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# Clonal Forestry: Who are you Kidding?

**Abstracts from a conference of the Nordic Group for the Management of Genetic Resources of Trees in Barony Castle, Scotland, 4th-7th September, 2002**

# Klónaskógrækt: ertu að grínast?

**Samantekt erinda frá ráðstefnu Norræna Skógerfðafræðihópsins í Barony Kastala, Skotlandi, 4.-7. september 2002**

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# 1 INNGANGUR

*Þröstur Eysteinsson (ed.). 2003. Klónaskógrækt: ertu að grínast? Samantekt erinda frá ráðstefnu Norræna Skógerfðafræðihópsins í Barony Kastala, Skotlandi, 4.-7. september 2002. Rit Mógilsár Rannsóknastöðvar Skógræktar nr. 17/2003. 52 s.*

Norræni Skógerfðafræðihópurinn er hópur fræðimanna á Norðurlöndunum öllum ásamt Skotlandi, Eystrasaltslöndum, Írlandi og Komi héraði í Rússlandi, sem eiga það sameiginlegt að stunda rannsóknir á erfðafræði og kynbótum trjáa. Hópurinn, sem er einn af svokölluðum fastahópum SNS, stendur fyrir árlegri ráðstefnu sem haldin er til skiptis í aðildarlöndunum og hefur hver ráðstefna þema sem gestgjafarnir ákveða hverju sinni. Í þetta skipti var röðin komin að Skotlandi og völdu þeir að fjalla um notkun klónaðs efniviðar í skógrækt.

Fjölgun úrvalstrjáa með klónun, þannig að útkoman verði tré með því sem næst sömu eiginleika og móðurtréð, hefur lengi verið talinn áhugaverður kostur til að ná fram markmiðum kynbóta í skógrækt hraðar og með öruggari hætti en hægt er með hefðbundnum kynbótum og frærækt. Fljótlega kom í ljós að mörg ljón voru í veginum, sérstaklega varðandi barrtré, og ýmis tæknileg, fjárhagsleg og jafn vel siðfræðileg vandamál sem við var að glíma. Með fáum undantekningum, s.s. víðir og ösp, er skógrækt með klónuðum efniviði enn ekki stunduð svo nokkru nemi á norðurslóðum. Markmið ráðstefnunnar var að meta stöðu mála varðandi rannsóknir, tækniþróun og notkunarmöguleika á klónuðum efniviði í skógrækt á Norðurlöndunum og nágrenni.

Í stuttu máli var niðurstaðan sú að talsverðar framfarir hafi átt sér stað á s.l. 10 árum, sérstaklega á sviði vefjaræktar og langtíma varðveislu efnis við mjög lágt hitastig, en að enn væri ýmislegt eftir, s.s. að auka framleiðni ná niður verði í plöntuframleiðslu. Það var niðurstaða flestra að ótímabært væri að gefast upp á klónun sem tækni til að ná fram markmiðum trjákynbóta. Í þessu hefti er að finna samantektir af erindum sem flutt voru á ráðstefnunni.

Ráðstefnan var haldin á vegum rannsóknastöðvarinnar í Roslin í Skotlandi, sem heyrir undir rannsóknasvið bresku skógstjórnarinnar. Gestgjafar voru þau Sam Samuel, Steve Lee, Allan John og Esther Ker og eru þeim færðar bestu þakkir fyrir árangursríkan fund.

SNS, Samstarfsnefnd um Norrænar Skógræktarrannsóknir, styrkti för Norðurlandabúa á ráðstefnuna og fær hún kærar þakkir fyrir.

*Lykilorð: Klónaskógrækt, trjákynbætur, skógerfðafræði*

## 2 INTRODUCTION

*Eysteinsson, T. (ed.). 2003. Clonal Forestry: Who are you Kidding? Abstracts from a conference of the Nordic Group for the Management of Genetic Resources of Trees in Barony Castle, Scotland, 4$^{th}$-7$^{th}$ September, 2002. IFRS report, 17/2003. 52 pp.*

The Nordic Group for the management of Genetic Resources of Trees is a network of researchers in the Nordic countries, Scotland, the Baltic States, and recently Ireland and the Komi Republic, Russia. The group, which is one of the continuous networks under SNS, the Nordic Forest Research Co-operation Committee, holds annual conferences that alternate between the participating countries. Each conference has a specific theme decided by the host country. It was Scotland's turn to host the conference this time and the theme was clonal forestry.

Clonal propagation of select individuals, in order to get offspring with the same characteristics as the parent tree, has long been of interest as a method to achieve tree improvement goals faster and with more predictable results than with traditional breeding and seed production. However, the promise of clonal forestry has not been achieved for a variety of technical, financial and even ethical reasons. With few exceptions, such as poplars, clonal forestry has not caught on as a viable alternative in Northern Europe. The aim of the conference was to review the status of clonal forestry and related research in the Nordic and neighbouring countries.

This volume contains abstracts of the papers presented at the conference.

The conference was hosted by the *Northern Forest Research Station*, Roslin, Scotland, operated by *Forest Research*, an agency of the British *Forestry Commission.* Thanks to our hosts, Sam Samuel, Steve Lee, Allan John and Esther Ker, for a productive and enjoyable conference.

The support of SNS, the Nordic Forest Research Co-operation Committee, in providing travel funding to participants from the Nordic countries is gratefully acknowledged.

*Key words: clonal forestry, tree improvement, forest genetics.*

# EFNISYFIRLIT / CONTENTS

# 3. OVERVIEW: THE STATUS OF CLONAL FORESTRY IN THE NORDIC COUNTRIES – AN ANALYSIS OF QUESTIONNAIRE RETURNS

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This presentation was a summary of the information gathered from a questionnaire distributed to each member country. Returns were received from Denmark, Estonia, Finland, Iceland, Lithuania, Norway, Sweden and UK.

For the purposes of the questionnaire, clonal forestry was defined as the commercial development of tested clones. This definition was sometimes loosely interpreted by some countries, the results however, do reflect the level of interest in clonal forestry across the Nordic countries.

Only three countries - Finland, Iceland and Sweden - are currently practising clonal forestry and this is restricted mainly to hardwood species such as aspen, hybrid aspen, poplar and birch. No countries are practising large-scale clonal forestry with conifer species. Sweden has a small (1k hectare) area of tested Norway spruce clones and around 10k hectares of untested clones. Finland also has a small (0.25k hectare) area of untested clones. Most countries are producing clones for research (i. e. non–commercial reasons) although in some cases these never leave the laboratory, but most countries also have some clonal tests established as field experiments.

Five countries – Iceland, UK, Denmark, Sweden and Finland – listed clonal forestry as a tree-breeding objective. The species involved varied from Poplar (Iceland) through Norway spruce (Sweden and Finland) and Sitka spruce (UK) to Christmas trees and Oak (Denmark). Reasons for pursuing clonal forestry as an objective included the perceived extra gain relative to the sexual route, packaging of rare traits to meet specific high-value demands, and uniformity and quality. Those countries not interested in clonal forestry as an objective list high development costs and environmental concerns as the main reasons.

The reason clonal forestry is not wide-spread across the Nordic countries is mainly due to technological barriers – retaining or inducing juvenility following testing, and methods to make large numbers of copies of the desires clones. Most interested countries are involved in research to address these problems and see the future along the lines of Somatic Embryogenesis (SE) and Cryopreservation. Little hope is placed in re-juvenation. Other main problems are acceptance by the public, certification (varies between countries) and national restrictions.

The next 5-years are seen as ones of technological development – finalising protocols for SE and Cryopreservation. It is unlikely that there will be large-scale planting of clonal material. Ten-years from now however, those countries interested in pursuing clonal forestry think they will be operational with their main conifer species. The next step for breeders could be one of educating the public, policy makers and budget holders.

## 4. KEYNOTE PAPER: THE TECHNOLOGY OF CLONAL FORESTRY OF CONIFERS

***Allan John***
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Clonal forestry is the planting of vegetatively propagated ramets, whose clonal identity is maintained, from known and tested ortets for the commercial production of forest trees. It is rare in conifer forestry. More common is vegetative multiplication, which is the vegetative propagation of mixtures of genotypes of known and tested genetic crosses where the identity of individual clones is not maintained.

Clonal forestry is rare because phase change from the juvenile to the mature phase precludes it, i.e. in the juvenile phase, vegetative propagation is relatively easy but the ortet is untested and, in the mature phase, the ortet is tested but vegetative propagation is difficult. Ways must be found to have the ramets of the ortet tested but also in the juvenile phase. This might be possible by rejuvenation of the tested ortet to the juvenile phase which seems unlikely at the moment or by maintenance of the ortet in the juvenile phase by, for example cryopreservation whilst the ortet is tested. The second is more likely of success.
A number of techniques are available for the vegetative propagation of conifers i.e. grafting, cuttings and tissue culture. Grafting is a very ex-

pensive process and would not be used in clonal forestry except under exceptional circumstances. Cuttings are used in vegetative multiplication systems. Their use in clonal forestry would require a constant supply of juvenile but tested ortets which is not feasible at the moment. Tissue culture, more specifically somatic embryogenesis combined with cryopreservation is probably the approach that will be taken in the future. Embryogenic tissues will be derived from immature and mature embryos of desired specific crosses. Part of the embryogenic tissue will be put into cryogenic storage and part will be used to generate plants that will be put through standard field progeny tests. Ortets shown to have all the desired qualities will then be generated from the stored tissues and clonal forests will be established.

Somatic embryogenesis will produce plants that are more expensive than those raised from seed. Some costs can be reduced by a degree of automation in media preparation but it is difficult to see how culture handling might be mechanised. The use of bioreactors would reduce costs significantly but embryogenic tissue, although it will grow in a bioreactor, will not mature to form somatic embryos in liquid culture. The new technique of temporary immersion is promising and is a compromise between a semi-solid agar system and a liquid bioreactor system.

The technique that will have the most dramatic effect on the selection and breeding of conifers and, through them, clonal forestry is marker aided selection. These are genetic markers that can be associated with particular characters or traits of the tree i.e. they are always present or absent when a particular character is expressed. It will take a huge research effort to find and test these markers and to prove their reliability and it is quite some time before this will be done. If their reliability can be proved, they will remove the necessity for any type of field testing of any genetic cross. Embryogenic tissue would be screened for a range of characters and, if the genotype was desirable, plants produced from the embryogenic tissue and used for the establishment of clonal forests.

