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Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future

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November 1-5, 2004

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North Carolina State University
Forestry Educational Outreach Program
Campus Box 8003
3028 Biltmore Hall
Raleigh, North Carolina, USA 27695
919-515-3184
susan_moore@ncsu.edu
www.ncsu.edu/feop

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FOREWORD

In November 2004, North Carolina State University hosted a joint conference of multiple working parties related to breeding and genetic resource management of IUFRO Division 2. The papers and abstracts that follow in this proceeding were presented at this conference entitled "Forest Genetics and Tree Breeding in the Age of Genomics - Progress and Future". This international conference brought together geneticists, breeders, applied and basic scientists, managers and professional foresters to exchange the latest information on forest genetics and tree breeding, with special focus on potential application of biotechnology and genomics in the future. Given that the topics were important, timely, and pertinent to scientists worldwide, a total of 231 people from 22 countries participated in this conference. The conference included invited, contributed presentations, and poster presentations. The main topics of the conference included:

- progress of major breeding and tree improvement programs,
- advances and challenges of clonal forestry,
- advances in forest biotechnology,
- advances in genomics and applications for tree breeding,
- genetic diversity and gene conservation,
- breeding strategies, progeny testing and selection strategies,
- advances in somatic embryogenesis technology and clonal forestry,
- social aspects of clonal forestry,
- genetic data analysis and modeling,
- advances in reproductive biology and seed orchard management,
- breeding for disease resistance,
- genetic gain modeling and prediction, and
- genetic improvement of wood quality,

Ninety-two presentations and 29 posters covered these topics. Some papers provided overview of the major tree breeding programs in the world and updates on the breeding strategies of advanced generations, clonal testing and selection strategies, top-grafting for accelerated breeding and genetic gain predictions. Other papers presented major breakthroughs in genomics and biotechnology research, such as genome sequencing, genotyping with markers (including SNPs, SSRs etc.), transcript profiling (DNA microarrays and RT-PCR), and metabolite profiling, genetic transformation and somatic embryogenesis for propagation. Genetic diversity and gene conservation were discussed for implications on breeding, biotechnology, deployment and forest resources management. Based on the up-to-date information, conference participants explored opportunities for integration of new genomics and biotechnology to major areas of breeding and genetic resource management.

As a part of the conference, an optional field trip was organized to visit MeadWestvaco Corporation and ArborGen. At MeadWestvaco, participants saw all aspects of breeding and tree improvement with loblolly pine (*Pinus taeda*), including breeding facilities, progeny and clone testing, vegetative propagation, greenhouse, seed orchard, nursery, and improved pine plantations. At ArborGen, forest biotechnology and genomics research was displayed, including genetic transformation, somatic embryogenesis, and gene discovery.

We express our appreciation to all invited speakers, moderators, contributing speakers, poster presenters, and conference participants for their contribution to a successful conference. We would like to thank our sponsors and the planning committee for their strong support to the conference.

Conference Organizers:

Bailian Li (Conference Planning Committee Chair)
Bailian_Li@NCSU.EDU
Steve McKeand (Local Organization Committee Chair)
Department of Forestry, Box 8002
North Carolina State University
Raleigh, NC 27695-8002
USA

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Concurrent Session III: Genetic Diversity and Gene Conservation
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A Look Back

Bruce Zobel[1]

It is a real pleasure and privilege to have the opportunity to welcome you to this conference. My first suggestion is that you relax. I am not going to recite a lot of data or complicated information but will just cover some of my observations related to nearly 60 years involvement in Tree Improvement. Unfortunately, I can cover only a few general principles relative to the application of genetics to forest trees.

In the 1950's and before, there was not much acceptance of forest genetics in forest management and there sometimes was outright antagonism shown by some foresters against the use of genetics. The common belief at that time was that a tree's characteristics and development were primarily the product of the environment in which the tree grew and its parentage was of little concern as a forest management tool.

The few concepts that I will describe are general and essentially apply to all species regardless of where the trees are grown.

1. All APPLIED and RESEARCH work involving genetics require FINANCIAL as well as OTHER SUPPORT. It is a FACT, that NO MATTER WHOEVER supplies the support WILL EVENTUALLY ASK THE QUESTIONS: "WHAT PROGRESS HAS BEEN MADE and WHAT ARE WE GETTING FOR OUR SUPPORT?" These questions will be asked by all organizations whether government, industry, education, individuals or other. The questions may come soon or they may be delayed, but they must eventually be answered if progress is to be continued. The answer is particularly difficult for forest genetics where many years may elapse before definitive results can be obtained no matter how good and intensive the research is.

Something new, such as forest genetics in the 1940s and 1950s, is often exciting with the initial support coming easily. This was especially true in applied tree improvement; in those years, after one overcame the basic resistance of the foresters to the use of genetics in forest trees, some supporters of genetics then became very enthused and acted as if genetics was to be a 'cure-all' for all forestry problems. A stage was reached where one no longer had to beg for support. However, it was then essential to carefully state, in effect, to the enthusiasts: "Down, boy, down!" and then carefully explain how genetics could be applied to forest management.

When I started the Texas Forest Applied Tree Improvement Program in 1951, it was evident that no positive results from the use of genetics would be quickly obtained. Therefore, in order to have some early results for our supporters, I designed the programs in Texas and later in North Carolina to heavily include aspects of wood properties that were valuable to the supporters of the genetics cooperatives. Results from working with wood could be obtained fairly quickly. Such things as wood density variation among trees, species and sites, its developmental trends and within tree patterns were of great importance to our supporters. Also of major interest was

[1] Professor Emeritus, Forest Management, N.C. State University, Raleigh NC.; Senior Associate, Zobel Forestry Associates, Raleigh N.C. Box 37398, 27627, Raleigh NC.

how this information could be used to improve forest products. After plantation forestry became widespread, special interest was also related to the presence and characteristics, as well as use of, juvenile wood. Our supporters were pleased with the early specific wood information and it allowed the time necessary for some genetic results to be obtained.

Too often, forest geneticists overlook the necessity for rapid information for their supporters and find that they no longer will supply the necessary funds and help to keep the program viable over a long period.

2. The second generalized need is TO KNOW YOUR SPECIES. This key fact is also often overlooked. One cannot really get to know a species unless 'one lives with it'. Once known, it is then possible to predict what will happen when action is taken or how the trees will respond to certain treatments. (One cannot get this necessary information by looking at a computer screen or by observing the species for only a short time). We had to do a lot of work and observation before we could properly handle our major species, loblolly pine and the eucalypts. (Some of my co-workers commented that: "He talks loblolly"). Many of our early activities were obtained from predictions made possible from knowing the species. Luckily, most predictions turned out to be at least partially correct, even though few facts were initially known as to what would happen when the work was done.

3. Related to knowing the species, is the necessity to TAKE ACTION BEFORE POSITIVE RESULTS are known. In forestry, TIME is MONEY. Whether or not facts are already known or proven, one is forced to act and cannot afford to sit around and wait for proven answers before action is taken. Such a policy is dangerous and 'chancy' but must be done if progress is to be obtained in a reasonable time.

A prime example of our first predictions was wood density. Knowledge of the variation patterns gave an indication that wood density was probably quite strongly inherited so we required it as a major selection criterion in all our seed orchards. My boss was 'horrified'! : "How can you include as mandatory a characteristic with unknown genetic response which will cost each supporter hundreds of thousands of dollars?" We were lucky on that one and wood density was found to be strongly heritable.

Sometimes ones ideas fail! Early in the 1940s we were very interested in rooted cuttings. We knew cuttings from mature pines were difficult to root while cuttings from juvenile trees often rooted well. I had the idea that perhaps if we grafted mature branches from mature trees onto seedlings, cuttings from the graft might have obtained some of the seedling's juvenile characteristics, so they would root easier. (Today physiologists laugh at such an idea but we had to try). Rejuvenation to the juvenile stage did not occur so the idea was a failure. However, since the grafted branches of mature trees on young trees maintained their physiological age just as if they were still on the original tree, we used this reaction to help in obtaining seed earlier from seed orchards. It was a major guide to seed production in grafts for seed orchards.

Another failed idea was related to yellow poplar (*Liriodendron*) which is insect pollinated, mostly by bees. In our studies we found that a whole hive of bees will work over a single tree by going from flower to flower on it, resulting in mostly selfed seed. We knew that

cross-pollinated trees grew better than the selfs. The question was how could we obtain a greater amount of cross pollination in the seed orchard. I had what I thought at the time was a great idea - we would graft several chosen good genotypes on the branches of individual seed orchard trees so the bees would do some cross pollination as they went from flower to flower on different branches. (This was after we tried the more usual methods like using pollen traps at the entrance to the bee's hive. This did not work with yellow poplar pollen). The method of grafting several genotypes to each seed orchard tree did not work because the grafts from the different genotypes grew at different rates and some produced few flowers or mostly males, and the grafts from different genotypes did not always flower at the same time. The hoped for great results from forcing cross pollination was a failure!

4. Always develop SIMPLE DESIGNS and REPORT RESULTS IN A SIMPLE WAY. There is a tendency, especially for young researchers, to develop complex studies and to report results in highly technical language. No matter how complex the study may be, it must be reported in plain language understandable to the supporters or to an audience. The study has to be designed to get a desired result. I have seen many good studies that were done correctly by competent people but were rejected because results were reported so they were not understood. One must report to the general audience and not to a few specialists that might be present. It is amazing over the years, how many good research projects I have observed that failed because results were reported in a highly technical manner that neither the supporters nor laymen could understand. Often this is done to 'snow' the audience as to the speaker's ability! After such presentations, I have had many people come and ask: "What was that person trying to say?" My answer usually is: "I do not know". Considerable of this type of thing will occur at this conference. The best advice is to adhere to the concept KISS - keep it simple, stupid! Enthusiastically tell what you have done and do not read your speech. (I always feel that I can read as well or better than the person on the podium).

Along with this is the presentation of results in tables or graphs. Many are unreadable. It is common to hear the speaker say: "Note the last line of the table which summarizes my results". More often than not, I sit in the audience and the last line is just a 'black line blob'. (Never project anything as small as typed material). Often the results are shown in graphs containing several study aspects so one cannot figure out what goes with what. This is especially bad when the speaker is in a hurry and takes the graph off the screen when one has seen only a part of it. Perhaps these errors are more evident at my age but they are common.

In establishing a study on seed orchards during my first years at Texas, I made the prime error! Being young and eager, I wanted to get as much information as possible for the work involved. I set up a seed orchard study involving (a) species, (b) kind of graft, (c) spacing, (d) fertilization, (e) cultivation, (f) sprays, (g) pruning and other. It appeared to me to be an excellent and economical test. About 10 years later, when I was in North Carolina, I had a phone call from Hans van Buijtenen in Texas, a good friend and my first graduate student who was then in charge of the Texas program. The conversation was short: all Hans said was "Bruce, I hate you". Later he explained that after all the work and care involved in the test, no usable results could be obtained. The test was so complex with so many variables and interlocking characteristics, that no usable results were found. That expensive multi-characteristic test should never have been established. It was humiliating! However, it is a common error.

6. I really should not start on a subject about past experiences because I could talk for hours, and days, about things learned in 60 years of intensive observation. However, there is one characteristic more than those mentioned above. It is to be ENTHUSIASTIC and SHOW IT. It really does not matter if one makes a small error in presentation; what will be remembered is that he/she surely loved their work. This is the best way to get the audience excited and to get your subject accepted. Real enthusiasm is rarely shown while reading a speech!

The best of luck in the following conference. It should be an enjoyable time where old friendships are renewed and new ones developed, as well as ideas exchanged.

Eighteen Years Later: Breeding Strategy – Don't Underestimate Simplicity

P. P. Cotterill[1] and C. A. Dean[2]

[1]ENSIS, Private Bag 10, Clayton South, Victoria 3169, Australia.
[2]Weyerhaeuser Company, WTC 1B10, PO Box 9777, Federal Way, Washington 98063, USA.

We have been asked to revisit a paper titled "Breeding Strategy: Don't Underestimate Simplicity" that was written by Paul Cotterill for the *IUFRO Joint Meeting of Working Parties on Breeding Theory, Progeny Testing and Seed Orchards* held at Williamsburg in 1986. Today, 18 years later, we ask how relevant are the themes of this 1986 paper? We examine how breeding programs may have changed since 1986; particularly in connection with clonal forestry.

Cotterill's 1986 paper focused strongly on a few simple messages regarding breeding strategy —

- It is much, much, much better to do a good job of a simple breeding strategy than to make a mess of something elaborate. The KISS principal ? Keep it simple; stupid!
- Genetic gain per unit time is "king". Breeding strategies that differ dramatically in cost and complexity can produce the same gains per decade. Excessively long generations will give low gains per decade regardless of the sophistication of the breeding program.
- Good management and through planning are "queen". Few would build a house without first having drafted a plan. Yet many tree breeders attempt to build the genetic structure of a species without first having a well documented breeding plan.
- Maximising response from tree breeding is basically a matter of efficient selection and short generations. Breeders were advised to focus on these factors when: (i) Financial, labour and capital are tight, (ii) resources are limited, and (iii) resources are unlimited.

We are reminded that the above messages remain very true today. Breeders are asked how many generations have they completed in the past 18 years? Genetic programs using advanced technologies need to be built on a strong foundation of traditional genetic improvement.

This paper also examines what has changed since 1986, particularly the strong push towards clonal forestry as a way of maximising genetic gain. Breeding strategies focused specifically on cloning are discussed.

Somatic embryogenesis offers huge potential for capturing genetic gain but can only be realised if coupled with a low cost delivery system. The relatively long time frame for gains from tree improvement to be realised in the harvest are a challenge for even the best-run programs. We have to pay attention to cash flow because we have to stay in business until the high NPV expectations from the investment in genetic improvement in forestry actually pay off.

Delivering on the Promise of Tree Improvement in the Southeastern US

T.J. Mullin, B. Li, and S.E. McKeand

Professors, Department of Forestry, N.C. State University, Raleigh, NC, USA

Fifty years ago, forestry in many parts of the world took a giant leap, when forest genetics pioneers proposed the application of breeding for important plantation species. Up to that point, the application of forest genetics had been largely limited to delineating patterns of variation and identification of appropriate provenances. The entry into applied tree improvement was made with the promise that gains, such as those being realized through agriculture's "Green Revolution", could also be made in intensively managed forest plantations. In the southeastern US, delivering on this promise became the mission of cooperative improvement programs where forest industries, and later government agencies and private investment corporations, pooled their energy and resources to carry out region-wide selection, breeding and testing programs. The North Carolina State University—Industry Cooperative Tree Improvement Program, founded by Bruce Zobel in 1956, as well as the other southern tree improvement cooperatives, have had a larger impact on planted forests than any other tree improvement programs in the world. Today, over 1 billion loblolly pine seedlings are planted annually on about 600,000 hectares in the South. Virtually all of these are genetically improved and about two thirds are the product of NCSU-ICTIP breeding efforts. Presently, about half of loblolly pine planted in the South originate from rogued 1stgeneration seed orchards, where average levels of improvement over wild stand checklots vary between 10 to 15% for stand productivity. The remaining loblolly planting stocks are progeny of 2nd-generation orchards with average gains between 15 and 35% over wild stand checks. Many cooperators now achieve additional gains through block deployment of single, well-tested open-pollinated families. Beyond gains in stem volume, the program has achieved important gains in rust resistance and stem quality. Today, members of the NCSU-ICTIP are taking new steps into deployment of control-pollinated crosses, vegetative propagules and somatic seedlings, to squeeze even higher returns from their breeding efforts. The NCSU-ICTIP has been a success story of university-industry-government partnership and has been widely emulated as a model to deliver on the promise of tree improvement. About to enter its 6th decade, the NCSU-ICTIP continues to refine its cooperative breeding program, even as it embraces new technologies and prepares to deliver on new promises offered by tree improvement in the age of genomics.

Strategies to Improve Operational Gains in Modern Tree Improvement Programs

Claudio Balocchi

Manager Genetic Division, Bioforest S.A (Arauco)
Concepción, Chile

The ultimate objective of a tree improvement program should be **to increase gain per unit of time.** This gain is normally predicted by genetic tests, where material of different improvement levels is compared, and is called **Realized Gain**. This gain does not necessarily predict the organization's gain at harvest time. The prediction of gain expected at harvest time requires the calculation of a weighted average based on the genetic quality of each planted hectare. Consequently, the final gain will depend on the genetic quality available in the program, and on the number of hectares planted with a specified genetic material. This expected gain called **Operational Gain**, will allow managers to estimate the harvest potential for their land-base and to evaluate the impact of the Tree Improvement Program. Consequently, operational gain, for a given silvicultural regime, depends on the Realized Gain in combination with the propagation strategy. The proper balance of these factors will allow organizations to maximize gains at harvest time.

The objective of modern Tree Improvement Programs should be to measure success by Operational Gains rather than by traditional Realized Gains. There are many examples in the world, where Realized Gains are much larger than the gains being obtained from commercial plantations. A major reason for this anomaly is the lack of efficient propagation systems for bulking up the best genetic material available in the genetics programs. For example, McKeand *et al.* (2003) reported that in the US South, most of the loblolly pine plantations are established with open-pollinated families; only 0.4% is established with full-sib families and there are no commercial scale plantations with clones, even though the high expected gains that have been shown for loblolly full-sibs and clones.
Modern tree improvement programs need to develop strategies to transfer these large predicted gains to operational plantations, so that the difference between Realized gains and Operational gains is minimized.

This paper will present examples with radiata pine in Chile and loblolly pine in Argentina of strategies to increase Operational Gains through the development, site allocation and multiplication of full-sib families and clones.

Breeding and Genetic Resources of Pacific Northwest Conifers: New Information Uses for the Pacific Northwest Conifer Trials in Europe and Western North America

S. J. Lee[1] and J.N. King[2]

[1]Project Leader for Conifer Breeding, Forest Research, Northern Research Station Roslin, Scotland;
[2] Research Scientist, BC Ministry of Forests, Research Branch, Victoria, BC, Canada

Western North America is a region very rich in conifer species. Many of these conifers are planted extensively on suitable sites over the northern and southern hemispheres including right across Europe (from Ireland to Turkey); New Zealand and Chile. In some countries on the western edge of Europe i.e. Ireland, Great Britain, Denmark and France, conifers from the Pacific Northwest are the mainstays of their forestry industry. The IUFRO Unit 2.02.05 : Breeding and genetic resources of Pacific Northwest (PNW) conifers, amalgamates all the previous individual species working groups: Douglas-fir (*Pseudotsuga menziesii*), Sitka spruce (*Picea sitchensis*), lodgepole pine (*Pinus contorta*) and the true firs (*abies spp*). Primarily a network of provenance trials, these were of particular value to European countries in determining the most appropriate species and provenances to plant in reforestation and aforestation involving exotic conifers from the PNW. The rangewide provenance collections and trials were also valuable to North American members in establishing seed movement guidelines and were particularly valuable in identifying populations for unique attributes. An example is the resistance to the white pine weevil that has been identified for Sitka spruce.

