growth rate of radiata pine cuttings was slower than that of seedlings. Sweet and Wells (1974) had similar results in a nursery study for the same species. In the first experiment, cuttings were taken from six-year-old trees and in the second experiment, from variable age classes. Perhaps some of these slower growth rates may be attributed to the donors being more physiologically mature than the young seedlings. If cuttings had been taken from younger material or hedged material, the results might also have been different. Hood and Libby (1978) found that, initially, the more juvenile cuttings of radiata pine had faster relative height growth rates, but that the more mature cuttings had greater height growth rates than either the juvenile cuttings or seedlings of the same genotype. Roulund (1978) found that cuttings of Sitka spruce grew taller, showed less stem form variation, and had less variation in date of bud flush than seedlings. Although the initial stem form of the cuttings was poorer, within four years, the form was similar to that of seedlings.

WORK REQUIRED

Techniques are now available for rooting cuttings of most species. However, as with any new program being initiated, refinements must be made for the given set of conditions and species in use. Additional research in rooting procedures should have low priority compared to research in several other areas.

All of the biological problems mentioned in the preceding section require continuing research. The most pressing is the problem of controlling maturation. If this problem can be totally solved, mature selections can be effectively incorporated into a clonal program. This, in turn, will affect other areas, such as the need for early testing and thedevelopment of breeding strategies. In addition, many of the physiological and biochemical processes need to be better understood.

A neglected area of research is mechanization. Rooting is a labour intensive process. If several of the steps, such as the collection and setting of cuttings, could be mechanized, large-scale program costs would be reduced substantially. However, mechanization is only economical when the scale of operation is sufficiently large. Thus, we must first be committed to a clonal forestry program before we can justify the required expenditures on mechanization.

We also need to closely examine the breeding and deployment strategies being proposed. We must then set up a series of appropriate guidelines for various kinds of clonal forestry programs, thus avoiding problems in the future of our forests and opposition to clonal forestry generated by such problems from public interest groups.

CONCLUSION

Acceptance of new concepts is often difficult, particularly for those of us who have been trained in the traditional methods of practising tree improvement and forest genetics. However, the time has come to re-examine and re-evaluate many of the current programs, determine what they are capable of providing and then look at some different alternatives to see whether the results can be improved. One alternative that must be considered is the clonal option. I believe that for many species and in many programs across Canada, we will find that macropropagation and the clonal option will become a forestry reality.

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IN VITRO PROPAGATION OF CONIFERS

J.M. Bonga

Maritimes Forest Research Centre
Canadian Forestry Service
Fredericton, New Brunswick, Canada

ABSTRACT

Conifers are relatively easy to propagate by in vitro techniques from juvenile material. However, attempts to propagate mature conifers by in vitro methods have generally failed. We have obtained adventitious shoots, but no roots, in cultures of explants from mature Larix decidua and Pinus banksiana. In the Larix cultures, the shoots formed in slices of female cones collected during meiosis and in shoot tips collected during needle primordia initiation. In Pinus, the adventitious shoots were obtained from cultures of embryonic shoots excised from male buds at the time of microstrobili initiation. Some of the problems encountered in conifer tissue culture are discussed.

RÉSUMÉ

Il est relativement facile de propager les conifères par des techniques in vitro, à partir de matériel juvénile. Toutefois, les tentatives de propagation à partir de conifères d'âge mûr par ces méthodes ont généralement échoué. On a réussi à obtenir des pousses adventives, mais pas de racines, dans des cultures de boutures de Larix decidua et de Pinus banksiana d'âge mûr. Les pousses obtenues dans les cultures de Larix provenaient de tranches de cônes femelles prélevées lors de la méiose et aussi de bouts de pousses prélevées au début de la formation des rudiments (primordia) des aiguilles. Dans les cultures de Pinus, les pousses provenaient de pousses sexuelles males prélevées durant la formation des strobiles. L'auteur traite de certains problèmes propres à la culture des tissues de conifères.