# 5. SOMATIC EMBRYIGENESIS IN SITKA SPRUCE FROM A COMMERCIAL FORESTRY COMPANY PERSPECTIVE

***Fiona Harrington***
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**Introduction**
Somatic embryogenesis of conifers has been the subject of intensive research by a number of organisations over the last 15 years. The potential of somatic embryogenesis, to accelerate the production of genetically improved material, has been demonstrated for a wide range of species. In spite of the progress made however, the process remains largely at the laboratory scale.

Coillte Teoranta, The Irish State Forestry Company, manages a productive forest of 345,000ha, 64% of which is Sitka spruce. The company produces approx. 30 million Sitka plants/yr. in its nurseries and is a major supplier to private companies. Currently, 1 million genetically improved rooted cuttings are produced annually. Strategic company policy plans for the production of 6 million improved rooted cuttings by 2007.

In order to facilitate the scale up of improved material, 40,000 improved stock plants will be required annually. These plants will be produced via the vegetative propagation method somatic embryogenesis.

In order to progress this technology to a commercial scale the following questions must be addressed; Are somatic emblings comparable with seedlings and rooted cuttings during nursery development and field performance on a reforestation site? Coillte has put in place a long-term research programme to establish the fidelity of Sitka spruce somatic emblings, from embling acclimatisation to field maturity. Preliminary results from field trials are presented. Is a somatic embryogenesis production system capable of delivering emblings, to operational field planting, at an acceptable cost? Details of our present protocol and current yields are given. This information is vital when planning the production of 40,000 emblings.

**Material and Methods**
Immature embryos are initiated on a modified MS medium supplemented with 2,4-D 1.0mg/l, with sucrose 1%. Maintenance medium is similar except the sucrose concentration is 3%. Maturation occurs on a modified

MS medium with ABA 50mg/l and activated charcoal 1.25g/l. Matured embryos are germinated on hormone free medium. Plants are acclimatised under high relative humidity in the glass house. All plants used in field trials were produced using this protocol.

Two year old somatic embling plants were established on a reforestation site in Spring 2000. Somatic emblings and seedling controls from three genetically improved families were represented. Seedlings from families 251, 191, 574 and emblings from cell lines 251J, 251H, 191A and 574A were used. After 3 growing seasons, the plants were assessed in the field and the following growth measurements were recorded; total height, current season growth and diameter.

**Results**

Initiation of embryogenic cultures using immature embryos is approximately 30%. Not all embryogenic cultures are capable of proliferating and as a result up to 50% losses may occur. The use of different growth regulators and concentrations has failed to improve on these results. Four to five months are necessary to establish cell lines in culture.

The average maturation rate for Sitka spruce embryogenic cultures is 50 embryos/g of embryogenic tissue. Differences in maturation rates within cell lines and from month to month have been observed, which suggests there is variation in the system. Increasing the osmotic potential of the maintenance medium with the use of PEG or myo-inositol have no effect on maturation. Similarly use of the ethylene-action inhibitor, AgNO3, does not significantly improve results.

Germination rate of emblings is 80% after a desiccation step. First root and shoot development is visible after 1 week. 90% of emblings >1cm can be successfully acclimatised under conditions of high humidity.

After 3 growing seasons in the field no significant differences in height, current season growth, or diameter have been observed, between somatic emblings and seedlings from the same families (Fig.1 fig.2 and fig.3). Differences have been observed between families, particularly with current season growth measurements.

**Acknowledgements**

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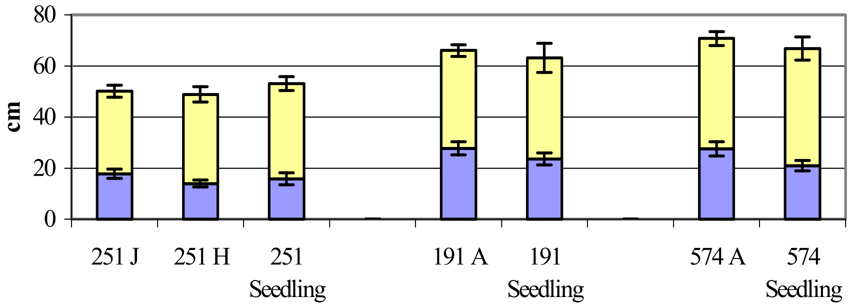


*Figure 1. Seasonal growth of somatic emblings and seedling family controls over two subsequent years*

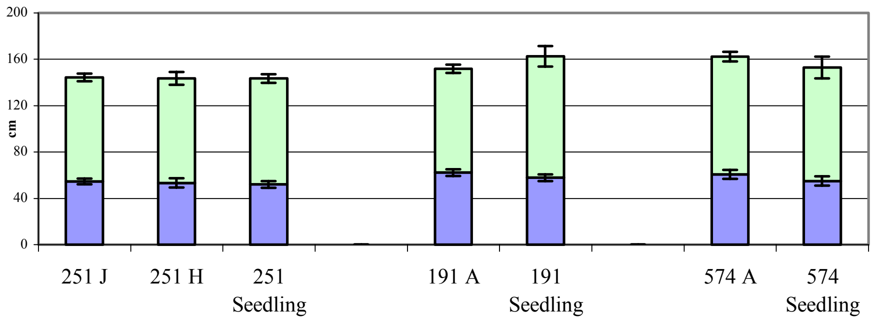


*Figure 2. Total height of somatic emblings and seedling family controls after the second and third growing seasons in the field*

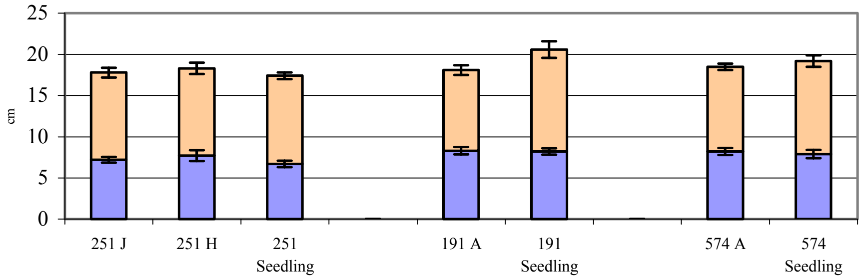


*Figure 3. Shoot diameter of somatic emblings and seedling controls after the second and third growing seasons in the field*

## 6. THE CRYOPRESERVATION OF SITKA SPRUCE

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The main objective of the study was to develop cryopreservation protocols for Sitka spruce embryogenic tissue and shoot tips that can be applied to a broad range of genotypes. Cryopreservation is the storage of living germplasm at –196C using liquid nitrogen. Cryopreservation can be used to maintain juvenility, to prevent a decline in morphogenic competence and to circumvent epigenetic and genetic change. It also avoids contamination and reduces the cost of maintenance of tissues in the actively growing state i.e. it is both time and space effective.

Embryogenic tissue from ortets of five families of Sitka spruce was cryopreserved by controlled rate freezing. The tissues were thawed and recovered on filter papers on Murashige and Skoog medium. The viability of the recovered tissue was tested with fluorescein diacetate vital stain, which fluoresces yellow green under violet light if viable tissues are present. Some ortets of each of the five families survived the process.

In vitro shoots of five full-sib families of Sitka spruce were available for cryopreservation. A number of techniques have been tried or are being developed. The objectives of the study were to develop an encapsulation and dehydration technique of cryopreservation and to use it to explore the role of dormancy and cold acclimation. No previously dormant shoot tips have been recovered but cold acclimation and abscisic acid and sucrose pretreatments have resulted in the first post cryopreservation recovery of Sitka spruce shoot tips.

## 7. SOMATIC EMBRYOGENESIS – USEFUL TOOL OR EXTRAVAGANCE?

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**Useful tool?**
Why is somatic embryogenesis (SE) considered as a technology that will be a useful tool? At least three features could be listed: 1) the cryopre-

servation option that allows storage of genotypes for a long time without losing vegetative propagation capacity, 2) rapid multiplication of a genotype for clonal forestry and 3) the prospect of automatic handling of somatic embryos, however not yet developed to commercial systems.

Reliable protocols have been developed and it seems unlikely that cryopreservation should be anything other than an advantage for maintaining juvenility where required. It is also a very space-efficient technology. For example, a cryo-vessel, 1 m high and 60 cm diameter can contain 3,600 cryotubes. Ten tubes per clone means that 360 clones can be stored on a surface of 0,5 $m^2$. A room with a net area of 10 $m^2$ can thus contain 20 vessels = 7200 clones. It can be argued that other micropropagation methods can use the cryopreservation option, but most of the development has been done on embryogenic cultures so far.

Norway spruce provides the following example of rapid multiplication by SE propagation. Starting with 10 mg tissue after 6 weeks of initiation treatment, and a proliferation rate that doubles the amount of tissue every second week, the amount of tissue 24 weeks after initiation would be $0.01*2^{(24-6)/2}$ = 5.12 g. Assuming 15 acclimatised somatic emblings per g tissue, there will be close to 80 plants produced after half a year. If proliferation is prolonged for two more months the amount of tissue will increase to 82 g, i.e., around 1,200 plants. To produce 1,000,000 plants under the above conditions would take 60 weeks from initiation, i.e., a little more than a year. These numbers are conservative and it may be possible to move faster with improved protocols or with other species.

No examples of mechanised handling of somatic embryos or germinants have so far been presented, but development is underway. It is too early to say whether this development will succeed. Large-scale plant production would benefit considerably from systems for mechanised handling of somatic embryos.

**Question marks**

Some studies have been published on genetic changes during propagation and after cryopreservation. For example, Isabel et al. (1996) reported somaclonal variation of 0.2% for two clones of *Picea glauca*. The deviating plants were identified by partially lacking chlorophyll (variegated needles) but only one of 250 RAPD markers correlated with this trait. In a study by Park et al. (1998) on the same species, high clonal correlation was found in field tests established after 3-4 years cryopreservation. Other studies point in the same direction and SE

seems to be robust from this point-of-view.
Whether genetic selection takes place during SE varies in importance depending on species and their respective success rates. A species like *Picea abies* shows rather high success rates when immature zygotic embryos are used as explants. No study has so far indicated any correlation between propagation characters and breeding traits, albeit the materials have been limited. Theoretical calculations show that the likelihood of an unfavourable selection is high in situations where a strong negative correlation is present in combination with low propagation rate and when a large proportion of the candidates must be retained after selection (Haines and Woolaston 1991). For other species the situation may be different. For example, some Pinus species have been more difficult to propagate by SE and strong selection might occur.