Currently though breeding programmes have moved on in their use of these species. In Europe many organisations have established first-generation breeding programmes and are self-sufficient in land-race seed often with extensive seed orchard programmes. These historic species and provenance trials are now being revisited to investigate their potential to satisfy new objectives and new information uses. Included in this is use of these large provenance collections planted across many sites to investigate and model climate change. This legacy has also provided unique populations to use genomics. Examples of genomics projects with Sitka spruce from the IUFRO collections are provided from both the UK Forestry Commission (Marker Aided Selection) and from a Genome Canada project at the University of British Columbia.

Genomics and Breeding of Low Elevation Mediterranean Conifers

Aravanopoulos F. A.[1], Bariteau M.[2], Isik K. [3], Schiller G. [4], Varela M.C. [5], Vendramin G.G. [6], Alizoti P.G. [1], Ducrey M. [2], Fabre J.P. [2], Fady B. [2], Isik F.[7], Korol L. [4], Lefevre F. [2], Ozturk H.[8] Pichot C. [2], Shklar G. [8], Siklar S.[8] and Teissier du Cros E.[2]

The state of the art of research regarding molecular genetics, genomics, as well as classical and molecular breeding in low elevation Mediterranean conifers is reported. In particular, pertinent research is reviewed in the following species and their interspecific hybrids: *Pinus halepensis* (aleppo pine), *P. brutia* (brutia or calabrian pine), *P. brutia* var. *agraphiotii* (agraphioti pine), *P. pinaster* (maritime pine), *Cupressus sempervirens* (common cypress), *Cedrus atlantica* (Atlas cedar), *C. brevifolia* (short-leaf cedar) and *C. libani* (cedar of Lebanon).

MATERIALS AND METHODS

Literature review was centered at the work produced in major forest genetics and breeding laboratories based on the Mediterranean basin. The collaboration of researchers from these laboratories who communicated the major findings of their work formed an integral part of this approach. Most of the published work that is reviewed herein has been extracted from searches using the Web of Science database and evidently has been published in eminent scientific journals of the field. The use of "gray" literature was generally avoided, except when no other information was available.

RESULTS AND DISCUSSION

Groups in Italy, Israel, Greece and Spain have studied *P. halepensis* extensively in terms of genetic variation using molecular markers. Population diversity, gene flow, major gene pools, migration routes and genetic mapping have been addressed. Most of the breeding research is centered in Greece (one provenance and two provenance-progeny tests, one seed orchard). Two clonal seed orchards exist in Turkey. In *P. brutia*, groups in Greece, Italy and Turkey have used molecular markers to address issues of population variation, gene flow and migration processes. A Greek group verified paternal inheritance of cpDNA. Extensive breeding programs exist in Turkey, where 47 seed orchards exist in addition to various provenance-progeny tests. In Greece, there are five provenance and one provenance-progeny tests. Three provenance tests exist in Israel, where the species has been introduced.

Two issues regarding Group Halepensis pertain to: (a) *P. brutia x halepensis* hybridization which has been studied and verified in natural stands in Greece and Turkey, while artificial hybridization was conducted in Greece; three parents-hybrid progeny trials and one hybrid seed

[1]Laboratory of Forest Genetics and Forest Tree Breeding, School of Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]INRA Unité de Rech. Forestières Méditerranéennes, Avignon, France, [3]Department of Biology, Akdeniz University, Antalya, Turkey, [4]Department of Natural Resources, Agricultural Research Organization, The Volcani Centre, Bet Dagan, Israel, [5]Estação Florestal Nacional, Oeiras, Portugal, [6]Plant Genetics Institute, Florence Division, National Research Council, Firenze, Italy, [7]North Carolina State University, Department of Forestry, Raleigh, North Carolina, USA, [8]Forest Tree Seeds and Tree Breeding Research Institute, Ankara, Turkey.

orchard exist in Greece, whereas elite hybrids have been verified with cpDNA markers and considerable heterosis has been reported, (b) the occurrence of a dwarf mutant variety with a high esthetic value and potential for growth under stress conditions (*P. brutia* var. *agraphiotii*) in Greece which was evaluated with genetic markers and a progeny test has been established. Regarding *P. pinaster* considerable research pertaining to within and among population variation, relationships, identification of major gene pools, gene and QTL mapping has been carried out with molecular markers in Spain and France, while substantial breeding work has been reported in Portugal (various provenance trials and seed orchards).

In *Cupressus sempervirens*, one of the most important Mediterranean conifers, genetic variation using molecular markers was studied in Greece, Italy and Turkey, while a gene and QTL mapping effort is underway in Greece; a marker tightly linked to a genomic region associated with tree form has been identified. Breeding has mainly focused towards resistance to the fungus *Seiridium cardinale*, in France, Greece and Italy. In Italy, various clonal trials for the evaluation of resistant material exist; some resistant clones are already in operational use. In Greece, three provenance-progeny tests, two clonal tests of resistant material and a seed orchard for the production of such material have been established. In France, one similar seed orchard and four provenance-progeny tests have been put in place. In the course of pertinent hybridization activities, a French group discovered for the first time paternal apomixis in plants, in particular in the relative north-African species *C. dupreziana.*

Regarding cedars (*Cedrus atlantica*, *C. brevifolia*, *C. libani*), molecular genetic variation has been studied in France and Turkey. Diversity parameters and taxonomical questions have been addressed. The most notable breeding efforts have been centered in Turkey where 11 seed orchards exist (*C. libani*). Various provenance-progeny tests exist in France (*C. atlantica, C. libani*), while in Greece two species-provenance trials (*C. atlantica*, *C. brevifolia*, *C. libani*) have been established.

CONCLUSIONS

Most of the reported work concerns genetic variation studies of the nuclear and cytoplasmic genomes (mainly cpDNA) with molecular markers, gene and QTL mapping (*Pinus pinaster*, *Cupressus sempervirens*, *Pinus halepensis*). Markers that can extend their use to genomic applications, such as ESTs and SNPs have been established in *P. pinaster* and *P. halepensis*. Relevant studies have shown a clear general trend: most of the diversity, major gene pools and presumed origin of the majority of low-elevation Mediterranean conifers lies in the south Balkan and Asia Minor peninsulas (mainly in the territories of Greece and Turkey). This is an area where selection for breeding and conservation of genetic resources must be a priority.

Genomic applications are limited compared to northern European or North American conifers, with the exception of *P. pinaster* and to a lower extend *P. halepensis*. There is an urgent need to extend these programs to the other low-elevation Mediterranean conifers given their characteristics and their importance in Mediterranean type ecosystems. Low-elevation Mediterranean species (especially pines) can form models for identifying candidate genes directly associated with adaptive diversity, particularly drought stress. In this respect, some

association was found between neutral and adaptive diversity, nevertheless no general trend has been observed.

Breeding programs are to a large extend depended on efforts at the national level. The are few exceptions regarding strong collaboration programs among Mediterranean countries, mainly through the realization of EU RTD projects; these programs proved to be highly successful and advanced pertinent research (e.g. FIREGENE, MED-PINES-CEDARS, FORADAPT). Breeding mainly focused on the species of Group Halepensis and improved seed is produced in most cases from 1st generation seed orchards. The process of parent selection for the establishment of 2nd generation seed orchards is underway for the maximization of realized genetic gain. It is concluded that more international collaborative research is needed, which is expected to create a high synergy among states and laboratories and accelerate breeding programs, while securing the conservation of genetic resources.

Swedish Tree Breeding for Norway Spruce and Scots Pine – Organization, Methods, Results

Per H. Ståhl[1] and Gunnar Jansson[2]

[1]Research director [2]Senior researcher
Skogforsk, Forestry Research Institute of Sweden, Uppsala, Sweden

The Swedish approach to long-term breeding is to integrate intensive breeding for general-purpose goals with gene conservation and preparedness for climatic changes.

Skogforsk, the Forestry Research Institute of Sweden, is responsible for all forest tree breeding in Sweden. The Institute provides interested orchard owners with orchard designs and suitable parent materials. The orchard owners then establish and manage the orchards, often with Skogforsk as their advisor.

A multiple breeding-population system is applied to spruce and pine. Meta-populations of more than 1000 parent trees are divided into some 20 breeding populations per species, each containing around 50 individuals. Each breeding population has a target area defined by photoperiod and climate. Long-term breeding is carried out in each breeding population. The selection of candidates is essentially on a within-family basis. A double-pair mating design is used and positive assortative mating is strived for. Progeny testing (for Scots pine) or clonal testing (Norway spruce) is used to increase the accuracy of the selections. The main goals of Swedish breeding are to increase the yield, adaptability and quality of the wood harvest, while safeguarding a necessary diversity in the breeding populations. The goals vary slightly from population to population

Research indicates that the breeding programmes are sustainable, robust, effective, and that the diversity losses are low. Based on analytical models and simulation studies, under normal genetic variation parameter assumptions, the breeding populations will for many generations retain large additive genetic variation for sustainable improvement as well as high level of allelic variation to meet unpredictable future changes in breeding goals and environment.

Genetic gain in production per hectare has been predicted to support decisions on the establishment of third-round seed orchards of Scots pine and Norway spruce. In general, the genetic gain of current seed orchards is 10-15%, and can reach around 25% in all new third-round seed orchards established with tested parents.

Current progress of tree breeding for *Cryptomeria japonica* in Japan

S. Kurinobu
Director of Breeding Division, Forest Tree Breeding Center, Japan

Abstract: Current progress of tree breeding for *Cryptomeria japonica*, a major plantation species occupying 45% man-made forest in Japan, is reviewed its research as well as operational tree improvement. The second generation plus tree selection has been started in genetic tests of around 30 years old with the involvement of private foresters to utilize rooted cuttings for a rapid realization of the operational use. They tend to select trees with very straight stem rather than general growth due to the priority trait to determine market price. Measurement in clonal tests of the first generation plus trees is still continuing to evaluate their growth pattern as well as their wood property to identify the best-suited clones to the long rotation forestry. The pattern of growth until 30 years of age was well agreed with the empirically known growth habit obtained through the long history of cultivation with local varieties. Wood quality of *Cryptomeria* is known to be considerably variable in module of elasticity, heart wood color and moisture content; fortunately for the breeders, they are generally under strong genetic control indicating a good potential of upgrading its wood quality through genetic improvement. Recently, the wide spread plantations of *Cryptomeria* has been recognized as a major cause of seasonal allergic disease (pollinosis); hence the breeding to develop the *Cryptomeria* with less pollen/allergen is strongly demanded. According to the current research, the selection of *Cryptomeria* with less pollen/allergen seems promising owing to its fairly large genetic variation in the first generation plus tree population.

Introduction

Cryptomeria japonica is an indigenous conifer species in Japan and has been planted for more than 400 years (Miyajima 1989). Sugi, Japanese name of *Cryptomeria*, means “Straight tree”. Straight bole, fast growth, good self-pruning ability and wide range of distribution enabled this species as leading plantation species in Japan. Total area of *Cryptomeria* plantation is around 4.5 million ha, 44% of man made forest. This means 20% of forest area or 12% of Japan is covered with *Cryptomeria* plantation.

Production purpose of *Cryptomeria* plantation is to supply sawn timber for house construction. Straight bole and good wood working property are the advantages with this species, however, it is variable in its durability and moisture content. These shortages have become the recent target of tree improvement. Attempts of use large laminated wood is getting popular to make full use of existing resources of *Cryptomeria*.

First generation tree breeding for *Cryptomeria*

Tree breeding for *Cryptomeria* was started in the early 1950’s by plus tree selection (Toda 1979). Plus trees were selected based on their pheotypic growth and tree form. Plus trees of *Cryptomeria* were selected in all of the five breeding region, among those the share of Cryptomeria is the largest in Tohoku, Kanto, Kansai and Kyushu. Total number of plus trees is 3,659 for *Cryptomeria* (Forest Tree Breeding Center 2003).

Clonal seed orchards were established with the grafts of plus trees at 151 sites of 392 ha. Scion gardens were established at 146 sites of 244 ha, however, many of them are not in use operationally except for those in Kyushu region. Plus trees were tested in the field with their rooted cuttings as well as progenies from the orchards. As of 2002, 1,182 tests of 1,453ha were established (Forest Tree Breeding Center 2003) and approximately half of them are clonal tests. Seed orchard improvement is now under way by roguing plus tree clones that produce poor progenies.

Clonal seed orchards and scion gardens that were established 30 years ago have reached a stage of full production. In spite of the increase in their production capacity, demand of seedling for reforestation has been decreasing in these 20 years. Current demand of *Cryptomeria* seedling for reforestation is around 14 million to cover 6,500 ha per year (Forest Agency 2003). The share of improved seed and seedling is increasing up around 70% of those used for the operational reforestation.

In order to establish base population for the second generation plus trees, controlled pollination has been conducted for these 20 years in each breeding region. Plus trees were grouped by target traits of improvement: growth, wood quality and etc., then they were mated with disconnected half-diallel or factorial design. Controlled pollinations are made in breeding orchard as well as potted rooted cuttings in green house. Approximately 50 tests have been established so far, however, the search for an appropriate test site is getting difficult due to rapid reduction in reforestation area.

Second generation plus tree selection

The second generation plus tree selection has been conducted in several genetic tests of around 30 years of age (Kurinobu and Chigira 2000, 2002). Experienced foresters were invited to the tests to choose good trees according to their own preferences. This is because the foresters are supposed to know the good trees based on their experience and they are also the users of improved seed. The selections by the foresters will be further examined on their wood properties, then those clones will be tested with their rooted cuttings in the field.

Foresters tend to select better growing trees, but the growth was not the first priority trait. Trend of the selection in three tests was examined with retrospective selection index. Stem straightness found to be the first priority traits, followed by d.b.h. and bottom stem crookedness (Kurinobu and Chigira 2002). This is probably because the log price with this range of diameter is much dependent on stem straightness.

Heritabilities were moderate ranging from 0.15 to 0.3 and no apparent difference among the three traits. Relative gains were around 10% for stem straightness, while the gain in d.b.h. seemed less than 10% (Kurinobu and Chigira 2000). Thus the foresters' intention to improve stem form would be achieved with this selection.

Selection until this stage is no more than a phenotypic selection. Breeding values should be predicted for each of the selected trees to evaluate their genetic potential. Large differences in BLUP were observed among the selections. Based on their BLUP and their pedigree records, trees to be used for the next generation of breeding would be determined.

Optimum selection age for long rotation forest management
Optimum selection age for long rotation forest management was examined from a different aspect of gain per year. The rotation age in Japan has become longer, currently more than 60 years from the previous 40 years, because of unfavorable economic situation in forestry as well as the demand to increase carbon sink function of the plantation. The differences in growth pattern among local cultivars have long been recognized empirically in Japan (Toda 1979, Miyajima 1989). For this reason, 30 years' trend of growth for each clone in three clonal tests was fitted to the Richards function of three-parameter model. The analysis was made to estimate upper asymptote (A), growth rate (b), while shape parameter c was kept as constant (Kurinobu and Toda 2000).

Clonal variations in upper asymptote (A) and growth rate (b) were both statistically significant. As a result of investigation on the relationship between the size of parameters and growth pattern in height-age chart, clones with high growth rate are generally better during the early period, but those with higher asymptote will surpass in the later stage of rotation. Therefore mild selection intensity would be recommended to retain plus trees of continuous growing type. Another 10 years' observation would be necessary to confirm the result of this case study.

Wood property study
Even with the straight bole and good wood working quality, *Cryptomeria* is known heterogeneous in module of elasticity and moisture content. Recent studies using logs from older clonal tests revealed that both module of elasticity and moisture contents are under strong genetic control; relatively high heritability and less genotype environment interaction (Fujisawa et. al 1994, 1995). Since genetic variations on both traits are sufficiently large in the plus tree population (Hirakawa et. al 2003), good amount of improvement could be achieved by selecting plus tree clones that satisfy criteria for the both traits.

Pollinosis problem caused by *Cryptomeria* plantation
Pollinosis caused by *Cryptomeria* pollen has become a serious social problem. It has long been recognized there is a large variation in male flowering ability among plus tree clones. To meet this social needs, plus trees with less male flowering were selected and they are recommended for reforestation (Senda and Kondo 1998). Measurement of allergen contents, direct cause of pollinosis, Cry J1 and Cry J2, are also under way (Goto et. al 1999, 2003, 2004), thus plus trees to meet social demand will be identified in near future.

Biotechnology research for *Cryptomeria*
RAPD, AFLP, CAPS and SSR markers are developed for clonal identifications (Hirao et. al preparation) and QTL analysis: wood property on MOE, wood specific gravities, percent of late wood (Kuramoto et. al 2000). Embryogenic tissue was induced from immature seeds, then germinates were obtained from the induced somatic embryo. GPF gene was successfully transformed to embryogenic tissue (Taniguchi et. al 2004).

Future directions of deployment
From the operational aspect, basic directions of tree breeding for Cryptomeria would be summarized as follows,
1. To develop second generation breeding population

Selection for the second generation plus trees should be continued in genetic tests and also in OP progeny tests until sufficient number of plus trees is secured in each region, then they are evaluated with clonal tests as well as predicting BLUP to choose mating parents for the third generation breeding population.

2. To complete measurements in first generation tests

Measurements on growth and wood quality in clonal tests and progeny tests would be conducted in another ten or more years to identify clones to meet various production purposes of forestry, the data obtained with this measurements would be used as basic information for evaluating second generation plus trees.3. To meet the Social needs

Clones to cope with pollinosis and CO_2 sink could be selected from the current plus tree population, while in the future it may be necessary to develop specific breeding population to attain those purposes.

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Clonal Forestry: Yesterday, Today and Tomorrow

W. J. Libby[1]

Abstract

Purposeful cloning of trees can be traced back 5,000 or more years. However, there is a difference between using clones and practicing clonal forestry. So-called "full clonal forestry" involves using well-known clones, thus allowing management to use that knowledge to adapt and prescribe details of propagation, nursery management, deployment assignments, silvicultural treatments and schedules, harvest sequence, and end uses for each particular clone or cultivar.

The first recorded example of purposeful forest-tree improvement and clonal forestry was in China, with Chinese-fir about 800 years ago. Local farmers established new plantings by cloning their best trees, thus developing excellent locally-adapted clones. This was followed by development of sugi cultivars in Japan beginning about 600 years ago, and plantation forestry with poplars beginning over 400 years ago.

Starting in about 1950, the principles of clonal forestry began to be applied in seed-orchards. Clonal plantations of Norway spruce, radiata pine and flooded gum soon followed in northern Europe, Australasia and Brazil. In the latter half of the 20th Century, technologies for clonally propagating forest trees advanced through a better understanding of maturation, and through the development of tissue-culture and somatic embryogenesis.