INTRODUCTION

In vitro cloning techniques have reached a level of sophistication where large-scale propagation is now possible for several hardwood species (for forest trees, see Brown and Sommer, 1982; Mascarenhas et al., 1982; for fruit trees, see Hutchinson, 1982; Mascarenhas et al., 1982). Although some hardwood species (Spiegel-Roy et al., 1980; Mascarenhas et al., 1982) can be propagated from mature specimens, most can still only be propagated from juvenile material. Similarly, conifers, except Sequoia (Ball et al., 1978), can presently be propagated from juvenile material only (David, 1982). Although *in vitro* propagation of juvenile material can be useful (i.e. for rapid clonal production of plantlets from valuable embryos obtained in controlled crosses (Mott, 1981; Smith *et al.*, 1981)), the main requirement for forest improvement is propagation of selected mature specimens (Zobel, 1981).

IN VITRO PROPAGATION OF MATURE CONIFERS

The difficulties in propagating explants from mature trees arise as a result of the lack of organogenesis *in vitro*. Two reasons can be suggested for this: (1) there are no organogenetically competent cells in the explants; and (2) there are a few competent cells but these are prevented from entering organogenesis by correlative inhibitions imposed by neighbouring tissues (Henshaw *et al.*, 1982). These conditions may not be static. There are moments in the annual growth cycle of the tree when tissues programed to follow one type of development are switched into a different development plan. Examples of this are initiation of primordia, the change from sporophyte to gametophyte, and stomatal differentiation. It is possible that during such developmental changes, some cells temporarily become more organogenetically competent, or if already competent, are temporarily released from correlative inhibitions (Bonga, 1982b; Riding and Aitken, 1982). This possibility of a temporary improvement in organogenetic capacity at specific moments at specific sites in the tree has provided the impetus for much of our work over the last few years. So far, we have found three developmental stages in the annual growth cycle during which, at least in some trees, explants capable of adventitious shoot formation can be obtained. These three stages are female cones during meiosis, male shoots during microstrobili initiation, and vegetative shoots during needle initiation.

Female Cones During Meiosis

A few small adventitious buds were obtained from somatic tissues of immature female cones of *Pinus mugo* (Bonga, 1981). More success was achieved with the somatic tissues of female cones of one twenty-five-year-old *Larix decidua* tree. In the spring of 1981, female cones were collected from this tree, cut in transverse slices, and cultured. Slices of cones collected at about the time of meiosis produced adventitious shoots; slices of cones collected earlier or later did not (Bonga, 1982a).

This experiment was repeated the next year and again only the collections that contained cells in meiosis (all stages between megaspore mother cell and tetrad) were responsive. However, the origin of the adventitious shoots was different in the second experiment. These shoots, instead of arising from slices of the stalk of the cone, developed from slices containing the bracts and scales. They arose mainly from the cut surfaces of the young bracts (Fig. 1) but occasionally from the side of the bract. These shoots were separated from the bracts and subcultured. In subculture, the shoots produced callus at their base. This callus formed new adventitius shoots (Fig. 2), which could be separated and subcultured individually after a few weeks. In one of the adventitious

FIGURES

Figures 1-4	*In vitro* culture of slices of immature female cones of *Larix decidua*.
Figure 1	Adventitious shoots (s) are forming on the cut surface of a swollen cone bract (b). x 15.
Figure 2	Adventitious shoots are developing on callus formed by the adventitious shoots shown in Figure 1. x 6.
Figure 3	A needle of an adventitious shoot on a bract has swollen and is forming numerous bumps (b). x 15.
Figure 4	The bumps shown in Figure 3 have turned into numerous shoots. x 6.
Figures 5-6	*In vitro* culture of male shoots of *Pinus banksiana* collected when the strobili were primordial.
Figure 5	The shoot has produced a callus which is forming a few adventitious shoots (arrow). x 15.
Figure 6	Elongated needles of one of the adventitious shoots shown in Figure 5. x 15.
Figures 7-8	*In vitro* culture of vegetative shoots of *Larix decidua* collected at the time of needle primordia initiation.
Figure 7	The shoot has produced callus which is producing numerous adventitious shoots. x 8.
Figure 8	A shoot removed from the mass of shoots shown in Figure 7 and subcultured. One needle in this shoot reached a length of 43 mm. Scale on the right hand side is in millimetres.