Non-genetic effects caused by propagation are probably always present in all kinds of propagation, and certainly so in vegetative propagation. The so-called C effects are effects that are common to the clone but do not have genetic origin. If clone and propagation method interact, the performance at propagation might not reflect later performance in the field. In a study by Högberg et al. (2001), treatments during propagation affected plant growth during both the first and second growth periods. However, it is likely that protocol development will decrease such effects. Maturation is crucial for production of high quality embryos, in turn reducing the C effects. In practice, there will probably also be selection against clones performing poorly during propagation and growth in the nursery. There are few reports on C effects after cutting propagation of conifers. So far, no published study has shown that the C effects persist more than a few years in field. The situation for SE in a longer perspective is unknown.
Intraclonal variation (c effects) reduces precision in selection. Högberg et al. (accepted) showed that coefficients of variation for Norway spruce clones, derived from somatic embryos, after two growth periods in the nursery were higher than what has been reported for cutting propagation. By early intraclonal selection on lateral roots the variation could be brought down to the same level as cuttings or lower. The reasons for intraclonal variation could be many, but once again the most likely explanation is maturation differences. This can occur even within a cell clump if hormone and nutrient availability varies within the tissue. Improvement of protocol leading to formation of high-quality embryos and rapid root development will decrease the c effects.

**Extravagance?**
When foresters or nursery managers ask about SE, price is often first in line. It is easy to understand that SE as it is outlined today, with manual one-by-one handling of embryos or plantlets, is considerably more expensive than seedlings. SE could not even compete with cuttings when comparing pure propagation costs.

However, manual handling during early propagation stages is not crucial as the costs can be diluted by a large number of plants in the end. Of course, automated proliferation and maturation in bioreactors helps a little but is not significant. The costly one-by-one handling starts after formation of somatic embryos when the embryos are transferred from maturation vessel to desiccation vessel and thence to germination vessels. If the protocol includes early plant growth in vitro with the roots in liquid medium, a transfer of plantlets from germination medium to liquid medium vessel is required. Finally, plantlets are transferred, either from germination vessel or liquid medium vessel to containers for ex vitro acclimatisation. Three to four manual one-by-one handling steps during propagation will be costly and the expected price for a SE plant in large-scale production is £0.75-1.25. A protocol that reduces manual labour is necessary to reach reasonable cost levels.

Plant production logistics can not be ignored if you want to deliver a clonal mixture at a certain time. Proliferation rate varies between clones, and if you have to wait for slow-growing cell lines, a considerable amount of work will be needed for maintenance of the fast-growing ones. If you can tolerate imbalances within the clonal mixtures this factor will be less important. Another way of handling this would be to select for high and uniform proliferation rate, but then you must either accept lower genetic gain or include more clones in the tests.

To compare the cost for juvenility maintenance is very difficult as many parameters are involved. If only the running costs are taken into account, hedging can be estimated to cost £70-100/clone during a 10 year-period. With cryopreservation, the cost would be £55-70/clone including freezing, storage and thawing.

Mass propagation by cuttings taken from somatic embryo donor plants would bring the cost down. If you get 200 cuttings per donor plant, the cost per plant would increase less than £0.01 The overall cost for clonal plant production would probably be less than with cuttings because of the lower cost for juvenility maintenance, lower risk for losing clones because of ageing after storage and no risk for environmental disturbances (pests, climate) of donor plants during the field test period.

**Time aspect**

The most important factor affecting the time needed to get a production population into the forest in large scale is the duration of field testing. Another factor is the time needed to scale up the production population. SE, cuttings and a combination of SE and cuttings can be compared theoretically, with the goal set at 20 million plants and field test time set to 6 years. Multiplication rates and other variables are based on experience with spruce. With SE alone, 20 million plants can be ready two years after field test, whereas the cutting alternative lags behind two additional years. The combined alternative takes the longest time because of the time needed for donor plants to reach the proper size for cutting production. This is an example where ageing is not taken into account. If ageing occurs in the cutting alternative, clones will be lost and there will either be fewer high-ranked clones available, or the gain will be lower when lower ranked clones must be used to keep the number of plants in the production population.

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# 8. CLONAL PROPAGATION OF SCOTS PINE – EXPERIENCES IN ALL THE METHODS TRIED: A REVIEW

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Scots pine (*Pinus sylvestris* L.) has been considered recalcitrant for vegetative propagation. In this presentation, the techniques developed until today for clonal propagation of Scots pine, i.e. rooted cuttings, micropropagation through organogenesis and somatic embryogenesis, are compared with each other, and their characteristics including material used as explants, propagation efficiency, quality of cloned plants, and specific problems together of new developments or potential solutions are reviewed.

**Rooted cuttings**
Different types of material, such as long shoots, needle fasicles as such or after pruning the branches or after chemical treatment of stock plants have been used as cuttings in Scots pine. The rooting percentages achieved have varied 0-100% depending on stock plant genotype and age, and growth regulator treatments applied. The material used as cuttings in the Finnish Forest Research Institute's (FFRI) experiments has been fascicular shoots induced in 2-year-old seedlings by cytokinin spraying. The amount of cuttings by genotype was on the average 40-60, the maximum being 150. The rooting treatments include auxin (IBA) application, and inoculations either with *Agrobacterium rhizogenes* or mycorrhizal fungi. The auxin treatment was needed for rooting the fascicular shoots, and it was further enhanced by agrobacteria, with different bacterial strains affecting pine genotypes differently. The roots that were formed following bacterial treatment were, however, not transformed. Also the application of ectomycorrhizal fungi increased the rooting percentages. With different treatments, on the average 30-50 % of the cuttings rooted, up to over 80 % in the best genotypes. The quality of the plants produced was, however, not good: only 40 % of them were orthotropic.

**Tissue culture through organogenesis**
The explants used for organogenesis were cotyledons excised from germinating embryos. In the protocol developed in FFRI, tissue culture medium used was half-strength GD gelled with agar/gelrite during shoot

formation and with agar during rooting. The growth regulators used for shoot induction were 5 μM BA and 0.05 μM NAA, and repeated pulses of 2.7 μM NAA were required for rooting. Application of the described protocol resulted in formation of adventitious shoots in 13-60% of embryos, the average multiplication rate being 3-15 shoots per embryo with the maximum of 35. Of these shoots, however, only 6% rooted. Moreover, the quality of the produced plants was poor, the majority of them being plagiotrophic and ramified. Interestingly, Scots pine plants produced through organogenesis were found to be early-flowering, producing megasporangiate strobili at the age of three years and microsporangiate strobili at the age of four years.

**Somatic embryogenesis**

For somatic embryogenesis, even younger explants than for organogenesis, i.e. immature female megagametophytes including zygotic embryos are needed. In the FFRI protocol, the tissue culture medium used is DCR with 9.1 or 13.6 μM 2,4-D together with 2.2 μM BA for induction and proliferation of cell masses. For embryo maturation, activated charcoal, 32 μM ABA, PEG8000 and 60g/l of sucrose are applied, the embryos being germinated with 20 g/l of sucrose. With this protocol, 28 % of the randomly chosen seed families showed potential for somatic embryogenesis, and within the reacting families 3 % of the explants produced embryogenic cultures. The average multiplication rate was around 50 somatic embryo per maturation cycle, varying from 10 to 200 depending on genotype. The greatest problems were in germination of cotylenodary somatic embryos, but it was found that application of mycorrhizal fungus, *Pisolithus tinctorius*, promoted *in vitro* root development in Scots pine somatic embryos resulting in germination frequencies of 48-83 %. Also the cryopreservation of the embryogenic cultures is possible, 75 % of the tested lines surviving cryostorage.

Somatic embryogenesis of Scots pine is still under development, and the results achieved by other research groups are comparable with the ones obtained at the FFRI. It can be concluded that at the moment none of the vegetative propagation techniques developed for Scots pine are applicable for producing clonal material for reforestation. Also for clonal testing in either tree breeding programmes or research projects, several factors, especially initiation frequencies, multiplication rates and quality of produced plants should be improved. Of the different techniques, somatic embryogenesis shows the greatest potential for this.

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# 9. HARD PRUNING AND LONG DAY TREATMENT OF STOCK PLANTS IMPROVE ROOTING AND EARLY GROWTH HABIT OF NORWAY SPRUCE CUTTINGS

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Nearly 20 years ago, we suggested using rooted cuttings as a means to bulk up well-tested families or other valuable Norway spruce material for reforestation purposes in Norway (Johnsen 1985). There was, however, a need to use containers to make plant production compatible with the nursery production system in Norway. Cuttings were rooted and then grown for one season without lifting, and thereafter sold on the market. We intended to maintain stock plants as hedges. However, cuttings looked different from normal seedlings, and problems with temporary plagiotropism became apparent.

We decided to look more carefully into stock plant treatments, and how twigs could be invigorated to improve rooting, root quality (higher number of adventitious roots per cutting) and attain a normal growth form after rooting. Stock plants were severely pruned on top and side branches, and kept 30 – 40 cm tall at the age of 7 – 8 years. Cuttings from hedged plants rooted much better, number of roots per plant increased, and plagiotropism was reduced compared to the controls. Pruning reduced variation among years and families. Hard pruning could maintain stock plant in use for at least 8 - 10 years.

Despite extensive pruning, temporary plagiotropic growth still appeared to some extent, and was obviously under genetic control (Johnsen and Skrøppa 1992). This finding led to a hypothesis that day length given to the stock plants could influence rooting and growth habit of rooted cuttings. If long days (22 h) were given to stock plants before and during collection of current years shoots, cuttings rooted better and grew taller and more normal than cuttings from stocks grown at shorter days (19, 16 and 15 h). The provenance of southern origin rooted better and grew taller than the northern provenances on all photoperiods. Two factors probably explain why this happens. Long days produce longer, thicker and more vigorous shoots with a larger area of tissue, which could form adventitious roots. Short days induce growth cessation and trigger the process that leads to dormancy, a drawback when cells should actively divide, form meristematic zones and differentiate to functional adventitious roots. Because needle primordia are formed partially before and under the rooting process, and because functional roots reduce stress and improve nutrient uptake, faster and better rooting will improve growth the first growth season after rooting (Johnsen and Tronstad 1998).

We conclude that stock plants must be grown under long days and cuttings should be collected before the dormancy process had started. Under these conditions acceptable rooting and growth will be attained, which is a prerequisite for a commercial production of Norway spruce cuttings.