Recognizing that these advances in understanding and technology were making cloning available for many more species, the Canadian Tree Improvement Association focused its 1983 meeting on clonal forestry. It was argued during that meeting that this would revolutionize forestry. This idea spawned a two-volume book on clonal forestry, summarizing the underpinning science and its applications, and several books and meetings on propagation technology.

It has recently occurred to me that what was new and revolutionary was not cloning, but forestry. Prior to a few hundred years ago, when the English and French began to grow trees for naval timbers, forestry as recently understood and taught was rarely practiced. "Foresters" were in reality mostly game managers, tending the forest for the recreation of the rich and noble. In an apparent return to this pre-forestry period, after about a century of offering professional forestry degrees, many universities have changed the names and foci of the relevant departments and schools from "forestry" to things like "wildlife biology" and "environmental science".

In the field, many decisions concerning forests are now made by wildlife biologists, hydrologists, archeologists, and recreation specialists. Organizations such as the Sierra Club, composed mostly of rich and noble people, are succeeding in focusing the mission of the U.S. National Forests back onto recreation and wildlife. Meanwhile, people continue to need and use wood and products made of wood. It appears that wood will be increasingly supplied from intensively managed plantations. It is to such plantations that clones bred, selected and/or engineered for better, more efficient wood production will be deployed. Are these best managed by foresters, or by a new breed of agronomists?

In the foreseeable future, whether we call it forestry or agronomy, clones will become the norm for intensive plantation management of many important forest-tree species. This will be supported by continuing advances in propagation technology and by ongoing tree-breeding programs, and may be catalyzed by the ability to genetically engineer specific clones.

Expanding on the Abstract

Given the relatively short time for this talk, I'll expand on just two of the items covered in the Abstract.

Chinese-fir

Chinese-fir, a redwood relative, has long been one of China's most valuable tree species. Like redwood, it sprouts from the stump. Like the early fruit, nut and oil domesticates, cuttings from these sprouts readily root when inserted into moist soil in appropriate conditions. Starting about 800 years ago, farmers in southeastern China would take note of the better Chinese-firs on their land. After those trees were cut, vigorous sprouts would originate from the stumps. The tops of those sprouts could be successfully planted as unrooted cuttings, while their already-rooted basal portions could be detached and then planted. The farmers would thus establish new groves on their lands from their best trees. In this manner, excellent locally-adapted clones were selected and used in their respective local regions, across 16 Chinese provinces with greatly different environments. As far as I know, this is the first recorded example of purposeful forest-tree-improvement and clonal forestry, although the Chinese farmers of eight centuries ago called it neither.

During the 1950s and 1960s, the politically inspired theories of the Russian plant physiologist, T. D. Lysenko, influenced Chinese forest policy. The use of those long-selected well-tried clones was officially discontinued, and some were lost. Politically-correct forest biology insisted that "more natural" seedlings be used for reforestation of Chinese-fir. Luckily, many remote farmers ignored orders from authorities in Beijing and today most of the clones are still available and again being appropriately used.

This is an excellent example of damage done by political orthodoxy intruding on forest science and forest practice. The politically mandated seedling plantations have grown unevenly with variable wood quality, substantial insect and disease damage, and reductions in harvest productivity. Where farmers such as the local Miao peoples of Hunan Province ignored or defied political biology, their recent clonal plantations continue to have excellent wood, and no observed insect or disease damage.

Clonal forestry?

As I was exploring the history of clonal forestry, it was soon abundantly clear that the history of cloning trees goes back several thousand years. It dawned on me much more slowly that what was new was not the cloning of trees, but forestry.

I'm not sure how far back forestry as we commonly understand it goes in places like China, but what records I've come across indicate that it was farmers, not foresters, who first began practicing with Chinese-fir what today we call "clonal forestry". We tend to trace the history of western forestry to western Europe.

By the 12th and 13th Centuries in Europe, local officials were regulating how much and where the peasants could harvest firewood. Gamekeepers tended the forests as places of sport hunting and other recreation for the rich and noble landowners. In the 16th Century, the beginnings of applied forestry practices began and evolved, and government agents began to reserve outstanding trees for such things as naval uses.

Modern forestry, as defined by management of forests for the continuous production of goods and services, didn't appear in Europe until after 1800 and, a half-century later, precursors to modern forestry began in North America. However, hunting-and-gathering, which for food had long been replaced by agriculture in western Europe and the Americas, remained common for wood in the forests. Trees that were useful were sought and harvested; those that were not useful were left, often to become the parents of the subsequent forest. Such highgrading, or creaming, remains the case in the majority of Earth's forests today.

Historically, as a culture ran short of wood, a common response was to acquire the wood elsewhere, often by conquest. This response was practiced, for example, by Babylon's King Hammurabi 4,000 years ago. An interesting exception was practiced in Egypt 2,300 years ago, when the Ptolemaic Dynasty built government nurseries and sponsored massive tree-planting programs. Legal protections were given to game and some trees by the Roman Empire, but it seems unlikely that foresters administered those laws. For many centuries, clones of easily rooted poplar and willow have been planted in Asia, the Middle East and around the Mediterranean. Their purposes were for erosion control, basketry and to provide fodder and bedding for animals. Those plantings were not managed by foresters either, and it would be a stretch to call those generally incidental plantings "clonal forestry".

In recent centuries, various countries have embarked on tree-planting programs and have established plantations. In the 1700s, England was planting and growing oaks for naval timbers. In the 1800s, Germany in particular established large plantations of conifers to offset regional depletion of native forests. In southwest France, 800,000 hectares of maritime pine plantations were established, both to create a resin-tapping industry and to stabilize coastal sand dunes then encroaching on agricultural lands. In the early 1900s, several Southern Hemisphere countries, most notably South Africa, Australia, New Zealand and Chile, began establishing extensive plantations of non-native tree species to avoid depletion of their native forests, and to generate export industries. In mid-20th Century, pine plantations were replacing cotton, tobacco, other agronomic crops, and harvested "old-field" pine stands in the southeastern U.S.; Douglas-fir and pine were being planted in the western U.S. to reforest after clearcutting or stand-replacing wildfires. It is when and where such planting programs are established that the possibility of using clones becomes attractive.

It is generally agreed that increasing percentages of the world's wood will be supplied by intensively managed plantations, while wild or extensively managed forests will primarily serve

such other human goals as producing high-quality water and air, as wildlife habitat, and for aesthetics and recreation. In recent decades, much of the power affecting on-the-ground decision-making in extensively managed forests in the western U.S. passed from the logging boss, then resided briefly with professional foresters, and then quickly moved on to hydrologists, modern gamekeepers (now called wildlife biologists), and modern forest stewards (now called park managers and recreation specialists). Many universities have abandoned forestry curricula, or they have morphed and renamed their curricula to serve environmental science, wildlife biology and similar interests. These changes reflect the interests of the students as well as a hard-eyed view of the future of forestry by faculty and deans.

Do foresters have a role in intensively managed plantations? Or would these plantations be better established and managed by people trained in such disciplines as agronomy and horticulture? Does the use of clones in such intensively managed plantations qualify as clonal forestry, or might it better be described as an important component of a relatively new and intensive form of agriculture? With respect to the production of wood to meet future human needs, I think clones are here to stay, but forestry may have had its honorable but brief run.

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[1] William Libby is a senior associate with Zobel Forestry Associates, Cary, N.C.

Clonal Forestry with Radiata Pine

M.J. Carson[1], S.D. Carson[1], J.D. Hayes[1], and C. Te Riini[1]

Carson Associates Ltd.[1], 34 Parawai Rd., Rotorua, New Zealand.

True clonal forestry with radiata pine, and with pines in general, is only just becoming an operational reality. Although cloning of pines has been practiced for many years, the high costs of propagation, coupled with problems associated with retaining juvenility during the clonal testing period, have inhibited progress in the application of full-scale clonal forestry. Recent large investments in both somatic embryogenesis (SE) and cryopreservation technologies have led to the development of reliable clonal storage systems, capable of holding clones in juvenile condition almost indefinitely. Also, SE either alone, or in combination with other forms of vegetative propagation, can now be used to reliably propagate and multiply selected, performance-tested clones from a full range of elite families, at a cost that is increasingly competitive with other, seed-based options.

The potential benefits of clonal forestry of pines have been recognized for many years, including: direct selection gains in yield, log and wood quality and health traits, indirect selection gains from optimal matching of clonal attributes to site and silviculture, and perceived gains from added uniformity of clones when grown in pure stands. However, the requirement to justify marginal investment in clonal forestry at an estate and stand level has necessitated a much greater emphasis both on researching the impacts of genetic change on regime profitability and wood value, and using the traditional tools of investment and risk analysis to determine and quantify the impacts of clonal gains. In general, these analyses have confirmed the feasibility of large-scale clonal forestry.

Clonal forestry poses some interesting challenges for radiata pine growers. Maintenance of genetic diversity against pests and diseases will trade off against the pursuit of selection gains in deployment decisions concerning numbers of clones, and whether to deploy them in sets or as pure stands. Models for predicting growth rate and quality will need to be validated and/or adjusted for clones, and for clonal uniformity, but there should be rewards in increased repeatability and predictability of clonal performance, particularly for high-heritability log and wood quality traits. In addition to delivering higher genetic gains from conventional breeding, clonal forestry will provide an ideal platform for applications of new biotechnologies, including marker-aided-selection and gene transfer. There is potential for clones to greatly enhance the profitability of pine plantations, with the cautionary note that this can only be achieved with excellent establishment and management practices, and well-defined breeding objectives.

Advances and Challenges in Clonal Forestry with Rooted Cuttings of Loblolly Pine.

Barry Goldfarb[1], Anthony LeBude[2], Fikret Isik[3], Steven McKeand[1] and Bailian Li[1]

[1]Professor, [2]Research Assistant, [3]Research Assistant Professor,
Department of Forestry, North Carolina State University, Campus Box 8002, Raleigh, NC 27695-8002 USA

Implementing clonal forestry for loblolly pine using rooted cuttings requires several necessary elements: (1) the gains from selecting and deploying clones must be large enough to justify the additional costs; (2) propagation systems must be reliable and cost-effective; and (3) the juvenility of clones must be maintained for the time necessary for selection, multiplication and deployment.

To investigate genetic gains, a clonal field-test experiment, with 450 clones from eight, unrelated full-sib crosses was established in 1998. Heritabilities for clone genetic values are moderately high for volume and height and very high for fusiform rust resistance. Heritabilities for form traits, such as number of forks, branch angle and bole straightness were also moderately high. Predicted volume gains (based on fourth-year results) from within-family selection were substantial, approaching 40% above the overall clone means, depending on the number of clones selected. Additional field tests to verify the clonal rankings obtained from the first test are underway.

To make the rooting process more reliable and cost-effective, we have been studying environmental variables and cutting physiology. Results to date indicate that moderate levels of water deficit (xylem water potential) stimulate rooting in loblolly pine cuttings. For large-scale production in different rooting environments (greenhouses, shadehouses and nursery beds), controlling irrigation according to environmental sensing represents a good possibility for automating propagation procedures. Vapor pressure deficit (VPD), which can be monitored automatically by measuring temperature and relative humidity, was closely correlated with rooting success in two experiments. Experiments are underway to empirically test misting regimes in response to VPD.

Results from a long-term clone maturation experiment show that rooting performance undergoes an initial decline two to three years after seed germination. However, only a slight decline is seen thereafter, through nine years of age. On average, cuttings from clones of a good-rooting family rooted at commercially acceptable levels nine years from seed. Rooted cuttings from clones two through eight years of age were planted in a field trial. After two growing seasons, differences in height among clone ages were small, although there was a suggestion of growth depression with increasing age. If rooting ability or growth rate declines below acceptable levels in older clones, cryopreservation of somatic embryogenic cultures could be used for juvenility maintenance and generation of stock plants, combined with a rooted cutting system for producing reforestation stock, if this proves to be the most cost-effective option.

Genotype ´ Environment Interaction in Clonal Tests of Slash x Caribbean Hybrid Pine

M.J. Dieters[1], D.A. Huber[2] and R. Peters[3]

Abstract: In Queensland, the testing and deployment of *P. elliottii* × *P. caribaea* var. *hondurensis* is based on the testing and multiplication of clones within elite families, using vegetative propagation via cuttings. A new series of clonal tests is initiated every 4-5 years from control-pollinated seed of elite hybrid families, seedling ortets are raised as hedge plants with shoots collected and set as cuttings for establishment of field tests and clonal (hedge) archives. Hedges are intensively managed to restrict maturation while clones are being evaluated in field tests. There are currently four Series of clonal tests (each Series initiated with the best available hybrid families) established in Queensland: the first field tests of each Series were planted in 1986, 1994, 1999 and 2003, and contain approximately 250, 480, 1200 and 2000 different clones respectively. Clones selected in the Series II tests are being deployed across approximately 4000 ha/year in southeast and central Queensland. Operational use of the Series II clones will be phased out from 2006 when elite clones from the Series III tests will become available for deployment. Optimisation of this testing and selection process is important to minimise costs and maximise economic returns from clonal forestry in Queensland.

The Series II clonal tests were established from 1994 – 1998 across a total of 22 sites in Queensland and northern New South Wales, encompassing a broad range of sites with a view to investigating the stability of clonal performance across sites. For the purposes of this paper we focused on analysis of data through to 6 years of age from 12 tests established between 1995 and 1997, which are located on near-coastal sites in southeast and central Queensland. These 12 tests included the largest number of clones, were most balanced in terms of clonal representation across sites, ane are most representative of the primary target environments where hybrid pine clones are currently deployed. Across this range of test sites very little genotype × environment (G×E) interaction was found, indicating remarkable stability of clonal performance across the major plantation regions of southeast and central Queensland. The importance of G×E was greatest when the trials were young, but was virtually non-existent by 4 years of age. Results of from these analyses were used to examine key questions about the management and design of future clonal tests in Queensland.

Keywords: Clonal forestry, genotype x environment interactions, *Pinus elliottii*, *Pinus cariabea* var. *hondurensis*, hybrids

[1] Senior Lecturer, School of Land and Food Sciences, The University of Queensland, St Lucia, QLD 4072 Australia
[2] Co-Director CFGRP, School of Forest Resources & Conservation, University of Florida, Gainesville, FL, USA
[3] Technical Officer, DPIF-Forestry, Fraser Road, Gympie QLD 4570, Australia

INTRODUCTION

In early 1991, Queensland forestry embarked on a program to deploy the interspecific hybrid between *Pinus elliottii* Engelm. var. *elliottii* Little & Dorman (PEE) and *P. caribaea* Mor. var. *hondurensis* Barrett & Golfari (PCH) on all suitable sites of the exotic pine plantation estate in southeast and central Queensland. At that time, it was anticipated that the hybrid would be deployed as seedlings or cuttings of elite F_1 families. The difficulty and cost associated with the production of F_1 hybrid seed, meant that the scarce and expensive hybrid seed was usually multiplied vegetatively by cuttings for plantation establishment. However, due to the development of maturation, the initiation of roots on cuttings collected from family hedges declines progressively with increasing hedge age. This required the replacement of family hedges every 4 – 5 years. The combined effects of low production of hybrid seed and the need to regularly replace family hedges meant that there were never sufficient hybrid plants to meet the requirements for plantation establishment.

These problems with the implementation of family forestry, led to the consideration of alternative approaches to the deployment of hybrid pine in Queensland – in particular the deployment of tested clones, i.e. clonal forestry. The implementation of clonal forestry offered the combined advantages of: selection within the best families to capture additional gain over family forestry; greater uniformity in the plantations; potential to select for traits which are expensive to measure accurately such as wood properties; and, selection for propagation traits to reduce the adverse effects of maturation in the nursery (e.g. Haines 1993). Following a clonal forestry workshop in December 1993, Queensland forestry adopted a policy which aimed to move from family forestry with the F_1 hybrid between PEE and PCH to 100% deployment of tested hybrid clones on all suitable sites in southeast and central Queensland by 2002. Funding was made available to support a detailed research program to deliver elite clones for commercial deployment by 2002, but with the secondary aims of investigating many issues associated with the implementation of clonal forestry, such as: optimal age at which elite clones could be selected; the stability of clonal performance across sites; the effects of spacing and silvicultural practices on clonal performance; and, the effects of deployment as mixtures vs. monoclonal blocks. This lead to the establishment of the Series II clonal tests: 479 clones in the first two field tests in 1994 at Beerburrum and Tuan, with a subset of approximately 225 clones planted in a further 7 field tests in 1995, and 7 field tests in 1996[4]. The selection of these test sites spaned the likely range where the hybrid could be planted commercially from Byfield (lat. 23º40'S) to Casino (NSW, lat. 29º13'S), from low elevation sites near sea level to 'high' elevation sites at around 720 m. The aim was to provide a set of field tests that could answer two key questions (Walker et al. 1996): how stable is clonal performance across sites; and what is the optimal age of clonal selection?

This paper presents results of the analysis of data to 6 years of age collected in 12 Series II clonal tests located on near-coastal sites in southeast (SEQ) and central Queensland (CQ). These sites are the most representative of the primary target environments where hybrid pine clones are currently deployed commercially, included the largest number of clones, and were most balanced in terms of clonal representation across sites. Results of these analyses are then used to provide

[4] A further 6 tests were established between 1996 and 1998 using line plots or block plots of a smaller number of clones that had performed well in the earlier tests.

indications of the optimal design for future clonal tests – number test sites, number of ramets/clone/test, and numbers of families vs. number of clones/family. Analysis of the data was undertaken at the University of Florida (June – August 2002) under a travel grant provided by the Australian Academy of Science.

METHODS AND MATERIALS

Genetic composition of clonal tests: Seeds from a total of 85 hybrid families (78 F_1 and 11 F_2 hybrid families) were sown in spring 1992. These families were selected based on the known performance of the hybrid families or their parents in hybrid progeny tests, from amongst all hybrid families in the seed-store at that time. Hybrid families were not produced specifically for the purpose of establishing this clonal test series. From the 85 hybrid families sown, 479 clones from 48 families (39 F_1 and 9 F_2 hybrid families) were then selected (based on rooting ability of clones within families) for inclusion in the Series II clonal tests. Only clones with 100% root initation were included in the field tests, resulting in 4 to 16 clones representing each of the 48 families, with an average of 10 clones/family. As this was primarily an operational clonal test, aimed at the identification of elite clones for commercial deployment, the mating design was highly unbalanced, including up to 12 crosses on one parent, and both full-sib and half-sib (polycross) families.