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## 10. DIFFERENCES BETWEEN HYBRID ASPEN CLONES IN REGENERATION FROM ROOT CUTTINGS AND ROOT MASS PRODUCTION IN DIFFERENT GROWTH CIRCUMSTANCES

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**Introduction**

Wood properties of hybrid aspen (*Populus tremula x Populus tremuloides*), such as short fibers, suitable fiber form, low lignin content and white-coloured wood, make it very suitable for the production of high quality paper (e.g. Ranua 1996, Pulkkinen et al 1999, Tarvainen 1999). Many of these properties show large clonal variation (e.g. Farmer 1962, Schier & Zasada 1973, Zsuffa 1992, Pulkkinen et al. 1999, Yu et al.

2001). Since the paper industry favours uniform fibres, a promising way to exploit the genetic variation is through vegetative propagation of the best plants. Micropropagation of hybrid aspen is very effective, but also expensive. The use of stem or root cuttings does not require highly sophisticated laboratory techniques or skilled employees, enabling reduced propagation costs (Ahuja 1983, Vasil 1994, Salonen 1998, Pulkkinen 1998, Yu 2001, Yu et al. 2001).The root cutting technique seems to be suitable for hybrid aspen propagation (Pulkkinen & Herrala 2000, Mäntylä 2001). However, there seems to be high variation in the capacity of clones to produce root mass and regenerate from root cuttings (Mäntylä 2001).

The aim of this study was to investigate the ability of eight hybrid aspen clones to produce shoots and roots from root cuttings and to produce root mass in different media.

**Material and methods**

Twelve hybrid aspen clones have been selected for commercial micropropagation in the Haapastensyrjä Forest Breeding Station of the Finnish Forest Research Institute (Pulkkinen 2001). These clones have been effectively micropropagated and have good wood characteristics for paper. Of these clones, eight were selected for this study.

In May, 2001, the roots of the two-year-old stock plants were washed and 3 cm long cuttings were taken from roots with a diameter of 2 to 10 mm. The cuttings were placed horizontally in Plantek-64-cells filled with a 1:1 mixture of sand and fertilized peat. The depth of the mixture was about 0,5–1 cm. The root cuttings were grown in a greenhouse for eight weeks with frequent watering. Ground temperature was about 20°C and air temperature closely followed changes in the natural environment (range appr. 15–27°C). Relative humidity was over 90%.

In June of 2001, 100 plants of each of the eight hybrid aspen clones were planted into a greenhouse bed. The bed was composed of five different media: sand, sand + fertilized peat (2:1), sand + fertilized peat (1:1), sand + fertilized peat (1:2) and fertilized peat. The bed was divided into two sections by irrigation treatment (low and normal). In May of 2002, twenty plants per clone were carefully picked up. Their roots were washed with water and the fresh root biomass was weighted.

**Results**
The mean number of root cuttings per donor plant varied from over hundred to about two hundred. On average, one can get about 160 cuttings from one two-year-old donor plant (Table 1). The mean sprouting percent was 45. The lowest sprouting percent was slightly below 30 and the highest slightly below 60 percent (Table 1).

*Table 1. The number of root cuttings, mean number of root cuttings per donor plant, number of cutting plants and sprouting percent of cuttings by clone. (n = 93).*

| Clone | Number of donor plants | Number of root cuttings | Cuttings per donor plant | Plants produced | Sprouting % |
|---|---|---|---|---|---|
| 1 | 12 | 2626 | 219 | 1422 | 54 |
| 2 | 11 | 1649 | 150 | 855 | 52 |
| 3 | 11 | 1258 | 114 | 721 | 57 |
| 4 | 12 | 2325 | 194 | 1065 | 46 |
| 5 | 12 | 2084 | 174 | 705 | 34 |
| 6 | 12 | 1628 | 136 | 470 | 29 |
| 7 | 11 | 1449 | 132 | 490 | 34 |
| 8 | 12 | 2220 | 185 | 1265 | 57 |
| Mean | | | 163 | | 45 |

The results for root mass production were in close agreement with those from the root cutting experiment (Figure 1). Four of the clones (1, 4, 5 and 8) showed both a very large number of cuttings and high root mass. Only one clone of eight produced a notably small number of cuttings per donor plant and root mass (number 3) (Table 1, Figure 1). The root mass of the most productive clone was almost three times as large (mean=55 g) as the clone that produced the least root mass (mean=19 g) (Figure 1).

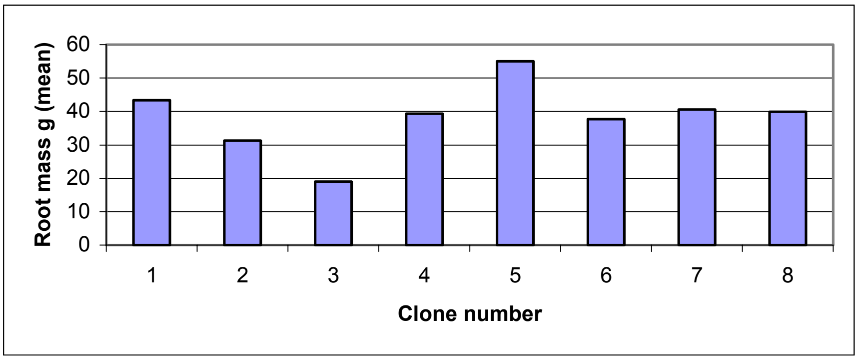


*Fig. 1. The mean root masses of plants of eight hybrid aspen clones after one growing season. (n = 160).*

The hybrid aspen plants responded very differently to the five growth media and the two irrigation treatments (Figure 2). Root growth was greatest in the normal irrigation treatment and in sand + peat (1:2), or just in fertilized peat. In the sand medium, which was poor of nutrients, the plants as a whole remained stunted. The mean root mass in the sand was only about 6 g fresh weight whereas in the best medium (sand + peat 1:2), it was over 7-fold, 45 g (Figure 2). In general, the lower level of irrigation was associated with smaller root masses. An exception to that was the sand + peat (2:1) medium, where the low irrigation treatment resulted in 1,5 times higher root mass per plant than the normal irrigation treatment (Figure 2).

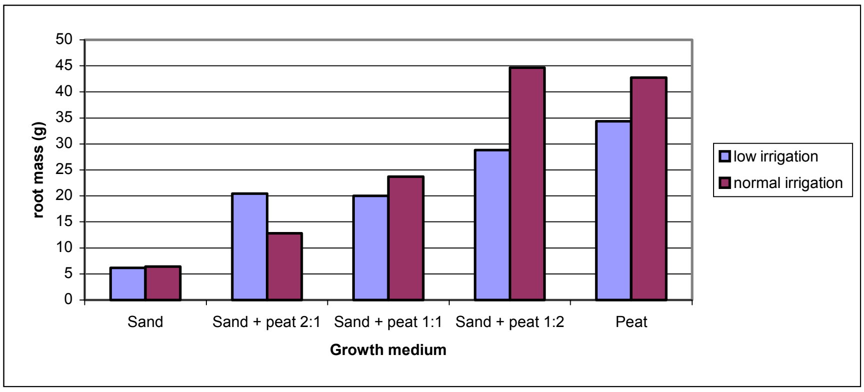


*Fig. 2. The mean root masses of plants of eight clones of hybrid aspen in five different growth media and in low and normal irrigation treatment. (n = 160).*

There were large differences in root mass among the most and the least productive clones in every growth medium. Only in the sand did all the clones perform almost equally. In the other media, the mean root mass of the most productive clone was 2–4 times higher than that of the least productive clone.

**Discussion**

Clonal variation in the production of root mass and the ability to regenerate from root cuttings were quite large among the eight hybrid aspen clones which had already been selected for micropropagation. Genotypic variation in the propagation capacity has also been found in micropropagation and stem cutting techniques (Salonen 1998, Yu et al. 2001). Therefore, it seems that clonal selection is an effective means to improve the supply of root cuttings. However, both root mass production and efficient sprouting need to be considered (Figure 4).

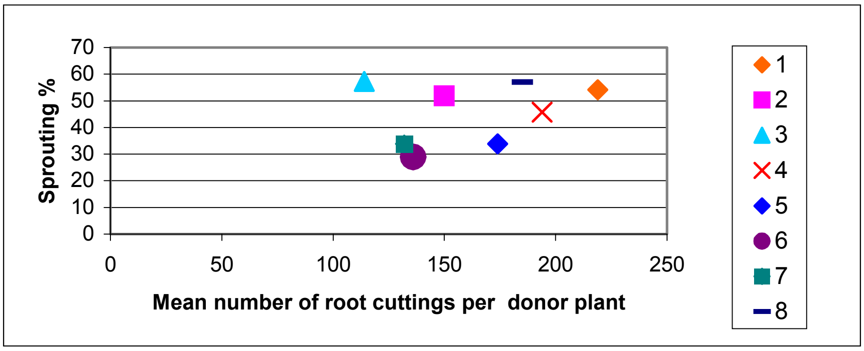


*Fig. 4. Mean number of root cuttings per donor plant compared with sprouting percent of cuttings in eight aspen hybrid clones. (n = 93).*

The clones near to the top right corner have the largest root mass and the best ability to regenerate from root cuttings. These clones are very suitable for commercial mass plant production. On the other hand, the closer to the base of the right corner the dot denoting a clone is located, the greater is amount of wasted work. Such clones produce lots of root cuttings, but the cuttings have a weak sprouting ability. The fewer root cuttings that are put in growth medium are lost, the lower are the production costs. Of these eight clones investigated, only four or five seemed to be suitable for mass propagation. The worst selection for commercial production appears to be clone number 5 which has a large root mass but a weak regenerating ability (Figure 4).

Furthermore, the growth medium seems to strongly affect root mass production (Figure 2). In the sanded media, the plants produced more roots since the sand improved water and root penetration. However, because the sand contained only a small amount of nutrients and no fertilisation was used, the plants could not grow large roots. The best growth medium seemed to be fertilized peat or a mixture of peat and sand at 2:1 ratio. The results indicate that the amount of irrigation is not as meaningful to the root growth as the composition of the growth medium (Figure 2). However, the irrigation treatments applied in this study might not have differed enough from each other, explaining the lack of notable response.