Experimental design and traits measured/assessed: The 479 clones included in the clonal tests were divided into 10 sets, with (on average) one clone from each family assigned randomly to each set. As the representation of clones within families was not balanced, the representation of families within sets also varied between sets. These ten sets were planted as separate plots of 48 trees within each replication of the Stage 1 tests planted in 1994 (Table 1). A single tree within each replication represents each clone within each set. Approximately 50% of the clones were included in the Stage 2 and 3 tests (Table 1), based on the performance of the clones in the earlier tests.[5] Therefore, the 10 sets were collapsed into 5 sets, by combining sets 1 and 2, 3 and 4, … 9 and 10. Again each set was represented by a plot of 48 trees in each replication at each site, with each clone within each set represented by a single tree within each replication. Further details of the experimental design, location of tests, and site details are summarised in Table 1.

The Stage 1 tests were measured more intensively than the subsequent Stage 2 and 3 tests (Table 2). All surviving trees were measured. At 6 months after planting the Stage 1 tests were measured for height and diameter at ground level (DGL), and then measured annually from 1 to 6 years of age for diameter and height. DGL was again measured at 1 and 2 years of age, and from 2 – 6 years of age diameter was measured at breast height (DBH, 1.3m above ground level). DBH and HT were used to calculate conical volumes (VOL) of each surviving tree (dm^3). A range of other traits were also measured in these clonal tests (e.g. frost damage, double leaders, branch size and angle, and stem straightness); however, we will only deal with the growth data in this paper.

Statistical methods and analysis of data: The analysis of the data from this series of clonal tests presented a number of difficulties. Firstly the families included a mixture of full-sib and half-sib families of both F_1 and F_2 hybrids. Secondly, many of the parents used were themselves related

[5] Note that slightly different clones were included in the stage 2 and stage 3 tests.

through previous generations of mating. And lastly, the clones were allocated to different sets within replicates. Therefore, analysis must account for clonal replication, allow full-sibs and half-sibs to be analysed in a single analysis, adjust the data for relationships among the parents and incorporate the set effects.

Table 1. Summary of Series II clonal tests in Stages 1-3 located on near-coastal sites in southeast and central Queensland.

Site No.	Stage	Region	Date Planted (mm/yy)	No. Reps.	No. Clones Tested	Slope Position	Annual Rainfall (mm)	Altitude (m ASL)	Latitude (ºS)	Longitude (ºE)
1	1	Tuan	04/94	5	479	MS-Ridge	1295	25 m	25º 42' 30"	152º 49' 30"
2	1	Beerburrum	05/94	5	479	Swamp	1665	25 m	26º 50'	153º 01'
5	2	Tuan	07/95	3	234	Ridge	1295	45 m	25º 43' 52"	152º 46' 45"
6	2	Tuan	07/95	3	231	LS-MS	1295	40 m	25º 44' 22"	152º 46' 30"
7	2	Toolara	07/95	3	236	Ridge	1340	60 m	25º 58' 45"	152º 52' 10"
8	2	Beerburrum	07/95	3	227	MS-US	1665	30 m	26º 58' 05"	152º 58' 25"
9	2	Beerburrum	08/95	3	229	Swamp	1665	16 m	26º 56' 05"	152º 59' 00"
12	3	Tuan	07/96	3	223	MS-US	1295	15 m	25º 41' 45"	152º 50' 10"
13	3	Byfield	07/96	3	222	Ridge	1650	40 m	25º 40'	150º 40'
14	3	Toolara	07/96	3	223	US-Ridge	1340	60 m	25º 58' 45"	152º 52' 10"
15	3	Beerburrum	07/96	3	221	Swamp	1665	10 m	26º 55' 35"	153º 02' 15"
16	3	Beerburrum	07/96	3	222	MS-US	1665	45 m	26º 57' 55"	152º 55' 10"

Note: The Byfield test (site number 13) is the only test located in central Queensland; Slope abbreviations – LS = lower slope, MS = mid-slope, US = upper slope.

Table 2. Summary of growth traits measured in the clonal test. A check mark (✓) indicates that all tests within a Stage were measured for diameter and height at the age specified.

	Measured traits/Age							
Stage	DGL 06	DGL 1	DGL 2	DBH 2	DBH 3	DBH 4	DBH 5	DBH 6
1	✓	✓		✓	✓	✓	✓	✓
2	✓	✓	✓		✓	✓		✓
3		✓		✓	✓			✓

Notes: Measurement age indicated as 06 = six months, 1 = 1 year, 2 = 2 years, etc.; DGL = diameter at ground level, DBH = diameter at breast height; Tree height was measured whenever diameter was measured; DBH and HT used to calculate conical volumes of each tree.

Preliminary analyses of the data from each site were undertaken in SAS (Proc VARCOMP) to determine if there was any reason to treat the F_1 families separately from the F_2 families – no significant effect of cross-type (i.e. whether an F_1 or F_2 family) was identified in the preliminary analyses of growth traits in the Stage 1 tests, therefore cross-type was ignored in all subsequent analyses. Secondly, the estimated variance components from SAS were compared with estimates

obtained from a new version of GAREML (developed by Dr. Dudley Huber), "NewREML10". This version of GAREML is suitable for the analysis of clonal data, and fits an additive relationship matrix so that full-sib and half-sib families can be analysed together, and to provide adjustments for estimates of the variance between families due to ancestral relationships among the parents. Estimates from GAREML provided comparable estimates to those obtained from SAS, and adjusted the variance components in a manner consistent with the known relationships amongst the parents. Therefore, all subsequent analyses of the data were conducted with this modified version of GAREML.

Single-site analyses were conducted for each trait at each site. Replicates were treated as fixed, and a random effect of set within replicate was fitted. GAREML estimates a pooled variance due to GCA (general combining ability) of the parents, and the SCA (specific combining ability) due to the interaction of male and female parents. As the data are from a hybrid population between PEE and PCH the standard genetic interpretation of the GCA and SCA variances (i.e. ¼ of the additive and dominance variances respectively) can not be made; nevertheless, the GCA variance estimates the pooled variance among the parental means, while the SCA variance estimates the variance due to deviations of family means from that expected from the average effects of the parents. The nomenclature of GCA and SCA variance will be retained with no further reference to the hybrid nature of the population; however, this is for convenience, and not meant to imply a genetic interpretation to these variance components. Finally, the model also included an effect of clone within SCA (i.e. clone within family) and a residual error. Other than the overall mean and the effect due to replicates (fixed), all other effects in the model were random. We also tested the significance of interactions between replicates and the genetic effects (GCA, SCA and CLONE(SCA)), and these interactions were not found to be significant. Therefore, these interaction terms were not included in any subsequent analyses. Analysis of the data in this way provided a pooled estimate of the clone within SCA variance across sets, and adjusted the data for environmental differences between sets. Since the means of each set were approximately equal (as would be expected from the random allocation of clones to sets, and sets to field plots), and sets were approximately balanced with respect to families, there were not expected to be any genetic differences between sets. Hence this appeared to be the most appropriate method of analysis – as opposed to nesting clones within sets.

Prior to conducting across-sites analyses, the estimates of the residual variance from the single-site analyses were used to standardise the data. Each observation was divided by the square root of the residual variance for that trait at that site, thus the transformed variables had a residual variance of 1.0, thereby allowing the data to be pooled without violating the assumption of equal residual variance across sites. The approach taken was to: a) initially put all 'similar' sites together, and then b) add or remove sites depending on the size of the genotype x environment (particularly clone x site) interaction. In this case 'ridge' sites and 'swamp' sites (mid to upper-slope = 'ridge', and mid to lower slope = 'swamp' sites, Table 1) in SEQ were initially analysed separately, then together, and finally the ridge site in CQ at Byfield (Table 1) was added. The alternative would have been to conduct pair-wise analyses (i.e. $\frac{1}{2} \times 12 \times 11 = 66$ pairs) for each trait measured at two or more sites, or over 1000 separate analyses of test pairs.

The model used for the across-sites analyses also included fixed effects for site and replicates within site, a random effect for set nested within replicates and sites, plus random effects for the

interactions of GCA, SCA and clone(SCA) with site. It was also possible to include interactions with rep(site) as indicated previously; however, when tested on 6-year volume this substantially increased the computational time required to complete the analyses, and did not significantly affect any of the variance components. Therefore the simpler model was used in all subsequent analyses.

Parameter estimates: Broad-sense heritability was estimated for each trait and age, from the variance components derived from the single-site and across-sites analyses as follows:

$$H^2 = \frac{s^2_G}{s^2_P} = \frac{2s^2_{gca} + s^2_{sca} + s^2_{clone(sca)}}{2s^2_{gca} + 2s^2_{gca*site} + s^2_{sca} + s^2_{sca*site} + s^2_{clone(sca)} + s^2_{clone(sca)*sote} + s^2_{error}}$$

where σ^2_G is the total genetic variance, and σ^2_P is the phenotypic variance estimated as the sum of all variance components (other than that for sets(reps)). For single-site analyses all components involving interactions with sites would be dropped from the estimate of the phenotypic variance.

The ratio between the variance due to clones within SCA (i.e. variance between clones with families) to the total genetic variance, σ^2_G was also estimated. This ratio is termed here C^2 and estimated as follows:

$$C^2 = \frac{s^2_{clone(sca)}}{s^2_G}.$$

Hence C^2 indicates the proportion of the total genetic variance between clones within family.

The variance components estimated from the across-sites analyses were used to calculate what are referred to as Type B genetic correlations. The Type B genetic correlation provides a measure of the importance of genotype by environment interaction – when there is no genotype × interaction then the Type B correlation is equal to 1. Type B correlations were calculated to measure the importance of the total genetic variance compared to the total genetic x environment interaction, as follows:

$$r_{G_B} = \frac{2s^2_{gca} + s^2_{sca} + s^2_{clone(sca)}}{2s^2_{gca} + s^2_{sca} + s^2_{clone(sca)} + 2s^2_{gca*site} + s^2_{sca*site} + s^2_{clone(sca)*site}}.$$

RESULTS AND DISCUSSION

Single-site analyses: The broad-sense heritability (hereafter referred to simply as 'heritability') estimates of the growth traits show a clear pattern of increasing heritability with increasing age (Figure 1). For example, H^2 of tree height increases from 0.18 at 6 months to around 0.5 at 5 – 6 years of age, and a similar pattern is observed for diameter. There are peaks in H^2 associated with volume at 2 years and the other traits at 5 years, where average estimates are obtained only from the data collected in the two Stage 1 tests. The heritability estimates from the Stage 1 tests

were generally higher than those from the Stage 2 and 3 tests, which was probably attributable to more intensive management of these two tests (better weed control particularly) leading to a reduction in unexplained environmental variation. Taking this into account it appears that the heritability of all growth traits plateaus around 6 years of age at 0.4 – 0.5.

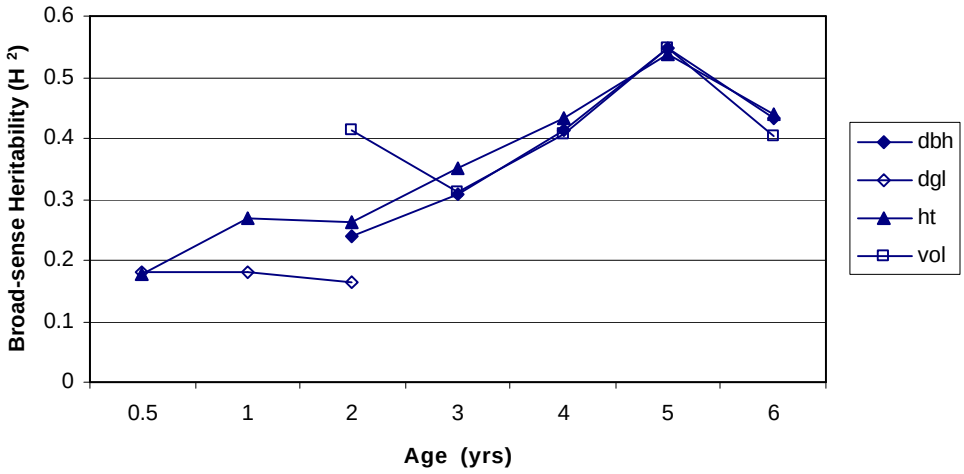

Figure 1. Changes in average broad-sense heritability (H^2) with age for growth traits, estimated from single site analyses of the Series II clonal tests. (Note: estimates for volume at 2 years, and all traits at 5 years only are only from the Stage 1 tests – refer Table 2.)

The proportion of the genetic variance between clones within families (C^2) demonstrates a similar pattern (Figure 2) to that observed for heritability (Figure 1), maximizing at around 4 – 6 years after planting. The remainder of the genetic variance (i.e. $1 - C^2$) is between families. This suggests a rearrangement of genetic variance within and between families with increasing age, which in turn implies that gain from within family selection is likely to maximise at around 4 years of age. This has a bearing on the relative amounts of genetic gain that than can be expected from family selection vs. clonal selection within families; however, this is not the whole story as the heritability of clonal means is often higher than that of family means, and the intensity of selection is also usually higher.

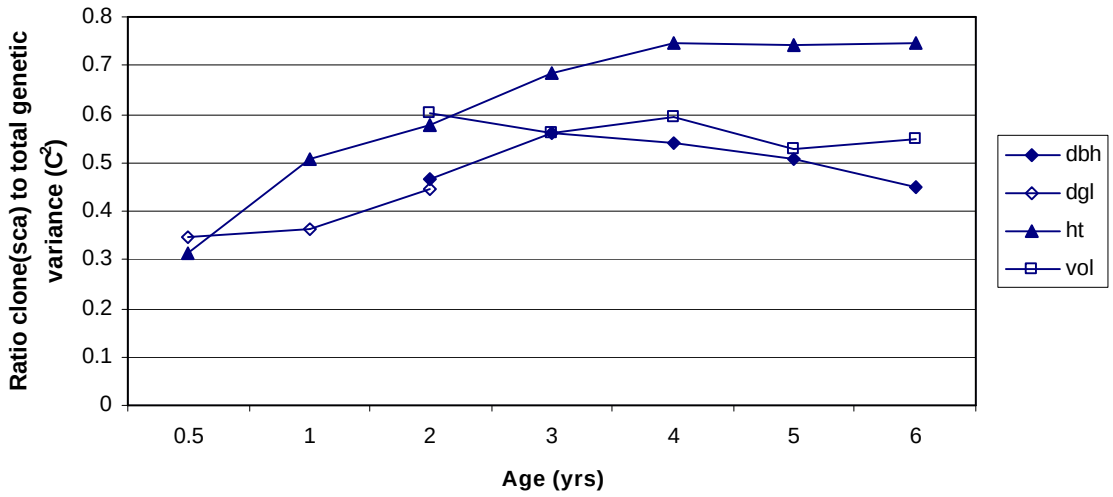

Figure 2. Changes in the average ratio of clone (within family) variance to total genetic variance (C^2) with age for growth traits, estimated from single-site analyses of the Series II clonal tests.

Across-sites analyses: The heritability estimates obtained from the across-sites analyses (Table 3) were similar to the averages from the single-site analyses (Figure 1). Typically the heritability of growth traits was lower across the swamp sites than across the ridge sites (Table 3). Poorly-drained swamp sites are usually more variable than ridge sites, so this trend to higher heritability on more uniform, well-drained ridge sites is not unexpected. These differences between ridge and swamp sites persisted to 6 years of age, but by 6 years of age the differences were relatively minor. The broad-sense heritability (H^2) estimates for the growth traits (Table 3) are considerably higher than those reported for loblolly pine (Paul et al. 1997; Isik et al. 2004), and the narrow sense heritability (h^2) commonly reported in the literature. Typically the narrow sense heritability of diameter, height or volume is around 0.15 – 0.25 in the parental species (Dieters 1996, Woolaston et al. 1990). This difference between the narrow and broad sense heritability represents an opportunity to make additional gain from clonal forestry – clonal forestry is able to capture the total genetic variation, while family forestry is only able to capture a portion of the additive and non-additive variance, and deployment via seed orchards only captures part of the additive variation.

The across-sites analyses indicated that there was relatively little genotype x environment interaction (G×E) among ridge and swamp sites when analysed separately (Table 3), so all sites in southeast Queensland were then analysed together. This analysis similarly indicated the presence of relatively little G×E, so the central Queensland (CQ) site (Byfield) was also added. The addition of the CQ site rather surprisingly tended to increase the Type B correlation estimates, especially at early ages (Table 3, Figure 3).

G×E interaction was relatively more important when the trees were young (Table 3 and Figure 3), but was of relatively little importance by 2 years of age on well-drained sites, and by 4 years of age on more poorly-drained swamp sites. This observed difference in the Type B correlations between well-drained ('ridge') and poorly-drained ('swamp') sites again is likely to reflect the within site uniformity of these site types, and the fact the early growth is usually greater on well-

drained sites. This contrasts with Paul et al. (1997) where the total genetic Type B correlation for height in loblolly pine across three sites, maximized at 2 years at close to 1.0, and dropped to 0.71, 0.74 and 0.80 at 3, 4, and 5 years of age respectively. However, since G×E includes components of G×L (i.e. GxLocation) as well as G×Y (i.e. GxYear) effects, as the trees grow, modifying their environment, and increasingly express their genetic potential (as demonstrated by an increased heritability as observed in Figure 1), it is likely that the genotype × location and × year effects are averaged out to some extent, leading to the observed reduction in the importance of G×E with time.

Although relatively little G×E interaction has been observed for the parental species across a similar range of sites (Dieters 1990, Woolaston et al. 1991), clones were expected to be more interactive with sites than families. For example Osorio et al. (2001) found very high levels of clone × site interaction in *Eucalyptus grandis* grown in Colombia, and variance components reported by Paul et al. (1997) indicate additive genetic Type B correlations of height in loblolly pine close to 1.0 from 2 to 5 years of age. Although the results reported here are somewhat surprising, they provide additional support for the continued implementation of clonal forestry in southeast Queensland. The fact that there is relatively little G×E indicates that the clones that have performed well in the clonal tests, can be subsequently deployed with confidence across a range of similar sites in southeast Queensland. Nevertheless, as we gather increasing knowledge about the performance of elite clones there may be scope to capture additional gain by deploying individual clones to specific sites.

Table 3. Estimates of broad-sense heritability (H^2) and Type B genetic correlations between sites (Clone = between clones, Total = total genetic) from analyses across all sites (11 sites in SEQ + Byfield), all southeast Queensland sites (11 sites), all SEQ ridge sites (7 sites) and all SEQ swamp sites (4 sites). (Slope positions as defined in Table 1.)

	All Sites		All SEQ Sites		SEQ Ridge Sites		SEQ Swamp Sites	
Trait	H^2	Type B	H^2	Type B	H^2	Type B	H^2	Type B
DGL06	0.26	0.76	0.12	0.45	0.21	0.53	0.11	0.40
DGL1	0.28	0.82	0.25	0.75	0.24	0.75	0.21	0.69
DGL2	0.22	0.98	0.22	0.98	0.20	0.97	0.22	0.97
DBH3	0.50	0.91	0.53	0.97	0.55	0.97	0.40	0.81
DBH4	0.58	0.93	0.63	0.99	0.63	0.99	0.55	0.95
DBH6	0.63	0.93	0.69	0.98	0.68	0.99	0.57	0.91
HT06	0.29	0.83	0.19	0.55	0.22	0.57	0.16	0.66
HT1	0.34	0.83	0.33	0.80	0.35	0.79	0.22	0.64
HT2	0.39	0.86	0.46	0.92	0.48	0.90	0.25	0.74
HT3	0.49	0.88	0.53	0.91	0.57	0.96	0.39	0.80
HT4	0.55	0.91	0.66	0.99	0.64	0.98	0.47	0.92
HT6	0.61	0.93	0.70	0.98	0.70	0.99	0.53	0.89
VOL3	0.50	0.91	0.27	0.93	0.53	0.94	0.41	0.84
VOL4	0.55	0.93	0.55	0.79	0.63	0.99	0.49	0.95
VOL6	0.55	0.91	0.59	0.79	0.61	0.97	0.49	0.89

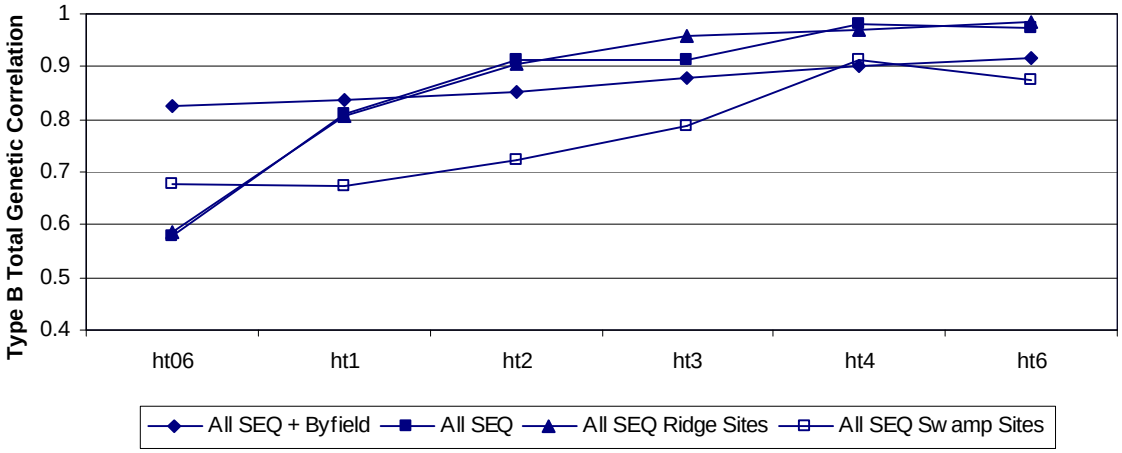

Figure 3: Changes in the Type B correlation between clones for tree height, for across sites analyses of different groups of Series II clonal tests.

Implications for design of future clonal tests: When establishing clonal tests it is highly desirable to have all the field tests established in the first year after the seed is sown. If all tests are to be established in the first year after sowing, there will be a limited number of plants (around 20 – 30 in this hybrid) that can be produced from each clone. What is the best way to use these few plants? How many test sites are required? How many replicates per test? Similarly there are limits to the number of clones that can be included in a clonal test series. This limit is determined by resources: money, people and infrastructure. Further, as the number of clones increase, the risk of mistakes increases rapidly. Therefore, assuming that the number of clones included in a clonal test is fixed, what is the best way to distribute these clones among families?

Using variance component estimates from this analysis it was possible to estimate the genetic gains obtained from varying levels of clonal testing: 1 to 12 sites, and for 1, 2, 3, 5 and 10 ramets/clone/site were compared to the predicted gain from infinite testing. The ratio of the predicted gain to that from infinite testing estimates the efficiency of different testing strategies. Using the variance component estimates for volume at 6 years of age across all sites, indicated that testing one ramet/clone/site was always less efficient than testing more ramets per site, and that testing on more sites always increased the efficiency (Figure 4). However, the there was relatively little additional benefit from using more than 5 or 6 test sites, particularly when testing 3 or more ramets/clone/site.

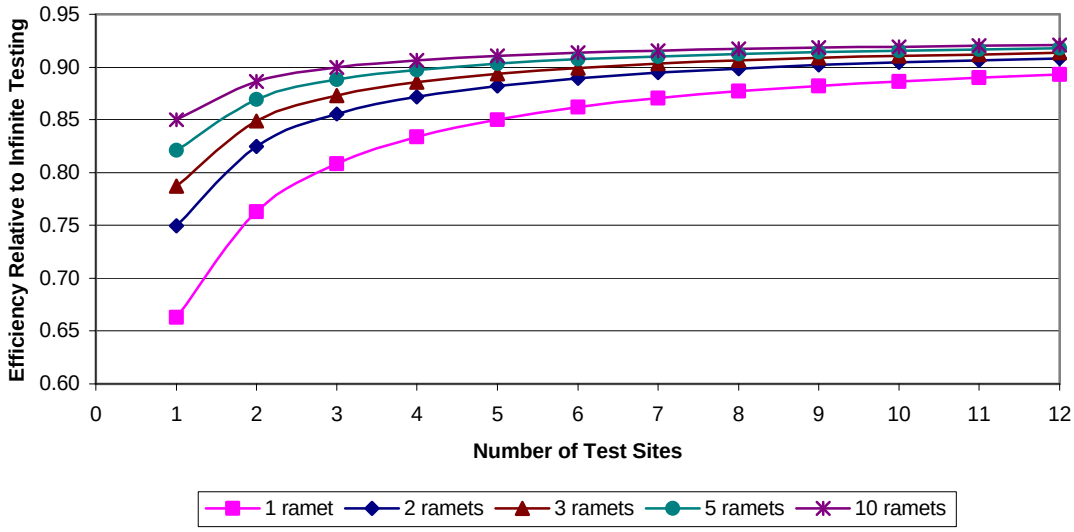

Figure 4: Efficiency of clonal testing for varying numbers of ramets/clone and test sites, relative to gain from infinite testing, using variance components estimated for 6 year volume across all southeast Queensland and Byfield sites.

Clearly, the various clonal testing scenarios examined in Figure 4, do not represent an equal investment in clonal testing. In an attempt to compare efficiency gains under a scenario of fixed resources, the maximum number of trees in tests was fixed at 10,000 with a maximum of 2000 clones in the tests. For example, for 2000 clones we could have 1 ramet/clone on up to 5 sites, thereafter the number of clones included must decline if we are to test no more than 10,000 trees. Under this scenario of fixed resources maximum gains were achieved under relatively low numbers of ramets/clone tested on only a few sites. However, there was no clear-cut anwer. To maximise gain from clonal testing under limited resources, it is necessary to balance: a) potential gain from increasing the replication at a site, and so improving the accuracy of estimating clonal performance at that site; b) testing more sites, and so being able to better predict clonal performance across a range of sites; and, c) testing as many clones as possible (to increase the selection intensity) without unduly compromising the accuracy of determining the performance of the clones tested. However, it is also usually more expensive to establish smaller tests on more sites, than to establish larger experiments of fewer sites. Further, the target population of environments in southeast Queensland broadly includes two site types (i.e. 'ridge' and 'swamp' sites), and three major plantation regions of Beerburrum, Toolara and Tuan. Hence the use of 6 test sites (i.e. 2 site types $\times$ 3 regions) would cover a large part of the variation in the plantation estate.

Three ramets/clone/site is probably the minimum number to ensure some level of replication at each site where survival is not complete. Therefore if we can reliably produce 20 plants/clone in the first year after sowing, the maximum number of sites that can be planted is around 6. This combination (3 ramets/clone $\times$ 6 sites) produced near maximum efficiency (Figure 4), and matches the site type $\times$ region variation in the southeast Queensland exotic pine plantation estate.

To check the validity of this proposal, we expanded the resources to allow the testing of 2000 clones and 36,000 trees under test (i.e. 2000 clones × 6 sites × 3 ramets/site). In this case, maximum gain can be achieved over a larger range of sites and ramets/clone/site, but still testing of 3 ramets/clone on 6 sites appeared to be optimal for testing of 2000 clones. Similar results have been reported by Russell and Loo-Dinkins (1993) for gains in the production population, based on simulation studies of clonal testing.

The finally we examined the balance between number of families and the number of clones per family under fixed resources, such that 2000 clones were tested with the aim of selecting 10 clones for deployment, under 4 scenarios: A) select in the top 10 families; B) 5 families; C) top 2 families; and, D) the top family. Scenario A would correspond to a situation where you wanted to maximise genetic diversity of the clones selected for deployment, and so selected the best clone in each of the top ten families. Scenarios B – D place progressively less weight on the importance of diversity among the clones, until in D all the deployment clones come from the single best family. Again, we predicted gains for 6 year volume, based on the variance component estimates across all sites, and the gains were then converted to percentages using the average 6 year volume and average residual standard deviation of these 12 tests. There were relatively small differences in the predicted gain from these four scenarios.

The scenarios modelled here do not adequately reflect the situation in the Queensland clonal forestry program. The advantage of clonal forestry over family forestry rests on the additional gain from selecting the best clones in the best families. Therefore, the predicted gain from family selection is not relevant since we could achieve this gain by simply deploying the best families without clonal forestry. However, quite clearly the gain expected from clonal forestry is highly dependent on the selection intensity that can be exerted at the clonal level. The more clones tested per family, the higher the predicted gain. But where resources are fixed, to increase the selection intensity within families it is necessary to reduce the number of families included in the clonal tests. This in turn means that we must be very confident about the choice of families for inclusion in the tests – this underscores the need for accurate data on the performance of families prior to their inclusion in clonal tests. This is important for two reasons: firstly, so that the number of families can be reduced (maximise intensity of selection within families by only including the best few families), and secondly, it is important to ensure that the families included have high means for the primary traits of interest as inclusion of sub-optimal families could substantially erode the potential gain from clonal forestry. Currently, the most promising families are identified based on the parental breeding values from hybrid tests, and these families are produced specifically for inclusion in future clonal tests. However, in the future, we anticipate pre-screening hybrid families prior to clonal testing to provide more accurate ranking of family performance, and identify families with high specific combining ability. There may also be scope to select families with both a high mean performance, and high within family variability.

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Clonal Forestry: Options, Deliverables and Benefits

Yousry A. El-Kassaby[1] and Ian Moss[2]

Abstract:

The sexual reproduction mode of the traditional tree improvement delivery systems has fallen short of packaging the maximum genetic gain attained by tree breeders. The system's intrinsic biological and/or operational limitations have individually or collectively contributed to the observed erosion of genetic gain. Bulking up elite genotypes through asexual reproduction (i.e., vegetative propagation) has been developed, tested and is being implemented on an operational scale by many organizations worldwide. Asexual reproduction methods used vary from the simple (large-scale rooted cuttings) to the sophisticated (somatic embryogenesis). Whichever vegetative propagation method is adopted, the user has ample opportunities and challenges. Opportunities include practicing large scale high-yield forestry, favourable returns on investment, strategic use of the forest estate, product uniformity, and strategic control of the genetic resources. Technical and operational challenges are identified and are under intensive research and development. Species-specific examples will be presented illustrating these opportunities and challenges.

Keywords: Cloning, genetic gain, economic evaluation, stand- and estate-level analyses.

INTRODUCTION

Forest tree improvement programs around the world have achieved substantial increases in growth and yield through careful recurrent selection and breeding. Genetic gains produced through breeding are packaged in seed orchards and delivered as seed to nurseries which act as factories, producing genetically improved seedlings for reforestation. To improve on the moderate gains captured in wind-pollinated seed orchards, many breeders have employed controlled pollination, crossing top-ranking (elite) parents for intensified selection for desirable traits. The seed produced is then used to grow zygotic seedlings (producing one seedling per seed) or for vegetative reproduction (each seed can produce many genetically identical seedlings).

The gains resulting from vegetative propagation of seed produced from crosses among elite parents have been planted over large areas with deployment methods called family and clonal forestry. The genetic gains achieved through family forestry are derived from the average values of the elite parents used in the mating design, and are thus higher than bulk seedlots collected from the seed orchards they represent. One limitation of family forestry is that it does not allow for within-family selection to further increase genetic gain because donor plants age during the time required for evaluation, and will be less amenable to vegetative propagation.

[1] Department of Forest Sciences, Faculty of Forestry, The University of British Columbia, 3616-2424 Main Mall, Vancouver, B.C. Canada V6T 1Z4
[2] ForesTree Dynamics Ltd., 1458 Kingsview Road, Duncan, B.C. Canada V9L 5P1

Implementing high-yield plantation forestry is expected to rapidly increase productivity of managed forests. This is clearly within reach: 5% of the global forest area in 2000 produced approximately 35% of the industrial roundwood (FAO 2001). Seed orchard-based tree improvement programs have a theoretical productivity maximum, constrained by biological factors. Scientists are exploring new technologies to increase these maximum values through cloning.

This paper provides a discussion of the methods and utility of cloning, and analyses of the benefits of cloning in a forest management context. Case studies are described illustrating applications of cloning to deliver genetic gains at the stand and estate levels, supported by economic analyses based on local environmental and market parameters.

CLONING METHODS AND APPLICATIONS

Cloning technology for conifers

Conifers can be vegetatively propagated using three techniques: macropropagation, micropropagation (organogenesis) and somatic embryogenesis (SE). Macropropagation is widely used in many conifer species, for example to produce rooted cuttings, but works best when tissue is physiologically juvenile. In micropropagation, growth regulators are applied to physiologically juvenile tissue segments, but success varies among species. SE produces embryos asexually from somatic embryonic tissues *in vitro*.

Clonal testing and its relevance to mass propagation

Genetic segregation and recombination produce the genetic variability, or within-family variation which can be exploited by controlled crosses among elite parents. Cloning selected genotypes enhances the breeder's ability to detect and optimize within-family variation, enabling them to capture maximum genetic gains. When elite parents are crossed, each seed produced has a unique genotype, each representing a potential source of clones. Before initiating large-scale production of a particular genotype, clonal testing is essential. For example, if 50 clones were produced for one cross, 100 – 200 copies of each clone are needed to accurately compare their performance across multiple sites to identify superior genotypes. These outstanding clones are then multiplied for large-scale production and thousands, or even hundreds of thousands of copies are produced for reforestation.

Cloning delivers higher value: case studies

Case Study 1 – Stand level: economic benefits of planting weevil-resistant Sitka spruce

Background

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is the fastest-growing species on many sites in the wet temperate coastal forests of British Columbia (B.C.). Despite its excellent performance, it is seldom planted due to the high risk of damage or mortality by the white pine shoot tip weevil

(*Pissodes strobi* Peck) on many sites. The discovery of naturally weevil-resistant Sitka spruce individuals and populations has allowed foresters to replant the species on suitable sites (King et al. 2004). Somatic embryogenesis (SE) technology is an ideal reproduction tool to produce required numbers of weevil-resistant planting stock representing the genetic contributions of many parents.

El-Kassaby and Moss (2001) analyzed the economic framework quantifying the benefits of using SE to deploy weevil-resistant Sitka spruce seedlings, compared to the other recommended alternative species for two site types in coastal B.C. The provincial ecosystem classification scheme situates the Coastal Western Hemlock biogeoclimatic zone (CWH) along the low-elevation outer Pacific coast, with the subzone modifier "vh" indicating a very wet hypermaritime climate, and the site series modifier reflecting the moisture and nutrient status of the site: 04 is less moist and rich than 08.

Methodology and model assumptions

1) The B.C. Ministry of Forests growth and yield model (TIPSY; Table Interpolation Program for Stand Yields, Ministry of Forests, 2000) was used to project log quantity and quality distributions that would be produced by rotation age assuming 1200 seedlings per hectare and a production volume "fall-down" of 5%. For Sitka spruce predicted volumes were further reduced by a factor denoting the susceptible proportion of the stand to weevil attack. Susceptibility levels were characterized as causing 0, 6, 9, 12 and 15% loss in stand volume.

2) Log values, representing the median or 50^{th} percentile of the range of prices observed during the 20-year period from 1980 to 1999, were based on Vancouver log market prices.

3) Logging costs were estimated using B.C. Ministry of Forests (2001) appraisal procedures. Road construction costs were estimated based on industry estimates (see El-Kassaby and Moss 2001 for details).

4) Costs of seedling production and planting 1 year after harvest were $0.12 and $0.25 per tree, respectively, for a total of $0.37 per tree or $444 per hectare, which does not include added costs of SE. No other silviculture was included in the model.

5) Soil expectation values (SEV, the initial value of a perpetual series of even-aged rotations, beginning from bare ground immediately after harvest) were calculated for each combination of management regime, site, species, Sitka spruce susceptibility rating, discount rate (2, 4 or 6%), and rotation age determination method (6% discount vs. maximum internal rate of return, IRR).

6) Stand value of establishing Sitka spruce with various levels of resistance to weevil damage were assessed relative to the value accrued by planting alternative species at a given site, all other factors being equal.

Results

Maximum mean annual increments (MAI) were always substantially higher for Sitka spruce than any other species, even with 15% of stand volume impacted by the weevil, relative to the other species. For example, MAI of 14.1 and 12.8 $m^3 \cdot ha^{-1} \cdot y^{-1}$ were obtained for Sitka spruce and the next best alternative, hemlock-fir, respectively, in CWHvh/08 sites where both species have equal site indices. This implies that even though the stands may be at the same height at age 50, Sitka spruce will produce more volume. This is expected since Sitka spruce is less shade tolerant than any of the other species (Krajina et al. 1982). SEV for all Sitka spruce scenarios was always the highest relative to the alternative species (Table 1, Figure 1). Based on this data, establishing weevil-resistant Sitka spruce plantations will produce a positive return when a 2 to 6% discount rate is applied.

Table 1. Maximum soil expectation values ($ ha^{-1}) (El-Kassaby and Moss 2001).

	Species[1]	**Ss**					**Cw**	**Hw**	**HB**
		Weevil susceptibility (%)							
Site Series	**Discount Rate**	**0**	**6**	**9**	**12**	**15**			
CWHvh/04	2 %	14,415	13,506	13,051	12,596	12,142	2,795	2,550	2,726
CWHvh/04	4 %	2,417	2,242	2,154	2,066	1,979	166	84	134
CWHvh/04	6 %	396	345	320	294	269	-245	-274	-256
CWHvh/08	2 %	11,539	10,807	10,440	10,074	9,708	5,987	4,602	4,828
CWHvh/08	4 %	1,750	1,614	1,547	1,479	1,411	722	528	586
CWHvh/08	6 %	174	136	117	98	80	-52	-130	-96

[1] Ss: Sitka spruce, Cw: western redcedar, Hw: western hemlock, HB: hemlock-amabilis fir mix.