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# 11. SUPERIORITY OF NORWAY SPRUCE CUTTINGS – NOT ONLY A MATTER OF GENETICS

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Norway spruce cuttings of selected clones generally perform better in the field than standard seedlings. The superiority of the cuttings is a result of genetic selection of the clones, but also of the propagation method itself. Most comparisons between cuttings and seedlings are confounded by genetic differences between the plant types, but there are a few where the genetic effect has been kept under more or less control.

Cuttings differ from seedlings in many aspects (e.g. Kleinschmit 1978, Roulund 1974). They are produced from twigs and are thus structurally and morphologically different from the start. A young cutting is often more sturdy and has a higher biomass than a seedling of corresponding height. The bark structure and presence of needles at the stem base differ between the plant types. Cuttings are ontogenetically older than seedlings, which may affect their growth rhythm and frost hardiness. Cuttings from old clones may suffer from plagiotropic growth. Differences in the root system may affect plant establishment and stability of the trees.

A field study of cuttings and seedlings, planted as 1.5-year-old unselected stock from the same seed source, was presented by Gemmel et al. (1991). Eight years after planting, the cuttings were significantly taller (15%) than the seedlings, as an average over the 20 sites established in southern Sweden. Survival of the cuttings was also higher. Other experiments point in the same direction. For example, Hannerz & Wilhelmsson (1998) found that cuttings were 6% taller than seedlings of comparable, unselected, origin after 14 years in a field test in central Sweden. Roulund et al. (1986) compared cuttings of selected clones with standard seedlings, and found them to be 18% taller at 13 years age in a Danish field test, despite that they were shorter than the seedlings at planting. However, Karlsson et al. (2001) showed that the magnitude of the ”cutting effect” may vary randomly between propagation batches. They found that seedlings and cuttings of the 2nd vegetative cycle grew similarly, but cuttings of the 3rd vegetative cycle were superior to seedlings.

A few experiments have showed differences in frost hardiness between cuttings and seedlings. Hannerz & Wilhelmsson (1998) found that cuttings were significantly less injured by spring and autumn frosts than seedlings of comparable origin. Hannerz (1994) also found that cuttings were much less damaged by winter desiccation than seedlings. The better hardiness may be explained by ontogenetic aging, since the cuttings may harden earlier and deharden later than the seedlings.

Spruce cuttings have a lower risk of being damaged by pine weevil. Hannerz et al. (2002) compared cuttings and seedlings with the same initial stem base diameter planted in 5 field trials in southern Sweden. They found that cuttings were less attacked by pine weevils, and of those that were attacked, fewer were girdled. On the “average” site, pine-weevil induced mortality was almost twice as high for seedlings compared with cuttings. Other experiments and testimonies from practical forestry have supported the findings of the study (e.g. Nyström 2002, Jan Weslien unpublished). In a laboratory experiment, seedlings were 5 times more eaten than cuttings, and it was observed that the pine weevils visited the cuttings at the same extent as the seedlings, but they did not feed on them (Göran Nordlander, unpublished results).

Roots are differently formed in a cutting, since adventitious roots are formed from the cut surface, while a seedling starts with a primary root system. There has been some fear that the root system may differ and affect the stability of the clones in a negative direction. However, controlled experiments in 15-year-old field tests showed that cuttings and seedlings had the same number of roots, an equal vertical and horizontal distribution of the roots and the same risk of root deformation (Hannerz & Lindström 1998). A Czech study where 25-year-old cutting and seedling roots were dug up came to the same conclusion (Mauer & Palatova, 1994).

In conclusion, there is evidence that cuttings usually have an advantage over seedlings. They establish quicker and grow better, at least initially.

They also seem to sustain frost damage better and are less attacked by pine weevils. There are still many questions to be answered about the basis of the “cutting effect”. For example, what is the effect of ontogenetic aging on e.g. growth and frost hardiness? Why are cuttings less attractive to pine weevils? How do we exploit the superiority of the cuttings?

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# 12. NON-ADDITIVE VARIANCE IN *PICEA ABIES* AND ITS INFLUENCE ON TREE BREEDING

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**Summary**
This study presents results from half-sib & full sib varieties (clones) of Norway spruce (P. abies). The selection of ortets was based on early height growth in the nursery. Two series of field-tests were established, one with half-sib varieties and one with full-sib varieties. In total 10 tests were established. In each test a design based on complete randomisation and single tree plots was used. Height growth was assessed after 10-11 years. In the estimation of variance components the ASReml software was used.
Overall, non-additive variance was substantial but smaller than additive variance. Non-additive variance was affected by origin and test environment (”frost”). Tests with cuttings are suitable in a breeding strategy based on general combining ability (GCA)- if non-additive variance is moderate. To increase trait heritability and selection accuracy a more distinct definition of the trait ”growth” may be needed.

**Introduction**
Typical questions that tree breeders may ask are: what is the level of additive & non-additive variance for early height growth in our tests? Are variety tests with cuttings suitable for a breeding strategy based on general combining ability, then assuming that the level of non-additive variance in relation to additive variance is low. Can we affect the level of non-additive variance? For example, do the range in origins that we are testing affect the level of non-additive variance and do the site conditions effect the level?

**Material**
Two sets of material were used: half-sib and full-sib. Selection of ortets was based on early height growth in the nursery. Two series of field-tests with propagated cuttings were established, one with half-sib varieties and one with full sib varieties. Two ortets were selected in the nursery in each half-sib family and the selected ortets were divided into southern and northern origins, Six field tests were established with cuttings from these ortets, The southern origins were planted on four of the southernmost test sites and the northern origins were planted on the

northernmost test sites (four tests). The northern and southern tests overlapped such that both southern and northern origins were represented in 2 tests. All tests were assessed after 11 years. In the full-sib material, three to seven ortets were selected in the nursery in each family originating from a partial diallell crossing scheme. Four field tests were established. All tests were assessed after 10 years. In all field tests a design with complete randomisation and single-tree plots were used. Approximately 2 cuttings / clone were planted in each test. In the half-sib material additive (VA) and non-additive variance (VNA) was estimated by using the statistical parent (mother) component of variance (σp2) and the clonal component of variance (σc2). Additive variance = 4σp2, non-additive variance = σc2 - 3σp2. The stand component of variance was considered a "provenance" effect and therefore not included in the additive variance. In the full-sib material additive and non-additive variance was estimated by using the parent component of variance, (σp2), the family component of variance, (σf2) and the clonal component of variance (σc2). Additive variance = 4σp2, Total genetic variance = 2σp2 + σf2 + σc2, Non-additive variance = total genetic variance – additive variance.

## Results

*Table 1. Results of the half-sib material divided into overall (6 tests), southern (4 tests), northern (4 tests) and two mid-tests.*

| | Overall | Southern | Northern | 2 mid-tests |
|---|---|---|---|---|
| **Families** | 824 | 441 | 383 | 824 |
| **Clones** | 1603 | 854 | 749 | 1590 |
| **Clones/family** | 2 | 2 | 2 | 2 |
| **Cuttings/fam** | 12 | 11 | 13 | 6 |
| **Height, cm** | 196,9 | 207,7 | 185,9 | 192,9 |
| **CVA** | 0,09 | 0,07 | 0,12 | 0,12 |
| **h2** | 0,07 | 0,04 | 0,11 | 0,11 |
| **H2** | 0,18 | 0,17 | 0,19 | 0,23 |
| **VNA/VA%** | 74 | 207 | -1 | 24 |

*Table 2. Results of the full-sib material divided into overall, sites with “no frost” (2 tests) and “frost” site (2 tests).*

| | Overall | "No frost" | "Frost" |
|---|---|---|---|
| **Families** | 141 | 140 | 141 |
| **Clones** | 642 | 635 | 631 |
| **Clones/family** | 5 | 5 | 4 |
| **Cuttings/family** | 31 | 16 | 15 |
| **Height, cm** | 133,4 | 147,9 | 118,5 |
| **h2** | 0,06 | 0,09 | 0,06 |
| **H2** | 0,10 | 0,11 | 0,11 |
| **CVA** | 0,10 | 0,12 | 0,09 |
| **VNA/VA%** | 51 | 17 | 95 |

**Discussion**

In this study, so called C effects, non-random environmental variances common to members of subgroups within a population, are assumed to be negligible or absent, which must be viewed with caution. Furthermore, the number of individuals selected in each family in the half-sib material was only two. To compensate for this, the total number of families is instead large.

Overall, the level of non-additive variance in relation to additive variance (VNA/VA) was substantial but seemed to be at a moderate level i.e. VNA/VA clearly below 100% (table 1 and 2). Non-additive variance appeared to be affected by latitude of origin as the southernmost origins in the half-sib material contributed largely to the to the amount of non-additive variance observed (VNA/VA 207%). If less than 5% of the families, the southernmost families, in the southern material were excluded the ratio of VNA/VA dropped to below 50%. Furthermore, non-additive variance appeared to be affected by test environment. The test sites with full-sib material vaguely defined as ”frost”, i.e. sites where frost damage has been observed, showed large amounts of non-additive variance (VNA/VA 95%).

If the largest proportion of the genetic variance for traits in a population is additive, then a breeding strategy based on GCA is usually preferred. Such a strategy aims to exploit the additive genetic variation by selection to increase the frequency of the alleles causing the favourable genotype.

If the largest proportion of the genetic variance for traits in a population is non-additive, then breeding strategies using clones, inbred lines or based on selection for SCA will have higher gains.

Variety tests with cuttings seem to be suitable in a breeding strategy based on general combining ability as the level of non-additive variance observed here was moderate. Based on the results in this study the level of non-additive variance can be affected by the latitudinal range of material that is tested and by environmental conditions at the test site. The results imply effects of differences in growth rhythm on the level of non-additive variance, as both origins and frost related problems, especially late autumn frosts are related to each other.

An hypothesis is that NAV in height growth is influenced by genetic variation in other traits i.e. frost hardiness. Buds are more frost sensitive in genetic material with a long growth period, but this material also shows a higher growth potential. Occasional bud damage due to frost may be included in the trait ”height growth” causing larger variation. This variation in height is then interpreted as non-additive variance. To increase trait heritability and selection accuracy a more distinct definition of the trait ”growth” is needed.