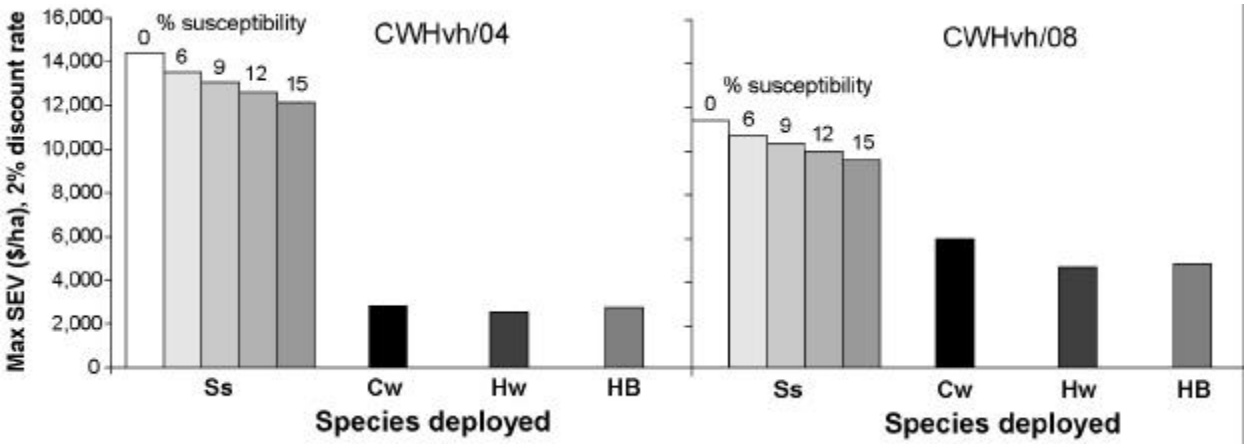

Figure 1. Soil expectation values associated with deploying Sitka spruce with a range of weevil susceptibility (0 to 15%) relative to alternative recommended species (See Table 1 for species codes) at a 2% discount rate on drier (left) and wetter (right) sites in coastal British Columbia (after El-Kassaby and Moss 2001).

Summary

The preceding analysis clearly demonstrated the great benefits of investing in weevil-resistant Sitka spruce SE. These benefits remained at a substantial level even after incorporating susceptible seedlings into the planting program to avoid excerting any selection pressure on the insect population.

Case Study 2 – Estate level: Long-term cashflows from loblolly pine plantation management

Background

Stand level economic analyses are used to facilitate decision making by evaluating the costs and benefits of applying different silvicultural options on a specific tract of land. Many stand level analyses must be integrated over space and time to effectively assess the impact of factors across the estate or landscape level. Estate level analyses are frequently able to highlight benefits and options not evident from stand level analyses. For example, stand level analyses may underestimate the value of a particular silvicultural investment when the effect is considered over the entire estate.

The following hypothetical example illustrates the utility of comparing the stand and estate levels of analysis. Loblolly pine volume growth is modeled and compared for two management regimes: a high-yield plantation regime incorporating SE, genetic gain of 40%; and a conventional seed orchard-based regime, genetic gain 13%.

Model parameters and assumptions

1) The hypothetical estate was in the Lower Coastal Plain Region with all stands planted at 723 trees per acre, over three site types (site index = feet at age 25): good (80), medium (65), poor (45).

2) Four product classes were produced: pulp, chip-n-saw, sawlogs and large sawlogs yielding average stumpages of $39.60, 79.20, 211.20 and 316.80 per unit, respectively.

3) The conventional regime was susceptible to canker which affecting 15% of the stand; high-yield regime reduced the impact to 7.5% (Li et al. 1999), producing less low-value pulp.

4) Corporate income taxes equalled 28% of net annual income.

5) Seedling and planting costs were $109 per acre ($0.15 per seedling). Incremental SE costs were assumed to equal 50% of the difference in the maximum net present value (NPV) (6% discount rate) prior to inclusion of the costs, so expected benefits from SE were split evenly between the purchaser and the producer.

6) Growth and yield outputs from the Georgia Pine Plantation Simulator (GaPPS v 4.2, Bailey and Zhou 1997) were programmed into a Stand Management Financial Analyst program (SMFA, I. Moss, unpublished) to estimate projected volume changes due to tree improvement according to Lambeth (1980).

7) Optimum rotation ages were then determined using two methods: a 6% discount rate, and maximum internal rates of return (IRR).

8) Forest level cashflow was determined for each site class, assuming that the area harvested and planted each year was constant for each site class and rotation age combination (e.g., if the rotation age was 20 years, $1/20^{th}$ of the area in that site class would be harvested and planted annually). Projected annual cashflow was then discounted in perpetuity at a 12% interest rate, assuming this equalled the cost of capital. The proportion of land required for each site class managed under the high-yield regime was estimated to maintain the original cashflow level calculated for the conventional regime.

9) Additional costs including land taxes, overhead, annual maintenance fees, etc. were not included in the model.

Results

Cashflows and rotation ages according to the management and rotation estimation methods are given in Table 2. Areas required to achieve the same cashflow as given by the conventional regime under different management strategies and site indices are given in Table 3 and Figure 2.

Table 2. Stand level discounted cash flow and maximum internal rates of return (IRR), financial rotation age, gross cashflow (annual harvest) and forest level discounted cashflow (NPV).

	Conventional Regime (13% gain)			**High-Yield Regime (40% gain)**		
Site index (ft @ 25 years)	80	65	45	80	65	45
Rotation calculation method						
Method 1: NPV @ 6% discount						
Stand level discounted cashflow ($/acre)	987	384	-86	1237	537	-46
Rotation age (years)	29	29	37	30	35	38
Cashflow (0% discount, $/acre)	6620	3351	1468	9512	6970	2233
NPV (12% discount, $/acre)	1902	963	331	2642	1659	489
Method 2: Maximum IRR						
Maximum IRR	13.1	9.5	5.0	11.6	9.3	5.6
Rotation age (years)	18	24	39	19	24	39
Cashflow (0% discount, $/acre)	2214	2179	1674	3416	3053	2383
NPV (12% discount, $/acre)	1025	757	358	1498	1060	509

Table 3. Equivalent areas required to generate the same forest level cashflows assuming all stands are proportionally annually harvested by stand level financial rotation age and replanted, as detailed in the text.

	Conventional Regime (13% gain)			**High-Yield Regime (40% gain)**		
Site index (ft @ 25 years)	80	65	45	80	65	45
Rotation calculation method						
Harvest at financial rotation age,	100	198	575	72	115	389

6% discount rate						
Harvest at financial rotation age, maximum IRR	100	135	286	68	97	201

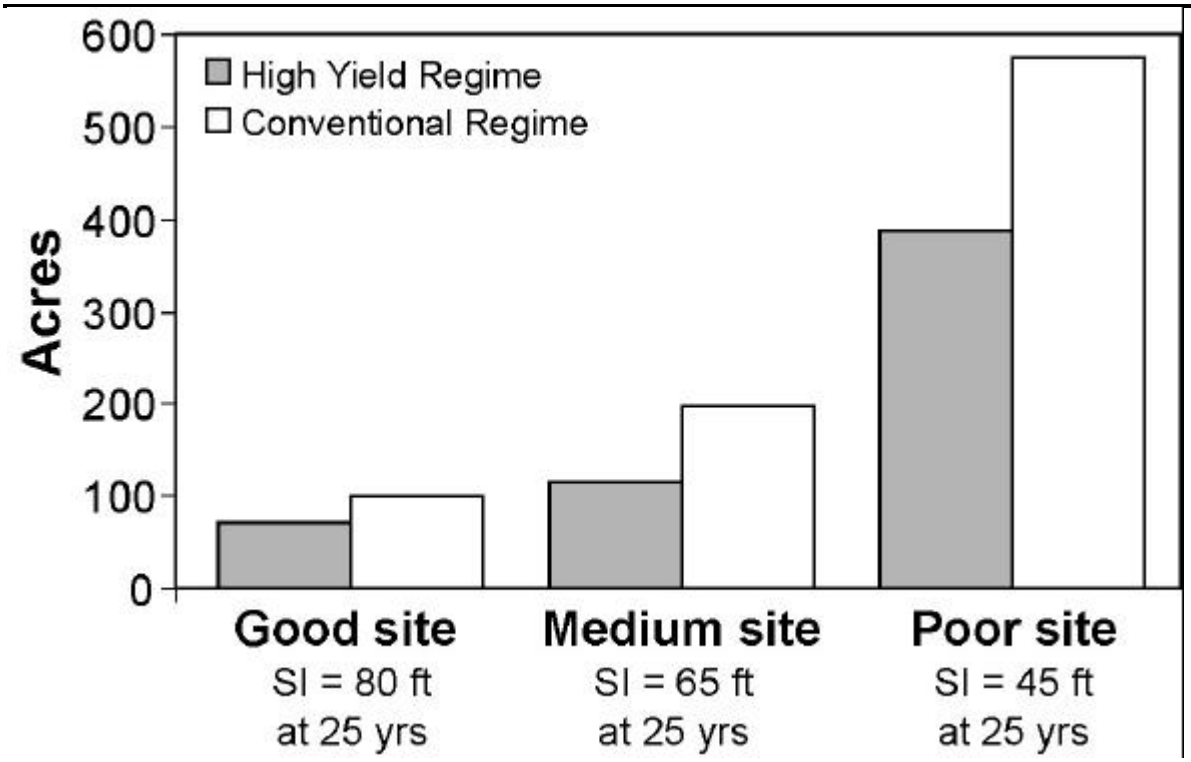

Figure 2. The equivalent area of forest land in three different site classes required to produce an equivalent level of cashflow if managed according to either High Yield or Conventional regimes. It is assumed that stands are managed on a financial rotation age using a discount rate of 6%.

To illustrate one comparison, to maintain a constant, forest estate level cashflow, an area equivalent to 13% of that managed under the conventional regime on poor sites could instead be managed under the high-yield regime on good sites. This figure is calculated from 331/2642 (Table 2) and 72/575 (Table 3). This may be compared to 17% of the land required if one were to use poor sites instead of good sites, and use only the conventional regime (100/575, Table 3). The results indicate that the most efficient strategy to incorporate SE would be to deploy this technology on good sites.

The models show that 100 acres of high-productivity sites produce the same revenue as 575 acres of poor lands (Table 3). Including SE would mean only 72 acres would be required, resulting in a potential for 223 acres of low-productivity land to be released from the management regime for every 100 acres high site lands managed under the high-yield regime (from Table 3, $575/72 \times 100 - 575 = 223$).

One management opportunity that may arise when such efficiencies occur is to dispose of some property that is no longer needed to maintain the baseline level of cashflow. Depending on the age class distribution and availability of timber, this property might be disposed of even before

the investments in SE mature. Another management option would be to increase harvest levels once the investments in SE begin to mature. Prior to deciding, the forest manager must weigh many factors: land and timber market conditions, investment alternatives, debt, assets and liquidity, and the current forest age class distribution, which constrains scheduling.

Summary

The benefits of investing in SE may be realized earlier than suggested by a standard stand level investment analysis because SE expands the forest land and timber management options available to the owner, which become apparent when, examined using an estate-level analysis. There may be further synergisms available between SE and intensive silviculture options, which must be evaluated on a site-specific basis and integrated into an estate-level model to estimate the benefits accurately.

CURRENT CHALLENGES FACING CLONAL FORESTRY

Major challenges of clonal forestry pertain to cloning technology itself, and plantation management using clonal seedlings and family forestry. The former will only be briefly discussed here since it is covered in depth elsewhere (e.g., El-Kassaby and Krakowski 2004, this Proceedings).

Technological challenges

Biological obstacles include variable genotypic propensities to cloning and maintaining physiologically juvenile tissues in storage. Technical hurdles occur both in the research and development phase, e.g., high numbers of clones are needed for accurate testing and selection for each cross, and in the production phase, where the various steps of the cloning process have inconsistent success and efficiency rates, and the ability to mass produce clonal seedlings, where many different factors affect capacity.

Plantation management challenges

1) Age of selection: long rotations create uncertainty if clones are selected very early; however, age-age correlations indicate that the time needed for testing could be reduced substantially with a modest trade-off in risk for earlier or larger returns.

2) Scope of testing: significant genotype-by-environment interaction for many important traits in forest trees necessitates extended testing, even within relatively environmentally homogenous planting zones. This allows the identification of specialists as well as generalists for deployment.

3) Family size: selection efficiency depends on family size. Its impact is more pronounced during multiple trait selection when the traits are negatively correlated.

4) Clonal representation: deploying clonal mixtures to mitigate potential risk has been suggested, although this decreases the benefits linked to product uniformity.

5) Clonal block size: blocks should be large enough to capitalize on product uniformity, but compatible with the prevailing forest management regime.

6) Arranging clonal blocks: planting the same clone in adjacent blocks must be avoided through rigorous GIS-based temporal and spatial planning.

7) Balancing genetic diversity and gain: the number of parents and clones per parent are critical to optimize the amount and distribution of genetic variation within and among plantations, while ensuring genetic gains. Incorporating a sufficient number of parents in seed orchards provides evidence that foresters are ensuring long-term sustainability by offering a compromise between reduced gains and higher genetic diversity.

CONCLUSIONS

Recent advances in cloning techniques have made high-yield clonal forestry more economically viable. Clonal forestry can maximize the economic benefits of conventional tree improvement by providing rapid delivery of high genetic gains via forest plantations. A supplementary benefit is the cost savings from product uniformity. The economic benefits of product uniformity are difficult to quantify for temperate zone species due to the lack of available data; however, the experience from tropical and sub-tropical zones' eucalyptus planations shows great flexibilities and returns. Estate level analyses reveal greater benefits and flexibility were realized than were apparent from stand level analyses alone. Cloning still faces some technical and logistical challenges, which can be solved through continued research, development and testing. Production capacity enabling delivery of large volumes of elite genotypes, clonal evaluation, testing and deployment methods, and assessing the dynamics of both genetic gains and diversity are critical areas for improvement.

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Advances in Biotechnology and its Application in Forestry

Maud Hinchee

ArborGen LLC., PO Box 840001, Summerville, SC, USA

Over the next 50 years, human demand will put extreme pressure on our natural forests unless wood harvests can be shifted towards highly productive forest plantations. Biotechnology will be an important tool in the technology toolbox for sustaining the world's forests. Improved genetics, provided through clonal forestry and biotechnology, together with improved silvaculture and plantation management practices, will be required to meet the wood demands of the future. Genomic research is expanding the foresty industry's capabilities to identify and utilize molecular technqiues toward tree improvement. ArborGen, a forest biotechnology company dedicated to improving the sustainable productivity of plantation forests, is developing plantation forestry species with improved wood properties, growth and stress tolerance. Transgenic tree product development requires multiple competencies, and ArborGen has invested several years towards developing the following platforms: 1) elite genotypes as a genetic base for transgenic products, 2) elite clone transformation capabilities, 3) gene licensing or discovery for introduction of valuable traits, 4) tree performance assessment in the greenhouse and the field, 5) quality assurance and regulatory safety, 6) commercial level scale up of transgenic tree products, and 7) marketing and public acceptance. ArborGen has made significant progress and effort in all of these key areas, with an expectation that we will develop improved plantation forestry trees that can benefit the forest industry but also positively impact on the environment and the world's natural forests.

Applications of Biotechnology to Plantation Forestry with Pines

M.J.Carson[1], S.D. Carson[1] and C. Walter[2]

Carson Associates Ltd.[1], 34 Parawai Rd., and NZ Forest Research Institute Ltd.[2], Rotorua, New Zealand.

The application of biotechnology to forestry, and in particular to plantation forestry in pines, holds considerable promise to: provide increased genetic gain, make forestry operations more economic, yield higher returns, and provide environmental benefits (including protection of natural forests). We briefly describe the current status of the technologies, outline a vision for future plantation forestry, and discuss the potential for, and obstacles to, implementing these new technologies to their full capacity, including the technical, regulatory, organizational, and social constraints that limit progress. We review the discussions around modern biotechnology, including concerns about GE and the debate about potential impacts on the environment. We also outline the benefits of using this new technology, discuss the estimation of potential financial gain, and, finally, discuss the institutional barriers to implementation.

Biotechnology of Wood Properties

Vincent L. Chiang

Forest Biotechnology Group, Department of Forestry, College of Natural Resources,
North Carolina State University, Raleigh, NC 27519, USA

This paper summarizes our previous and current research on genetic engineering of lignin biosynthesis for the purposes of improving wood pulping and bleaching efficiency. For these purposes, our targets were to produce transgenic trees with low content of lignin that is also chemically reactive (high lignin S/G ratio). Using aspen as a model species, we have characterized the biochemical functions of various genes and the kinetic properties of these gene products involved in monolignol biosynthetic pathway. The results of these characterizations proved strong evidence for a principle phenolic flux leading to the formation of monolignols. Biochemical evidence further demonstrated that, in this principle flux, 4CL could be the enzyme limiting total lignin accumulation, whereas CAld5H might control the lignin S/G ratio. These propositions were fully supported by the *in vivo* functions of these enzymes. Transgenic trees with inhibited 4CL enzyme activity exhibited 5 to 45% reduction in lignin contents. The chemical structure of the resulting lignin remained essentially unchanged. More importantly, the lignin reduction was compensated for by a concomitant increase in cellulose content. When antisense *4CL* and sense *CAld5H* genes were simultaneously transferred into aspen via *Agrobacterium*, transgenic trees expressing each one and both of the transgenes were produced. Lignin reductions up to 55% were achieved in antisense *4CL* plants and up to 3-fold S/G increases were observed in sense *CAld5H* plants. These effects were independent but additive, with plants expressing both transgenes having less lignin and higher S/G ratio. Consistent with our previous results, lignin reduction has always resulted in an increase in cellulose content. These transgenics could be potentially valuable for pulp production. But more importantly, these benchmark transgenics are rich sources of information for functional genomics and metabolic engineering, allowing the generation of the ultimate raw materials for wood pulp production.

While lignin quantity and quality can now be manipulated in trees through biotechnology, the knowledge of how to tailor other important wood qualities, such as density, cellulose/hemicellulose properties, and microfibril angle is still lacking. Unlike the control of lignin biosynthesis that can be achieved at levels of individual genes, the control of these other wood properties is likely at levels of gene network regulation. The growing knowledge of gene network regulation together with the enormously important genome sequence of a tree species represent an opportunity not available before to uncovering the underlying regulatory networks that control these wood properties. Some of our preliminary results on the regulatory genes associated with wood formation will be discussed.

Biotechnology Research in the USDA Forest Service

G.S. Foster[1] and F.E. Bridgwater[2]

Abstract: The Forest Service Research and Development (FS R&D) is conducting research in a wide variety of arenas. Biotechnology is itself an important research area and provides important tools in other more traditional research. FS R&D has traditionally been involved in four major areas of forest biotechnology research: vegetative propagation, molecular marker applications, genomics/bioinformatics, and genetic transformation. Each of these has different priorities for private and public-sector clients. Research that promises to increase productivity and profitability will primarily benefit forest industry and non-industrial private landowners while research on ways to sustain ecosystems, restore species, and preserve threatened and endangered species has more direct application on forests owned by the public and non-governmental organizations. FS R&D supports all of these clients, and prioritizes among the four research areas to reflect the needs of all. FS R&D is applying genetic tools to answer questions about biodiversity, conservation, genecology, pest resistance, forest productivity and forensics.

INTRODUCTION

The mission of the USDA Forest Service is to sustain the health, diversity, and productivity of the Nation's forests and grasslands to meet the needs of present and future generations. Historically, the Forest Service has conducted basic and applied genetics research and managed tree improvement programs to meet land management needs. As natural resource management needs on federal lands have shifted, needs for genetic information and tree improvement have shifted (Friedman and Foster 1997). Forest Service Research and Development (FSR&D) scientists carry out basic and applied research in support of this mission for both public and private forest landowners and other clients interested in forest resource issues. Forest Service R&D conducts research on a wide variety of topics. Research is organized into four major areas; 1) Resource Valuation and Use, 2) Science Policy, Planning, Inventory and Information, 3) Vegetation Management and Protection, and 4) Wildlife, Fish, Water, and Air. Biotechnology research is a significant component of the last two areas. For the purposes of this discussion, we will address those areas of biotechnology that deal directly with genetics or its applications.

[1] USDA Forest Service, Research & Development, Vegetation Management & Protection Research Staff, Washington, District of Columbia 20250
[2] USDA Forest Service, Southern Research Station, Forestry Sciences Lab, Research Triangle Park, North Carolina 27709

FOREST SERVICE R&D CLIENTS AND THEIR NEEDS

Forest Service R&D serves a variety of clients. These include forest industry, the National Forest System (NFS) and other government organizations, non-government organizations (NGOs), non-industrial private forest landowners (NIPFs) and the state organizations that serve them. Although there is significant overlap in genetic research needs and interest areas among these groups, each group places different priorities among those interest areas.

Application of biotechnology tools in FS R&D can be divided into six broad interest areas: biodiversity, conservation, genecology, pest resistance, productivity, and forensics. Biodiversity research is fundamentally aimed at maintaining or enhancing biological diversity in an environment as indicated by numbers of different species of plants and animals. Biotechnology tools are often used to discern phylogeny or species differences and interrelationships. Conservation research includes research that would help to protect species of interest including threatened or endangered species or to restore species to environments that they formerly occupied. Genecology includes research to understand genetic variation and adaptation in forest populations to provide planting guidelines and research to predict how these populations might change with changing environments. Pest resistance research and development is aimed at managing indigenous and exotic forest pests. Productivity research includes research that would help to maintain or enhance outputs from the forest. This category includes outputs from non-traditional forest products as well as outputs of commodity crops and is primarily aimed at enhancing the global competitiveness of forest industry and the economy in general. In forensics, biotechnology tools are used to identify individuals or species of organisms (often animals) for management or legal purposes.

We assigned research priorities to the six broad interest areas and assigned our perceived priorities for each client group (Table 1).

Table 1. Forest Service R&D clients and perceived priorities on genetic issues

Interest Areas	Industry	NFS	NGOs	NIPF/States
Biodiversity	L	H	H	M
Conservation	M	H	H	L
Forensics	L	M	H	M
Genecology	H	M	H	M
Pest Resistance	H	H	M	H
Productivity	H	L	L	H

Note: L=low, M=medium, and H=high; NFS=National Forest System, NGO=non-governmental organizations, and NIPF=non-industrial private forest landowners.

Clearly there is significant overlap among groups and research interest areas. For example, pest protection research clearly has an element of productivity. Similarly, genecology research is of

interest to private industry because it provides guidelines for planting commodity crops on sites to which they are best adapted and to NGOs who want to predict the potential impacts of global warming on current ecosystems. However, it seems clear to us that private industry and the NIPF/States groups favor research that promises to increase productivity and profitability while NGOs and the NFS favor research in issues that will help them holistically manage the entire forest ecosystem.

BIOTECHNOLOGY TOOLS

We classify the tools of forest biotechnology into four categories: genetic transformation, genomics, vegetative propagation, and marker applications. We then indicated in which of the six categories of interest areas each tool would be favored, at least in FS R&D (Table 2).

Table 2. Biotechnology Tools Applied to Genetic Issues

Interest Areas	Transformation	Genomics	Vegetative Propagation	Marker Applications
Biodiversity				+
Conservation	+	+	+	+
Forensics				+
Genecology		+		+
Pest Resistance	+	+	+	+
Productivity	+	+	+	+

We believe that marker applications and genomics research have the broadest applicability across the six interest areas we enumerated.

FOREST SERVICE R&D IN BIOTECHNOLOGY

Eight Research Work Units in the FS R&D are involved in biotechnology research that employs genetics. Four of these (Corvallis, Oregon; Placerville, California; West Lafayette, Indiana; and Saucier, Mississippi) are units whose mission is genetics research. The remaining four locations (Delaware, Ohio; Moscow, Idaho; Rhinelander, Wisconsin; and Missoula, Montana) have units with broader missions but employ a significant component of genetics in their research. Current research at each location represents a large range in objectives from applied tree improvement to fundamental genomics research on genome organization and function (Table 3). Species under study include trees, other plants, insects, diseases, and animals.

Table 3. Forest Service R&D research employing biotechnology

Genetic Issues	Corvallis	Delaware	Missoula	Moscow	Placerville	Rhinelander	Saucier	W. Lafayette
Biodiversity	+		+		+		+	
Conservation	+		+		+		+	
Forensics			+				+	+
Genecology	+			+	+	+	+	
Pest Resistance	+	+		+	+		+	+
Productivity	+				+	+	+	+

The different types of research conducted at the eight locations are reflected in the kinds of biotechnology tools they employ (Table 4).

Table 4. Biotechnology emphasis of Forest Service R&D Research Work Units

Biotechnology Research Areas	Corvallis	Delaware	Missoula	Moscow	Placerville	Rhinelander	Saucier	W. Lafayette
Marker applications	+	+	+	+	+	+	+	+
Genomics	+				+		+	
Genetic transformation								+
Vegetative propagation								+

PARTNERSHIPS

The Forest Service is cooperating with a wide variety of partners to accomplish its genetics research and development. This is especially true in the area of biotechnology where the various Research Work Units collaborate with universities, private industry, private genetics companies, tree improvement cooperatives, other federal agencies, Forest Service National Forest System and State & Private Forestry. One current example is the Loblolly Pine Genome Project (LPGP) that is currently being planned (http://dendrome.ucdavis.edu/lpgp/, University of California at Davis, last accessed on 10/21/2004). Dr. David Neale, Pacific Southwest Research Station, Davis/Placerville, California, has provided leadership for the LPGP that has six committees and involves universities, private industry, private genetics companies, tree improvement cooperatives, and other Forest Service Research Work Units.

There are a host of other such biotechnology research projects involving Forest Service scientists. In fact, this line of research is typically complex and the level of funding required is quite large in order to make significant progress; hence, it lends itself to cooperative efforts.

CONCLUSION

The USDA Forest Service is keenly interested in using genetics as a tool to solve both basic and applied problems. A wide range of biotechnology research is being conducted by at least eight Research Work Units with a large number of species of trees, other plants, animals, fish, insects and diseases. Much of this research and development is conducted with partners. Numerous other Forest Service R&D, National Forest System, and State & Private Forestry work units contract and collaborate with genetics labs to use biotechnology to solve management issues. Biotechnology is a significant part of our society and is used to solve a myriad of problems and to create opportunities in natural resource management. Given this fact, biotechnology R&D in the Forest Service will continue to grow in the future.

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Maize Genome Organization and its Implications for Applied Genetics

S.V.Tingey, Research Fellow
Crop Genetics Department, DuPont Company, Wilmington, DE, USA

Crop improvement fundamentally affects culture, health and quality of the environment. We are now entering a new century of crop improvement, and sound science is building an unprecedented view of gene content, genome organization, and historical selections contributing to yield. The seed is a superb vehicle for the creation and dissemination of the benefits stemming from research and product development. However, current applications of biotechnology require investments into basic and applied research hitherto not undertaken in agriculture. If biotechnology is to continue to drive crop improvement, gene discovery efforts need to focus on loci which are not only necessary, but also sufficient to modify phenotype.

A genetic discovery paradigm will be presented, which has as its primary objective the normalization of a large crop genome. Historical recombination and selection within a maize breeding population have been used to create a genetic framework, which when aligned with a physical representation of gene order and genetic diversity, provide an efficient discovery platform for genes that control agricultural traits.

Recent revelations, coming primarily from the application of genome science technologies, have shown that maize gene content is perhaps less diverse than previously anticipated. In contrast, comparative genome studies demonstrate that DNA sequence micro-colinearity between maize varieties can be very different both with respect to gene content and intergenic sequence composition. These observations reveal that the cultivated maize genome has gone through extensive modification with respect to genic positions, and may have also been the product of several independent genome expansions over the last two million years.

Implications for maize crop improvement will be presented, based on a rapidly changing picture of maize evolution and historical improvement.

Genomics to Breeding in Loblolly Pine

David B. Neale[1], John M. Davis[2], Timothy L. White[2], Timothy A. Martin[2], Jeffrey F.D. Dean[3], Sarah F. Covert[3], Lee H. Pratt[3], Marie-Michele Cordonnier-Pratt[3]

[1.]Institute of Forest Genetics, USDA Forest Service and the University of California, Davis, [2.]University of Florida, [3.]University of Georgia

The Allele Discovery for Economic Pine Traits (ADEPT) consortium was developed with the goal of bringing genomic technologies to application in the breeding of loblolly pine. The association genetics approach was used to discover relationships between naturally occurring allelic variation and phenotypic variation for a suite of economic traits. This approach has been pioneered in human genetics and is leading to the discovery of genetic variation causing complex and common disease. The ADEPT project has focused initially on discovery of alleles responsible for quantitative resistance to fusiform rust and pine pitch canker and response to water deficit. A large clonal study from the University of Florida Forest Biology Research Cooperative forms the discovery population. The University of Georgia was responsible for gene sequencing and bioinformatics. The Institute of Forest Genetics and UC Davis conducted allele discovery by high-throughput re-sequencing and single nucleotide (SNP) genotyping. Results will be presented that demonstrate that individual genes controlling for quantitative traits can be identified and the effects of individual alleles on phenotypes can be estimated. It is expected that a genomic approach to quantitative trait improvement in loblolly pine can be realized in the very near future.

Population Management in the Age of Genomics

Rowland D. Burdon[1] and Phillip L.Wilcox[1]

Abstract: Continued population improvement will remain the foundation for future genetic gain in forest trees. Any gains that can accrue directly from genetic engineering, and be captured through propagation technology, will be superimposed on those from population improvement. Efficient population management will need to be directed not only at pursuing recognised breeding goals, but also in preparing for addressing new, unpredictable breeding goals. Both these goals are served by maintaining genetic diversity.

Genetic diversity depends initially on the population base. For characterising the base, genomics is a powerful adjunct, in conjunction with common-garden experiments. Within populations there are various possible measures of genetic diversity, involving polymorphisms for neutral markers and functional genes, and numbers of alleles and allele frequencies. Calibrating marker diversity against functional diversity is not simple, but will become easier with functional genomics in which the phenotypic impacts of nucleotide polymorphisms may eventually be identified. A need exists to counter the tendency for continued selection to erode diversity.

An associated, but not identical issue is controlling levels of inbreeding, which is not wanted in commercial material and yet may serve as a breeding tool. A customary approach is to pursue intensive ongoing genetic gain in a pedigreed breeding population underpinned by very broadly based gene resources. Use of genomics can relax some requirements for population management, notably the need for pair-crossing in order to maintain pedigree. Related to that, genomics can serve to retrieve historical losses of pedigree information, even overturning the tenet of production populations being genetic dead-ends. It can also correct potentially costly misidentification of material.

Genomic information has the widely hailed application of marker-assisted selection, although this has encountered various problems with forest trees. It is a promising tool in the quest not just for disease resistance but resistance that is durable. This requires a diversity of resistance mechanisms, yet the presence of some resistance genes can mask the presence or absence of others, unless the latter can be detected genomically.

With future knowledge of functional polymorphisms the new understanding of the nature and origins of genetic variation should allow far more efficient management of populations in order to both exploit genetic diversity and preserve it for the longer term.

Keywords: Population management, genetic diversity, genomics, DNA markers, pedigree

[1] New Zealand Forest Research Institute, Private Bag 3020, Rotorua, New Zealand

INTRODUCTION

Genomic information on forest trees can be used for several purposes, apart from being part of the advancing front of fundamental knowledge. Of main interest for this paper is how the availability of genomic research tools and the resulting information can serve the underlying objectives of population management which, in turn, is all about capturing cumulative genetic gain while maintaining the genetic diversity that safeguards the future genetic gains. For capturing genetic gain, genomic information can be used for marker-assisted or marker-based selection which exploits linkage disequilibria between assayable polymorphisms and ones that underpin phenotypic variability. Such selection can be refined into gene-assisted or gene-based selection if it has become possible to identify actual nucleotide polymorphisms that govern quantitative effects on traits. Much has been written about the issues of refining and quantifying the efficiency of selection based on use of genomic information, but for this paper that is of interest mainly for its ramifications for appropriate structuring of populations. Genomic information also has value for safeguarding the capture of genetic gains by verifying genetic identity. Of prime interest for this paper are the various more direct applications of genomic information for appropriately structuring populations.

The need for genetic diversity in forest tree breeding programs has never been seriously challenged, even if it has not always been honoured in how improvement programs are implemented. Long-term gain will depend on genetic diversity, which will tend to be run down by the process of intensive selection and associated maintenance of very finite breeding populations. Such limitations in long-term response to may not arise at all rapidly provided the same breeding goal continues to be pursued. However, breeding goals can change rapidly, owing to changes in market perceptions or biotic developments such as the emergence of significant fungal diseases or animal pests. Such changes can be substantial, and place heavy demands on available genetic diversity. While genetic engineering offers the prospect of conferring attributes that are not programmed by any of the available genetic diversity within species, the contributions of genetic engineering are widely seen as being properly superimposed upon a platform of genetic improvement resulting from classical breeding. For instance, engineering a new function from scratch can take a long time, and be a very uncertain venture compared with capturing the attribute from straightforward expression of existing genetic variation. On the other hand, genetic engineering technologies could also confer a narrow suite of attributes faster than existing breeding approaches can, although this may well impose added pressures of reduction in diversity, particularly in deployed populations.

Even without a specific or pressing problem, obtaining a meaningful measure of genetic diversity is highly desirable. Diversity can derive both from diverse population origins and within-population variation. Genomics can be used to indicate origins, of material of mixed ancestry. Traditional taxonomic tools, including chemotaxonomic ones, can be effective in this area, but genomic information has major advantages through often requiring limited tissue samples, providing quick results, and being independent of the multifarious environmental influences. Information on population origins needs to be complemented by information from common-garden field experiments which yield information on a range of traits that include growth potential, environmental tolerances, biotic resistances, and wood properties.

MANAGEMENT ISSUES

The issues will be addressed under the following categories:

- Characterising the genetic base (sorting out taxonomy and crossability; identifying ancestral populations, migration history, and past adaptive pressures)
- Quantifying within-population diversity
- Managing inbreeding and relatedness
- Selection using markers
- Assuring durable disease resistance
- The future (in the era of functional genomics).

GENETIC BASE

Knowledge of the genetic base that gave rise to one's population is always welcome, although not necessarily crucial or easily obtained. In the first instance, one may want to clear up the taxonomic status of one's material. Crossability, while an important but not absolute taxonomic criterion, is of immediate practical importance and something that needs to be addressed empirically. For addressing the general taxonomic issues, there are various classical herbarium-based criteria, which have come to be supplemented by phytochemical and cytological evidence, but genomic analysis is developing into a powerful new tool for the purpose. Even without a traditional taxonomic issue, it can be valuable, for instance, to know the geographic origins of a land-race population. For instance, the effects of sub-optimal origin may have been masked by either a shake-out of the neighbourhood inbreeding of natural populations, or by adaptive responses to selection pressures imposed by the exotic environment. Alternatively, a knowledge of the Quaternary migration history may warn one that a local population is likely to be significantly sub-optimal for commercial purposes. For addressing such issues, common-garden field experiments are likely to provide the best information, but genomic information may often provide a powerful adjunct, with the advantage of being far quicker to obtain.

Attempts to characterize different populations or even interfertile species for their collective diversity, without the benefit of common-garden field experiments, can be perilous. Apparent affinities among such entities can depend very strongly on the set of genomic markers chosen. Besides, the inherently neutral nature of many genomic markers, whatever their power for certain purposes, means that there is no *a priori* reason why they should indicate values for the adaptive or commercial traits that are of direct interest to the breeder. In a case that will not be named, a genomic study showed a particular tree species to be undistinctive with respect to the markers used. From that, the suggestion was made that the species could forgotten about for purposes of genetic conservation. This flew in the face of common forestry knowledge that the species concerned is highly distinctive in respect of certain ecological tolerances and silvical properties.

Indeed, indications are that neutral DNA markers can often be poor indicators of adaptive differences among populations within species (e.g. Karhu et al. 1996).

Where there is interest in preserving populations in a genetically pure state, genomics can serve as a new and powerful tool for detecting genetic contamination, quantifying it, and even culling individuals resulting from the contamination. This, however, will depend on identifying DNA markers that differentiate well between the populations in question.

ISSUES OF QUANTIFTING WITHIN-POPULATION DIVERSITY AND IMPLICATIONS

Apart from population origins being a source of diversity that can be both identified and characterized, there is the within-population diversity, which may often be the bigger general issue. There remains the issue of whether marker diversity parallels the functional diversity that is of real interest to the breeder. But there are also issues of what are the most meaningful measures of diversity for markers and functional traits respectively.

Various possible measures of diversity exist, and they can have different implications according to the intensity of selection and the time frame of interest. For both markers and functional alleles there are numbers of alleles at the polymorphic loci, the expected heterozygosity over such loci which will often be dominated by intermediate allele frequencies, plus the numbers of loci involved. For functional alleles, the magnitudes of their effects are also of obvious importance.

Numbers of alleles per locus and expected heterozygosity are two, largely complementary measures. Numbers of alleles are readily influenced by sampling effects. As such, they are very subject to founder effects or genetic drift, which can both occur incidental to intensive selection. They will also be affected by sample sizes in population surveys.

Percentages of functioning genes that are polymorphic are still conjectural, at least for the typical outbreeding forest tree species. Nevertheless, this is an area where knowledge will doubtless make rapid advances (see later).