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# 13. CLONAL FORESTRY WITH *POPULUS TRICHOCARPA* AND BREEDING FOR POPLAR RUST RESISTANCE

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Black cottonwood (*Populus trichocarpa* (Hook.) Torr. & Gray) was first imported to Iceland from Alaska in 1944. It fast became a very popular tree for use in gardens and parks, but only since about 1990 has it been used extensively in afforestation. Even though many black cottonwood clones have been imported over the years, almost exclusively from Alaska, only a handful were in general use before the 1990's. However, beginning with the first clonal trials in 1992, efforts have been made to increase the number of clones in use, aiming both at tree improvement through clonal selection and increasing diversity. By 2000, afforestation planting reached 280,000 rooted cuttings, making black cottonwood the $5^{th}$ most planted tree species in Iceland, comprising about 5% of planting. About 10 clones make up the bulk of planting stock. Black cottonwood is most often planted in mixtures with sitka spruce and in shelterbelts along with willows, almost never in monoculture plantations. The tallest tree in Iceland is a black cottonwood, over 22 meters, planted as a sapling in 1970.

Poplar rust (*Melampsora larici-populina)* arrived in Iceland in 1998 and has spread rapidly in southern Iceland since then. It is mostly a problem in gardens and parks where people do not want diseased trees. Severe infestation seems to be able to cause die-back of shoots through early autumn frost damage although that has not been a common occurrence in recent years. It is unknown how large the poplar rust problem will be in Icelandic forestry in the future.

Guðmundur Halldórsson, entomologist at Iceland Forest Research and Halldór Sverrisson, plant pathologist at the Icelandic Agricultural Research Institute, inoculated black cottonwood with poplar rust in existing clonal trials to study variability in rust resistance. In short, the outcome was that only 3 clones combined good rust resistance with good growth, form and survival. A decision was made to try to increase the number of available well-growing, rust resistant clones by doing a single round of breeding and selection. A total of 20 controlled crosses were made in spring 2002 between the 3 "good" clones and

14 other clones selected for good growth and/or survival and exhibiting at least medium rust resistance where known. The resulting 12,000+ seedlings belonging to the 20 full-sib families will be multiplied via cuttings in spring 2003 after which the seedlings will be planted in a single progeny archive. The cloned material will then be subjected to both early testing in the nursery and planted out in clonal trials in several places, where selection of new clones for future use will eventually take place.

## 14. KEYNOTE TALK: COMMERCIALISATION OF CLONAL FORESTRY

***David Thompson***
*Coillte Teoranta- The Irish Forestry Board, Research Laboratory Newtownmountkennedy, County Wicklow, Ireland*

This presentation attempted to answer the questions: 1) What propagation technologies will be needed to produce material for clonal forestry, 2) Can these technologies be integrated into existing plant production systems, and 3) What will it take to sell clonal forestry to the Industry? Clonal forestry in this presentation is defined by the use of tested clones as opposed to the use of proven or promising families or populations.

While clonal forestry offers the greatest potential to deliver the benefits of the best genotypes, it has several limitations. Interest in clonal forestry is based on the *Eucalyptus* model which does not have the same problems as conifers such as maturation problems, higher costs, questions of the levels of genetic gain and the length of time required to test and implement it. Many meetings on vegetative propagation and clonal forestry have taken place over the last 30 years and in spite of some very positive meetings (the “Clonal Forestry Workshop” held in New Zealand in 1989) little practical application of this technology seems to have taken place, apart from *Eucalyptus* and poplar, in spite of attempts in several countries.
Serious biological bottlenecks as well as economic and social/ environmental concerns have hindered progress with conifers. Advances in propagation techniques and cryogenic storage have bypassed most of the biological bottlenecks. Propagation by rooted cuttings costs less than somatic embryogenesis, but propagation rates are low, while with somatic embryogenesis high propagation rates are possible, tissues can be cryogenically stored, but plants have a high production cost. A hybrid system using somatic embryogenesis to pro-

duce stock plants from which cuttings for rooting are collected is perhaps the most effective and efficient approach and such a propagation system would fit well into existing nursery practices.

Unfortunately the lure of clonal forestry and its promises of greater gain, increased flexibility and uniformity tend to overshadow it's increased complexity and costs. It is assumed that these higher costs will be offset by higher gains, however, alternative systems such as "bulk propagation" of untested clones, "family forestry" or even "full sib forestry" can provide almost the same level of gain at a much lower cost. While many researchers believe that clonal forestry is used commercially in Australia and New Zealand, this is not the case and in reality it is also under test there. Therefore Clonal Forestry with coniferous species is not "just around the corner" and we should not claim that it is. In order to sell Clonal Forestry to the Industry we need to get the facts together about the true costs and benefits but we also need to seriously consider alternative systems (bulk propagation, family forestry and full sib forestry) before we make a decision on which line to follow. For Europe the spruces Norway and Sitka were proposed as species where clonal forestry would be most likely to develop in the near future.

## 15. CUTTINGS OF LODGEPOLE PINE IN SUCCESSFUL PLANTATIONS

***Anders Fries***

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In a cutting propagation program with lodgepole pine (*Pinus contorta* Dougl. var. *latifolia*) performed at our department, we developed a technique for production of rooted cuttings under greenhouse conditions. We propagated full-sib families for establishing clonal field tests. The full-sib families originated from controlled crosses between plus tree clones in Swedish seed orchards.

The program was initiated 1986, and the first relevant field tests was planted 1991. By successively larger clonal field tests until 1995, we now have more than 10.000 cuttings at 4 different test localities (6 test plantations). The test sites are located on normal and for lodgepole pine relevant sites 30-80 km north-west of Umeå (latitude 64°N, altitude 200-300 m).

One aim was to study whether the precision of full-sib progeny tests can be improved if each full-sib family is tested with clonal copies, i.e. if the cutting propagation technique introduces larger variation in growth performance than was gained by using clonal copies. To reduce the effects of initial ortet and scion differences, data on a number of ortet and cutting characteristics follow each individual cutting thorough the whole process. We also compare test designs by evaluating within and between clone variation in different plot types. Plots with one, two and three clones, respectively, and plots with all clones randomized are tested.

We started with 25 full-sib families from 5 latitudinal groups, but due to poor rooting for 4-5 families, we ended up with 20. Rooting percentage was 72%. Initial measurement was made at planting, and half of the material was measured after 7 years in field. So far, genetic parameters have not been evaluated, but survival at the four test sites was 90% (87-95%). Undamaged or only slightly damaged trees were 79% (53-91%).

The 7-year-measurement indicated similar variation in growth rate between plants from seedlings of the same full-sib family, as between cutting propagated plants within the same clone. This indicated that the possible reduction in variation by using clonal replicates might be compensated by variation introduced by the propagation technique.

If a technique for propagating Scots pine (*Pinus sylvestris* L.) by cuttings is developed, our field tests with lodgepole pine, which is considerably easier to propagate, may contribute with genetic parameters, and give information to be used when designing field tests. This advantage is emphasized by the fact that no stability problems due to poor root development have been observed. Moose damage did, in addition, not occur at all, although the surrounding Scots pine plantation had frequent attacks.

Contributors to the project: Assistant Prof. Anders Fries (project leader), Professor Dag Lindgren, Forest Technicians Stefan Löfmark, Lars-Göran Lejdebro, Bengt Olsson, Erik Walfridsson (now at The Forestry Research Institute of Sweden). The projects were financed by The National Board of Forestry, Sweden and Carl Trygger Foundation.

## 16. RESULTS AND EXPERIENCES FROM THE CENTRAL SWEDISH CLONAL FORESTRY PROGRAM

***Johan Sonesson***
*SkogForsk - The Forestry Research Institute of Sweden*

The Central Swedish Clonal Forestry Program was started in 1989 by four Swedish forest industry companies. The objectives were to test and select Norway spruce clones with high growth and survival rates and to create a base for propagating 9 million cuttings of tested clones per year. The clones were selected mainly in full- and half-sib families from phenotypically selected plus-trees. After a first selection of ortets in 2-4 year old nursery or farm-field tests, 4954 clones were established in field trials. Each clone was tested on at least three sites and the trials were assessed after six years in the field. The best 10% of clones were selected for propagation in the company nurseries.

The average survival in the field trials was 80% for the cuttings and 77% for seedling checklots. Average height after six years was 148 cm. Average BLUP-genotype values for the selected best 10% of the clones were 20% higher than for the average clone, 39% higher than seedling checklots of recommended provenance and 48% higher than seedling checklots of local provenance. The early selection in a farm-field test has proven to be efficient in a large proportion of the material used.

Less than 1 million cuttings from tested clones have been produced so far and interest has switched to bulk cutting propagation of families of high genetic quality. The main reasons for abandoning the tested clones were that the aged clones have lower rooting success and a higher degree of plagiotrophic growth than bulk propagated cuttings from juvenile seedlings. Other reasons were the high costs for hedging during the test-time and the legal restrictions on clonal mixtures. The estimated market price for a bulk cutting is 60% higher than for a seedling of comparable size, and the price for a cutting of tested clones is estimated to be 100% higher than the seedling. It is concluded that clonal forestry with Norway spruce will not be a realistic alternative until efficient methods of maintaining juvenility have been developed.

To produce enough seed of high genetic quality for bulk cutting propagation, an indoor seed orchard has been established. The best clones from the testing program will be used as parents in the orchard. They will also be used in conventional clonal seed orchards as well as in the Swedish breeding program.

# 17. CLONAL FORESTRY IN BRITAIN: WHAT'S IN IT FOR US?

***Steve Lee***
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The problems with Sitka spruce breeding today using the sexual route are that it takes too long, costs too much and provides a variable product which does not always combine together the required traits in an optimal manner. The objective should be plantations that are uniform and consistently good for growth rate and wood density. Clonal forestry provides the technology for this to happen.

Clonal forestry using tested clones is not yet being practised in the United Kingdom (UK), but remains a tree-breeding objective. Half-sib family forestry is operational (> 6 million cuttings planted per year) and plans are in-hand to develop full-sib family forestry. The genetic base of half-sib family forestry is currently high since seed are sold as mixtures of 20 half-families where the male input consists of an intimate mixture of 20 unrelated pollens. The genetic base of full-sib family could potentially be narrower depending on the number of families deployed. Economic traits under selection are growth rate, stem straightness, branching quality and wood density.

It is difficult to compose half-sib family mixtures that combine good growth rate with improved wood density. Even within a full-sib family with predicted gains that are positive for growth rate and wood density at the family-mean level, there will still be a wide variation about this mean, this variation is increased further as more full-sib families are added to the mix.

Clonal forestry allows breeders to select individual genotypes that combine traits which usually work against each other (correlation breakers) such as growth rate and wood density and then make many copies for deployment. Insight from existing trials consisting of just 6 full-sib families each containing 7 or 8 tested clones suggests the gains could be considerable. Selecting the best clone per family at one site shifted gain from +16% for diameter and –4% for density, to +15% for diameter +9% for density – which could change the economic value of the crop considerably if it were to satisfy a higher strength–class in the construction industry. Increasing the selection intensity (more full-sib families; more genotypes per family) would increase gains further.

Research continues into developing the tools required to deliver clonal forestry. Cryopreservation and Somatic Embryogenesis are seen as the two most important elements in arresting age-phase change and large-scale multiplication of selected genotypes respectively. We plan to have protocols in these techniques operational by 2005.

## 18. CLONAL PROPAGATION OF *ABIES LASIOCARPA* FOR CHRISTMAS TREE PRODUCTION. WHAT CAN BE GAINED?

***Harald Kvaalen[1)] and Ola Gram Dæhlen[2)].***
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**Introduction**
In 1989 the Oppland Forest Society started to test provenances, open pollinated families, full sib families, and clones of *Abies lasicarpa* for Christmas tree traits. Here we will present the most important results from the trials.

**Materials and methods**
Fourteen provenances were tested at two locations at Biri in 1989. One location is more exposed to frost. Progenies from controlled pollination were obtained from the *Abies lasiocrapa* seed orchard at Biri. From a subset of the full sib families, 322 clones were produced from rooted cuttings. The full sib families and the clones were planted at the frost exposed location. In 1997, Christmas tree quality was scored in three classes, where class 1 were trees that can be sold without pruning, class 2 were trees that must be pruned, and class 3 were trees that cannot be sold in any case. Aphid attack was also scored in three classes. The number of main branches was counted and the height was measured. The mixed procedure in SAS was used to estimate variance components and compute best linear unbiased predictors (BLUP) of breeding values for the various traits. The predicted breeding values were used to rank families and clones with respect to Christmas tree quality. To estimate the percentage of trees in the three quality classes, if the selected population(s) were to be used at large scale in commercial plantations, the five percent best were selected and the models

were run once again to estimate the phenotypic variance in the selected population. This variance was thereafter used to calculate the fractions of trees in the different quality classes.

**Results and discussion**

There was large variation in Christmas tree quality among the tested provenances. Some evidence for provenance x site interaction was also found. In terms of quality, the best provenance at one site was among the worst at the other site which is more exposed to spring frost. The narrow sense heritability of Christmas tree quality was 0.40 estimated from the male parent variance component in the controlled crosses. Based on all 322 clones, the broad sense heritability was 0.47. Keeping in mind that only six fathers were used for the controlled crosses, this may suggest that the additive variance is most important for Christmas tree quality. Selection at the provenance level is always necessary to get reasonably adapted material. The gains in quality are limited however as selecting the best provenance would only produce 22% class 1 trees. The reason is the large within provenance variability. Selection of the best full sib families gives considerably higher gains (49% class 1). However, large scale production based on full sib families would be an expensive and difficult alternative, if not combined with vegetative bulk propagation. Selection of the best clones gives a high fraction of class 1 trees (66%) and almost complete elimination of trees that cannot be sold. Most of the clones were from two full sib families that had lower than average quality. Even higher gains are thus expected from clonal propagation if the clones are selected within the best full sib families. For the other traits studied the broad sense heritability was 0.43 for height, 0.37 for branch number and 0.47 for aphid attack. The breeding values (BLUPs) of quality and height were not correlated. In contrast, aphid attack was negatively correlated with quality, both in the full sib families and in the clones. Selection for Christmas tree quality can therefore be done without increased risk for aphid attack or reduced height growth.

## 19. KEYNOTE TALK: PUBLIC ACEPTABILITY OF CLONAL FORESTRY

***Richard Worrel***
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The term “Perception is Reality” expresses the fact that for many important issues – such as clonal or GM forestry - an objective truth does not exist and people’s differing perceptions are equally valid. How people perceive things depends on their value systems. In respect of natural resources and the natural world, philosophers make a distinction between anthropocentric and biocentric value systems (see below).

Public viewpoints on forestry are often biocentric, whereas forest industry views and those of genetics researchers are generally anthropocentric. Forest scientists have tended to see themselves as being value-neutral because they are used to using value-neutral research methods, whereas in fact their views have clearly been strongly allied to industry. This alignment with industry tends to undermine credibility of scientists as providers of unbiased information. Tree breeders’ frame of reference for clonal forestry sees the transition from wild populations to clonal trees in terms of increasing usefulness to industry, whereas the public view focuses more on loss of naturalness. This leads to potential for problems in communication on the issue.

Problems with clonal forestry arise mainly in terms of public benefits and impacts. There does not appear to be anything *intrinsically* wrong with cloning trees (as opposed to e.g. humans) because it happens widely in nature. Possible negative impacts of clonal forestry include:

1. Loss of naturalness – i.e. loss of “natural” components of genetic diversity whether apparent (as in clonal poplar plantations) or “imagined” as might be the case with clonal mixtures (can’t see it but someone has told you about it). Actual effects depend on control measures but it can be argued that there is considerably increased risk of negative effects in the deployment of clonal material unless control is good.
2. Altered niches for associated flora and fauna due to changed phenology, physiology and morphology of trees .
3. Increased risk of catastrophic pest or pathogen attack if screening is unsuccessful.
4. Contribution to ongoing intensification of forest management and increasing “artificial-ness” of forests when social trends are fa-

vouring the opposite.

5. Contribution to loss of local identity if trees across entire regions are derived from a small number of parent clones.

Public interest benefits include:

1. Increased timber production.
2. Reduced need for timber production on remaining forest area.
3. Possible application to conservation problems e.g. an attractive method of producing improved material for certain native trees (oak).

Issues which arise from this consideration of impacts and benefits include:

1. One must not underestimate an interested public's liking of what they perceive of as naturalness and local diversity. Even if it can be proved that deployment of clonal material does not reduce genetic variation, or even increases it, some people will still argue that "natural" patterns are preferable. This is *not* an irrational view, but the logical outcome of a biocentric value system.
2. The degree of change away from natural patterns of genetic variation depends on various things i.e. number and characteristics of clones used, the number of copies per clone and the degree of use of clonal material compared with seedling material. Immediate impacts are probably small but progressive replacement of seedling forests by clonal material become more significant in the long term.
3. The public are likely to be less concerned about exotic species than native species.
4. Segregation of forestry into highly intensive plantations (wood farms) and larger areas of more natural forests is not seen as the best way of delivering public benefit. The use of clonal material in less intensively managed forests is an attractive possibility.
5. Public acceptability might be easier if the link between "clonal" and "intensive" was less strong.
6. There will be limits to productivity of clonal material imposed by the site and overcoming these by fertilising or irrigation for example raises wider questions of sustainability.
7. The argument that clonal forestry reduces pressure on natural forests has to be carefully considered and there are many circumstances where this seems unlikely.
8. Genetically modified (GM) material will probably be mainly clonally produced. meaning that "clonal" and "GM" will become

conflated in the public's mind.

The main conclusion of this analysis is that as far as public interest is concerned, effects of clonal forestry are reasonably balanced and that they are not as benign as researchers generally portray them. The way forward will include suitable control measures. These are most likely to be framed in a way which is acceptable to tree breeders if they have entered into a constructive public debate. A process or forum is needed which is:

- public and inclusive;
- recognises and accepts peoples'/groups' different value systems;
- addresses economic, environmental and social concerns;
- is based on consensus building and conflict resolution methods;
- employs a professional neutral facilitator.

In addition, tree breeders could consider:

- establishing a series of plantations with contrasting levels of genetic diversity, demonstrating potential acceptability of clonal material.
- employing a minimum of staff with a genuine competence in and empathy for nature conservation and biocentric perspectives.
- dropping use of the term "clone" as this is a damaged word due to association with human and animal cloning.

All these issues will be far more important as the debate on GM forestry develops in the coming decades.

Anthropocentric (people-centred) view:

- Human interests (rights) override the interests of all other species virtually all the time.
- Other species are valued according to the benefits they provide for mankind i.e. they are viewed as *resources*.

Some outcomes and associations:

- Forest management driven mainly by economic perspectives (i. e. economic use of *resources*)
- Strong reliance on scientific information
- Strong faith in technological solutions
- Modernist approach to aesthetics - modern, *functional* things are beautiful (high rise buildings, spruce plantations)

- Little interest in locality and local diversity.

Biocentric (life-centred) view:

- Interests of other species override some human interests some of the time
- Other species are valued both for their benefits for people, *and some form of intrinsic value* (independent of their value to people.)

Some outcomes and associations:

- Forest management driven mainly by environmental and community perspectives
- Less reliance on scientific information and more on cultural – i.e. history, literature, song, myth, spirituality
- Sceptical of technological solutions, preferring solutions based on "natural ", "ecosystem" or "traditional" models
- Post-modern or romantic approach to aesthetics – wild and natural things are beautiful (old traditional buildings, natural forests).
- Strong empathy with locality and local diversity.

## 20. ECOLOGICAL EVALUATION OF CLONAL FORESTRY WITH CUTTING-PROPAGATED NORWAY SPRUCE

***Johan Sonesson[1)], Per Stahl[1)], Richard Bradshaw[2)], and Dag Lindgren[3)]***

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The clonal habit is widespread in nature. Typically 3-15 genotypes are found growing together in stands which cover several to many hectares and can reach very high ages. Coniferous clones are however uncommon in nature.

While clonal forestry is a relatively recent phenomenon in Sweden it has a long history in other countries, e.g. the *Cryptomeria* plantations in Japan. Clonal forestry with Norway spruce could significantly increase the productivity of Swedish forestry.

The main ecological threats posed by clonal forestry are linked to the increased uniformity and productivity of plantations composed of single or a few clones. There is also an expected interaction with herbivore populations living on spruce. Most threats do not differ significantly from those posed by other methods of intensive forest production. Below-ground effects of increased root contact through root grafts and increased microbiological uniformity in the soil is a largely unresearched area that may give cause for future concern.

The Swedish legislation controlling clonal plantations is among the most restrictive in the world and is judged sufficient to safeguard forests and the environment, even with a fairly widespread use of clonal plantations. Today, clonal plantations are quite limited, perhaps one million cuttings are used annually as compared to some 300 million seedlings. If clonal plantations are mixed as demanded by Swedish legislation, the phenotypic variation in clonal stands will not differ from that of a seedling stand for any trait.

This study was requested by a number of Swedish forestry companies in connection to the revision of the Swedish FSC standard and was financed by Swedish Association for Forest Tree Breeding. Copies of the report can be obtained from:

SkogForsk
Uppsala Science Park,
SE-751 83 Uppsala
Sweden

## 21. LONGITUDINAL EFFECTS OF SCOTS PINE PERFORMANCE – A JOINT RESEARCH PROJECT BETWEEN SWEDEN AND RUSSIA

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Scots pine (*Pinus sylvestris* L.) is a major forest tree species in northern parts of Europe with large economic importance. Extensive breeding and seed orchard programs exist in both Scandinavia and Russia. A lot of knowledge about provenance variation and transfer effects, serving as a foundation for advanced breeding, has been gathered. In Scandinavia mainly latitudinal gradients, i.e. northward and southward transfers, have been investigated but also altitudinal gradients. Extensive transcontinental provenance experiments within the former USSR have also been established and evaluated. In addition, genetic variation and genetic parameter estimates, including genotype x environment (GxE) interactions, are now accumulating from progeny trials. The overall results show a potential to utilize both transfer of provenances and selection of individual trees to effect survival and growth. Further, the GxE interaction is not experienced to be large enough to cause major rank changes of families or provenances when grown within a limited geographic area, and hence the performance seems stable. The general explanation is that Scots pine, being a highly outcrossed species with extensive gene flow, has evolved a clinal adaptation to large scale climatic conditions, e.g. photoperiod and temperature of the growing area, but no specific adaptation to particular site conditions such as humidity, fertility, soil texture, etc. However, very little is known about the effect of longitudinal origin of the seed source, at least when it comes to the performance of continental origins at maritime sites and *vice versa*.

The overall aim with the joint project is to establish parallel field trials in Sweden and in the Komi Republic in Russia with identical material for future investigations of Scots pine performance outside today's testing and utilization ranges. This will increase knowledge regarding performance and stability in contrasting environments (genetic variation, GxE interaction, genetic correlations, etc.), and also improve the possibility to predict effects of possible global climate change. In addition, this will facilitate tree breeding co-operation between countries, including both genetic material and methods.
Results from a preliminary study that we carried out exposed distinct differences between Scots pine from Komi and from Sweden in damage levels after autumn freezing tests of one-year-old seedlings. Latitudinal clines were evident in both categories of material, where northern origins experienced less damage, but provenances from lat. 63° in Komi were as hardy as provenances from lat. 67° in Sweden. The results revealed essential differences in adaptation for this species, indicating that the longitudinal or continental origin adds an important factor, besides photoperiod, in evolving hardiness adaptation.

Four field trials have been established in Sweden and two in Komi. The remaining two trials in Komi will be planted in 2003. All trials include identical material, i.e. 43 provenance seed lots from lat. 60°– 65° in Komi and 58°– 69° in Sweden and Finland, together with 266 open pollinated plus tree families from Ust-Chilma (65.4°) and Usinsk (66.1°) in Komi and from lat. 65.5°– 67.8° in Sweden. The management, inventories, and evaluations of these trials will be jointly co-ordinated by Sweden and Komi.

## 22. DECENTRALIZED TREE IMPROVEMENT IN ECOLOGICALLY MANAGED FORESTS

***Hubert Wellendorf***
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A framework is outlined based on the assumption that natural regeneration prevails. From a breeding point of view, this implies that once a forest is established or regenerated by planting, further improvement must be performed "in situ," i.e. without inclusion of external genetic material other than the inevitable gene flow through migrating pollen clouds. However, optional one-time enrichment may be accepted.

Typical species in this category include beech and oak in Central and Northern Europe. The developed framework refers to Danish conditions, where natural as well as introduced provenances from Central, Western, and Northern Europe have been planted and where the present trend is to convert to natural regeneration and convert pure stands to mixtures with other species.

Components of the flow-chart presented framework are classification of forest into those that are aimed to be improved, and those that are aimed to be conserved. Further, a distinction is made between establishment of new forests and regeneration of already established forests.

In the afforestation situation, a one-time aggressive introduction of the best populations of sufficient genetic diversity is of paramount importance, as later enrichments are only possible in limited scale. Further improvement is then only possible through selective thinning until natural regeneration of sufficient amount occurs. This type of silviculture was a well-established art in classical forest management in a large part of Europe and it is strongly recommended, that this type of activity be maintained and further developed in present and future forest management systems.

If there is concern about whether sufficient genetic diversity is maintained after these strong, selective thinnings, a system of monitoring genetic diversity by using molecular markers is recommended.

## 23. DEVELOPING A PAPERWOOD IDEOTYPE FOR NORWAY SPRUCE

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Pulp and paper manufacturing industry has a demand for sustainable fibre producing systems. In a recently started research project, we aim at responding to this challenge by developing a model tree which optimally meets the requirements of modern paper manufacturing processes. The ideotype is defined as being one that is capable of producing large quantities of uniform fibres per unit area when grown at densely spaced stands with short rotations. Short rotations and the absence of thinnings are necessary conditions for the paperwood ideotype because the profitability of thinnings of small-sized trees is low. A short rotation period is also important in avoiding problems due to root-rot (*Heterobasidion parviporum*), a common decaying fungus in spruce stands of southern Finland. In our ideotype scenario, the trees are harvested before the rot has emerged or had time to spread to the stems. The paperwood ideotype thus combines characteristics that are valuable not only from the point of view of the paper industry, but also of economics of forest management.

Within this project, we investigate the potential of *Picea abies* f. *pendula,* a rare narrow-crowned form of Norway spruce, in meeting the various criteria that define the ideal paperwood cultivar. According to our initial hypothesis, the narrow-crowned spruce type comes close to the required standards. If we were able to find support for this hypothesis from our maturing field trials, the narrow-crowned spruce could be rapidly developed into a commercial cultivar since most practical difficulties associated with vegetative propagation have already been overcome**.**

Thus far, we have harvested a few sample trees from a 30-year-old field trial which contains both normal *Picea abies* and *Picea abies* f. *pendula* phenotypes at three intensities of inter-tree competition **(**weak / moderate / strong**)**, and measured a great number of biomass components on these trees. In future, we will collect more data to evaluate structural and chemical variation in fiber properties of trees with distinct crown types. Furthermore, we will study the impact of inter-tree competition on a number of traits, such as biomass partitioning, wood producing capacity, harvest index and the uniformity of fibre population. Provided that the pendula type of Norway spruce will display an adequate match to the modelled ideotype, further investigations are needed to formulate guidelines for economically optimal silvicultural management (e.g., stand density, optimal site types, rotation) of fibre production stands established using clonal material of pendula spruce.

## 24. FINAL DISCUSSION AND CONCLUSIONS

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During a short final discussion, participants put forward conclusions, ideas and proposals for future work under the three main themes of the meeting. These were noted and are summarised below. The items in the summary are simply in the order in which they were noted in discussion and do not suggest any priorities. A number have repetitive elements both within and across themes.

**Technologies**

1. There was a general consensus that the use of rejuvenation techniques to overcome phase-change effects was likely to be less successful than cryopreservation in both spruce and pine.
2. Somatic embryogenesis, whilst successful, is often quite genotype-specific and improvements in general applicability are needed.
3. Techniques now well established on test material and cell lines need to be applied to specific material coming from breeding programmes.
4. Several countries indicated that they are now ready to increase the use of clonal material in plantation forestry.
5. Cryopreservation is recognised as the most successful tool in circumventing the effects of phase-change and there are clear indications that the technology is almost developed to the point of routine use.
6. Now that technical details of somatic embryogenesis have virtually been mastered, developments incorporating greater automation and ensuring more predictable uniformity of the propagule will

need to follow to ensure more widespread use.

7. Micropropagation techniques are not currently seen to be relevant or realistic in pine.
8. Effort is needed to ensure that the successful products of somatic embryogenesis emerging from laboratory culture are taken through to field-stage evaluation.
9. Methods and systems need to be developed to make the best utilisation of current products of micropropagation; in particular systems of stock plant, hedge and donor management should be studied. These provide effective methods of hybridising propagation techniques to the greatest advantage.
10. Do not cry “Wolf!” Feed proven levels of the new technology into existing systems while further refinements are consolidated.
11. In the wider area of propagation techniques which could serve clonal forestry, the limited resources put into somatic embryogenesis now are likely to bring the most rapid advances.
12. It is recognised that problems remain in the transfer of emblings to soil; this area of research should now received priority.

**Commercialisation**

1. Trials of clonal material should be established to study the uniformity of growth and the expression of other traits.
2. It is already recognised that planting stock derived from cuttings is less subject to weevil attack by virtue of a more resistant root-collar area.
3. Somatic embryogenesis can feed into established methods of commercial vegetative propagation using cuttings, through its use in creating basic hedges. Hybrid systems of this type should be established and will bring faster development of more truly clonal stock.
4. The increased costs of plant production through vegetative propagation remain. Commercialisation will become a greater possibility when these can be reduced.
5. All opportunities should be taken to involve producers from the commercial sector where possible to sustain a momentum in the general acceptance of clonal forestry.
6. The potential gains evaluated by breeders need to be demonstrated through the establishment of networks of genetic gain tri-

als that include clonal material.

7. It is now relevant to pay attention to current and potential customer demand for clonal forestry and information on this should be studied.

**Acceptability**

1. Being able to demonstrate the virtues and the lack of disastrous consequences of clonal forestry is now important. Trials and demonstrations of a range of clonal material, with clear controls for comparison should receive high priority in all countries intent upon developing clonal forestry.
2. Collation of work carried out to date in a range of active countries could provide initial material to carry the interest in clonal forestry forward and encourage its acceptance.
3. Persistent public resistance is likely to remain a problem and much effort to increase the awareness of methodology, genetic variability etc. will be needed.
4. Nomenclature remains a problem in relation to public perception. Clone seems to imply activity at the fringe of the acceptable use of genetic material by the public. Variety is likely to cause less of this type of concern. Poplar clones are referred to in this way outside the scientific community.
5. Better knowledge of markets is needed and efficient techniques to test these need to be developed.
6. There is a need to proceed carefully without rushing and to find out the real questions and their importance before delivering the answers.

**Spruce breeding network**

An interest in further co-ordination of effort among those participants involved in developing clonal forestry as a product of their spruce breeding programmes was clearly expressed. No specific proposals were put forward but EU 6$^{th}$ Framework and the development of an email network were recognised.