Potential response to selection depends on functional diversity. The immediate functional diversity, and consequent short-term responses to selection, will tend to be governed by alleles of intermediate frequency – unless there is both very intensive selection and alleles of large effect for the trait(s) under selection. Longer-term selection responses, however, may depend more on alleles that are currently at low frequencies but will only contribute significantly to expressed variation when their frequencies increase. The ways in which low-frequency alleles contribute to functional diversity will be especially sensitive to the degree to which the effects of such alleles are recessive or dominant.

In functional genes, many polymorphisms are silent, since they involve synonymous codons. Considerable discrimination is therefore possible between identity by function and identity by descent, which may offer important insights into the way in which different populations may have come to diverge.

Another issue is to what extent the functional genetic variation is governed by polymorphisms in the coding or promoter regions of genes. Some recent findings for plant species other than forest trees (e.g. Morgante & Salamini 2003, Paran & Zamir 2003) have indicated that large phenotypic variations can be governed by polymorphisms in the regulatory region rather than in the coding regions of the genes concerned. Such genetic variation will therefore not be evident in protein polymorphisms, although it could be detected via variation in transcript- protein- and/or metabolite concentrations in specific tissues.

MANAGING INBREEDING AND RELATEDNESS

Apart from running down genetic diversity, long-term maintenance of closed, finite breeding populations inevitably incurs some level of inbreeding. Inbreeding is not necessarily the same thing as loss of diversity, because deliberate inbreeding can be practised to both maintain diversity and preserve options for subsequent outcrossing. Deleterious effects arising from inbreeding, namely loss in fitness and/or reproductive capacity via the effects of lethal or semi-lethal loci, could be managed using information from markers linked to such loci (e.g. Kuang et al. 1998). However, costs involved in establishing populations for detection of marker-trait associations are likely to be considerable relative to the gains from such selection, particularly in breeding populations. Nonetheless, as coancestry builds in such population, the imperative is likely to increase for more proactively dealing with the problem. Options range from avoiding certain allelic combinations to actively purging lethal genes.

For both managing inbreeding and maintaining genetic diversity, maintaining full pedigree information is extremely valuable. However, the traditional way of maintaining full pedigree has its costs, both direct and in achieving some of its aims. Making controlled pair-crosses, and keeping pair-crosses properly identified in replicated field experiments, are expensive operations, especially if the reproductive biology of a species makes pair-crossing difficult. In some cases, open pollination may even be preferred. Also, the size of fully pedigreed breeding populations may be constrained to levels at which loss of low-frequency alleles can become significant (Burdon 1997).

Even where full pedigree is nominally kept, misidentification can occur in various ways. That can compromise genetic gain, the more so the more advanced a breeding program is. It could also compromise control of inbreeding, and even maintenance of genetic diversity. However, simple-sequence repeat (SSR), or microsatellite, markers are proving a very powerful tool for verifying identity of both individual clones and parentage of progenies. Indeed, the misidentification rates being thus detected are quite embarrassing – something that is admitted much more freely by word of mouth than in print.

Genomic information, however, can allow retrieval of pedigree information. Lambeth *et al.* (2001) has advocated the use of DNA markers in order to allow retrieval of full pedigree information after saving costs by making polycrosses instead of pair-crosses. Earlier, Burdon (1997) had proposed use of DNA markers be used to make good a complete lack of pedigree information if it was necessary to select from unpedigreed commercial stands. This eventuality runs counter to a traditional tenet of commercial stands being a genetic dead-end in population

management. It could, however, arise in the case of a biotic crisis whereby only very rare trees were resistant to a new disease or pathogen strain. Such resistant individuals might be products of very rare favourable *de-novo* mutations, and finding them would likely depend on the sheer numbers of genotypes that would only be available in large commercial plantings. Pedigree reconstruction would allow the breeder to avoid selecting resistant trees all from one or a very few pedigrees with the attendant risks of both inbreeding and loss of genetic diversity. Having a finite set of possible or likely parents, as may be the case in seed orchards, would be a big advantage. That said, the challenges of achieving complete pedigree reconstruction may be formidable, but even partial success may suffice for achieving the underlying objective. Such a scenario may seem fanciful, but biotic crises have affected some very important tree species. For the breeder to tackle a biotic crisis head-on would also require powerful propagation technology and/or the lack of alternative species that are strongly competitive commercially.

Despite the obvious challenges in developing a sufficiently powerful set of markers for complete pedigree reconstruction, the sort of SSR marker diversity discovered in pines (Karhu et al. 2000) and and Douglas-fir (Slavov et al. 2004) augurs well.

SELECTION USING GENETIC MARKERS

The potential benefits of using genomics to detect and use quantitative trait loci (QTL) for marker-assisted or marker-based selection (collectively referred to as MAS) are well recognised. It is widely agreed that there are tree general areas where MAS could be especially beneficial: increasing selection intensity, earlier selection, and cheaper selection for expensive-to-measure traits. However, various methodological traps have come to light (e.g. Ball 2001), and much had already been written about both the potential advantages and the pitfalls of MAS (e.g. Strauss et al. 1992, Johnson et al. 2000). The magnitudes of QTL effects are subject to estimation errors, and some can be greatly overestimated, in what is widely known as selection bias. However, experience with annual (or other short-term) agricultural crops has indicated that where QTL of large effects are involved, as may happen with introgressing genes from wild relatives into domesticated crops, some major payoffs can accrue (cf Paran & Zamir 2003).

Overall, MAS can be a selection tool for both breeding and production populations. While use of MAS as such is in no way central to population management, questions have been raised concerning the possibility that its intensive use would incur risks of otherwise avoidable loss of potentially useful alleles. On the other hand, as information on functional loci becomes increasingly available, tools to track specific alleles will become more and more effective, facilitating quantification and/or avoidance of allele loss during selection.

With forest trees, which are typically outbreeders, there is usually not the linkage disequilibrium between non-genic marker alleles and QTL that is required for MAS. However, with fusion of well-differentiated populations, or with advanced hybrids between species (as in domesticated apple - Bus et al. 2000) – there may well be cases of the desired combination of large-effect QTL and strong linkage disequilibrium. Another exception is within known pedigrees, for which linkages have to be identified in each individual case. This area has been the focus of much effort over the past decade, driven in part by the development of within-family marker-assisted

selection. Such research has also resulted in the development of marker tools that can reveal much about population structure and existing inbreeding levels. However, these marker systems are largely based on polymorphisms in non-gene-associated regions, and may not adequately reflect the underlying functional variation.

More recently, research has changed focus from pedigree-specific populations, to non-pedigreed association genetics. One of the reasons for this is the desire to develop the option of among-family selection. The marker systems concerned would be more likely to be based on expressed genes that have a suspected role in trait variation. The benefit of assaying polymorphisms within and associated with expressed genes, together with non-structured populations for ascertaining marker-trait associations, is that key parameters such as gene and nucleotide diversity, heterozygosity, inbreeding and coancestry relationships can be calculated (Neale & Savolinen 2004). Therefore, the experimentation required for gene-assisted among- and within-family selection has the added benefit of revealing key information regarding those experimental populations.

A key constraint to the implementation of markers as selection tools, is the substantive up-front costs required to detect marker-trait associations (Johnson et al. 2000, Wilcox et al. 2001). As such the more likely implementation is in deployed populations as opposed to breeding population advancement, because of earlier commercial returns. If marker-trait associations are used for selection in the breeding population, a build-up of coancestry may be hastened because of unequal selection rates in specific pedigrees. Such a build-up would need to be judiciously countered in order to maintain diversity.

DISEASE RESISTANCE (DURABILITY)

A related issue is ensuring durability of disease resistance against genetic shifts in the pathogens (e.g. Burdon 2001). While there is no certainty that lack of durability will be a problem in any particular case, measures to ensure durability seem prudent. Durability typically appears to depend strongly on a diversity of resistance mechanisms. For large-scale deployment of individual clones, pyramiding a multiplicity of resistance factors within individual clones is a likely need. For heterogeneous populations, it appears that population resistance is not dependent on a multiplicity of resistance factors all being present in every individual. Either way, however, knowledge of the various resistance factors, how they operate, and how to detect their presence, is extremely valuable. It is one of the problems with disease resistance that some of the more spectacular forms of resistance are vulnerable to pathotype shifts and yet tend to mask the presence of other resistance factors that may be needed in order to ensure durability. For addressing this general problem, genomic information when combined with infection studies in genetic experiments can be extremely powerful, and its use is progressing rapidly with annual crops. This is partly because there are often individual resistance genes of substantial effects in situations where there are superficial appearances of polygenic resistance. With the loblolly pine/fusiform rust pathosystem progress is being made in identifying genetic factors involved in resistance (Wilcox et al. 1997, Schmidt 2003). On the one hand, the short period of the pine host susceptibility during the rotation may make for a relatively simple pathosystem, and one in which the pine is not unduly vulnerable to rapid pathotype shifts (Schmidt 2003); on the other

hand, acquiring good data is relatively slow and difficult. In the case of leaf rusts affecting poplars more rapid research progress is apparently being made (e.g. Yin et al. 2004). Apart from poplars having come to be treated widely as model species, the biology of the rusts is conducive to much more rapid acquisition of data.

With the search for resistance to blister rust on North American white pines much progress has been made by classical genetic studies. Various individual resistance genes have been identified, and the nature of their action (e.g. dominance or recessiveness) characterised (Sniezko et al. 2004). This, however, has been a long, drawn-out process. It is to be hoped that, with genomic tools available, future progress can be much more rapid, in both research and its applications.

For deployment of genetically resistant material the diversity of resistance mechanisms can, in principle, be used judiciously. Comprehensive pyramiding of resistance genes in individual genotypes cannot be expected to come quickly. But, even without such pyramiding, it should be possible to deploy mixes of tree genotypes that can be expected to show good population resistance.

THE FUTURE

Current population management is based largely upon various suppositions, which are often tacit rather than explicit, concerning the nature of genetic diversity and the ways in which it can be generated. The usual rule of thumb is for production populations to contain a (very) few tens of effectively unrelated individuals, breeding populations a few hundred, and gene resources a few thousands (e.g. White 1992). It tends to be assumed that mutation is a rare event at any individual locus, but that low-frequency alleles are potentially very important. Yet there are various long-term selection experiments, in which continued response has occurred for many generations in closed populations, which suggests that significant mutational or quasi-mutational events may not be rare. Since maintaining breeding populations and gene resources is expensive, and likely to become more so as progressive genetic gain increases the opportunity costs of maintaining such material, the question of requisite populations sizes and appropriate management systems is far from trivial.

Questions of both population management and capture of genetic gain for operational use will be increasingly addressed by extending genomics into the study of functional genes. There are various ways in which this can be done. First, the genes need to be identified and located, and then their polymorphisms can be studied. Starting with just with information on tight linkages still leaves very long sequences to investigate and interpret. However, with libraries of expressed sequence tags (ESTs), belonging to genes whose function has been identified in model species, there are now much more powerful tools for locating specific genes. The quintessential model plant species has become Arabidopsis which, despite not being closely related to conifers, shows remarkably high levels of orthology with the full range of forest trees (e.g. Kirst et al. 2003), even though much of the developmental physiology and morphology appear very different. However, with the availability of the full nuclear and organelle genomic sequences of poplar (*Populus trichocarpa*) (http://www.jgi.doe.gov/poplar), it is likely that this will become the model species of choice for certain purposes. Among conifers, there are not only the

orthologies but also some remarkably high levels of synteny (e.g. between loblolly and Monterey pines). Thus, genes of common functions tend to be located in essentially the same positions on the same chromosomes of the respective species, so genomic information obtained from one species can be easily used for studying another. With the identity and roles of various genes identified, the developmental significance of polymorphisms can then be ascertained. Tactically, that can allow the breeder to screen for particular alleles, or to identify alleles that might be transferred between species by genetic transformation.

The value of orthologies for probing the nature of polymorphisms has its limits, however, since the roles of particular genes in governing trait phenotypes can vary among plant species. Experimentally, the roles of different base-sequence alleles in governing phenotype can be probed or verified by using genetic transformation; however, some of the information may take a long time to come in with trees, particularly for traits that are expressed only at later ages and where no early indicator characteristics can be used.

Various strategic implications will stem from the basic information on the levels and nature of polymorphism, on the dynamics and nature of mutation and on gene transposition. Knowing the level and nature of polymorphisms involving gene function, as distinct from recent descent, should give better insights into the significance of numbers, at different stages in the life cycle, in population management.

We have various implicitly or explicitly pedigree-based measures of effective population size, which serve as indicators of changes in genetic diversity. These measures include Status Number (Lindgren et al. 1996), as well as more traditional measures (Caballero 1994). Although we have some intuitive appreciation of the value of different measures for different purposes, their exact practical significance is usually very uncertain. Hopefully, with knowledge of the level and nature of functional polymorphisms, and of mutational processes and transpositions, it will be possible to achieve not only better and more direct measures of genetic diversity but also much better interpretation of the measures of effective population size.

CONCLUDING

For many of those who are charged with population management, or see it as their responsibility, a strongly precautionary approach is indicated in the present state of genomic knowledge. At the same time, breeders may have to deal with people who want to see guarantees of returns from the direct costs and opportunity costs involved in population management, which can place them in a difficult situation. Better knowledge will therefore be extremely welcome. As yet we have no silver bullet, and we may never be able to majorly relax the requirements for sizes of the populations needed for continuing advance of breeding programmes. Nonetheless, the very rapid progress that is now occurring in functional genomic promises a much better basis for future decision-making.

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Genetic Architecture of Quantitative Traits and Commercial Application 0f Marker Aided Selection in Pines

S. D. Carson[1]

[1]Director, Forest Genetics Ltd, Rotorua, New Zealand

Reports of QTL detection experiments with pines concluding that traits previously thought to be quantitatively inherited are actually controlled by relatively large effect genes are accumulating in the literature. But does the data really prove this hypothesis? Can the data also be said to support an alternative hypothesis of only small gene effects? Results from simulation of QTL detection experiments and experimental data with *Pinus radiata* suggest the latter. Implications for commercial application of MAS (identified using any experimental method) will be discussed, and projections of gain from MAS for wood density and diameter in *Pinus radiata* will be presented.

Map-Based Assembly and Structural Characterization of the *Populus* Genome

Stephen P. DiFazio[1], Tong-Ming Yin[1], Nik Putnam[3], Gwo-Liang Chen[2],Philip F. LoCascio[2], Dan Rokhsar[3], Frank Larimer[2], and Gerald A. Tuskan[1]

[1]Environmental Sciences Division and [2]Life Sciences Division, Oak Ridge National Laboratory
[3]DOE Joint Genome Institute

The international effort to sequence and characterize the *Populus* genome is a pioneering event for woody perennial plant research. The genome was sequenced to 8.1X depth by the Joint Genome Institute of DOE, supplemented by end-sequencing of a 10X BAC library by Genome Canada. Assembly was accomplished using the JAZZ shotgun sequence assembly program, coupled with physical mapping data derived from BAC fingerprinting and genetic mapping using 688 microsatellite loci. The assembled genome consists of 19 map-based scaffolds (corresponding to all *Populus* chromosomes) containing approximately 307 megabases (Mb) of genome sequence. Physical distance was directly proportional to genetic distance, but with substantial variation across the genome. An additional 177 Mb of sequence is contained in nearly 22,000 unmapped sequence scaffolds, many of which consist of repetitive, noncoding DNA. We have characterized repeat composition of the genome using several independent methods, including an assessment of the frequency of 16mers in raw sequence reads, and all-vs-all blast searches of sequence scaffolds followed by clustering and multiple sequence alignment of repetitive elements. We have identified over 1000 putative transposable elements and over 25,000 uncharacterized repeat elements, comprising approximately 25% of the assembled genome. Repeat occurrence is inversely proportional to the size of sequence scaffolds. In addition, approximately 27% of the raw sequence reads remain unassembled, and repeat composition is higher than that of assembled reads. Map-based methods will be used to assemble the vast majority of euchromatic DNA, thus circumventing the difficulties posed by repetitive DNA for assembly of whole-genome shotgun sequence.

Challenges in The *Ex situ* Conservation of Tropical Trees

W. S. Dvorak

Director: CAMCORE & Professor, Department of Forestry,
N. C. State University, Raleigh, NC, USA

CAMCORE has worked to conserve 38 tropical and subtropical species representing 500 populations in the last 24 years. Based on criteria developed by the International Union for Conservation of Nature and Natural Resources (IUCN), the conservation status of these species can be categorized as: 37% low risk, 50% vulnerable, 11% endangered, and 2% critically endangered. Within species classified as "low risk" are tree populations that are threatened by encroachment from humans.

Eucalyptus urophylla, *Gmelina arborea*, *Pinus jaliscana* and several populations of *P. caribaea* var. *hondurensis* have been the focus of our conservation efforts in Southeast Asia and Mesoamerica in the last five years. Each species carries with it specific challenges for *ex situ* conservation either in the sampling phase in natural stands or the establishment of field conservation plantings. For *Pinus caribaea* the challenge is whether to sample isolated populations in the northern range of the species in Guatemala and Mexico or concentrate on more accessible stands in the central part of the natural distribution. For *Pinus jaliscana* it is learning how to grow the species as an exotic. Jaliscana pine has a tendency to die back at 1 m height in most countries where it has been tested. The reasons for this are still unknown. For *Eucalyptus urophylla*, *ex situ* conservation plantings are often contaminated with pollen from more abundant plantations of *E. grandis*. A myriad of questions exist on how to maintain pure lines of *E. urophylla* and the benefit for doing so. For *Gmelina arborea*, material collected from natural stands in South East Asia never grows as well as improved material from a Costa Rican seed orchard. The question becomes how to best incorporate unimproved material from conservation programs into advanced generation tree breeding programs. Conservation works best when users see the long-term benefits for their efforts.

Some of our oldest field plantings are more than twenty years of age. CAMCORE is embarking on the development of 2nd generation conservation strategy. The main challenge deals with developing a sampling strategy in first generation field plantings to maintain population integrity in the 2nd generation in a cost-effective manner. Details are provided in the presentation.

Conservation of Forest Genetic Resources: National and International Perspectives

Judy Loo

Ecological geneticist, Natural Resources Canada, Atlantic Forestry Centre, Canadian Forest Service; Coordinator, IUFRO Task Force 4, and Chair, Canadian delegation, NA Forest Genetic Resources Working Group; P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7, Canada

Abstract

Conservation of forest genetic resources is recognized increasingly as an important component of sustainable resource management, as well as an important aspect of conservation of biological diversity. Serious global threats to forest genetic resources include climate change, loss and alteration of habitat, and invasive alien species. The natural diversity within native gene pools contains valuable keys for responding to these threats, so erosion of genetic diversity narrows options for adaptation and mitigation.

In spite of increasing concern, the range of forest species under genetic management or conservation remains small. Resources for research, management, and conservation of genetic resources are more readily available for agriculture than forest species and little attention is paid to tree or shrub species that lack major commercial importance. The IUFRO Task Force 4: Management and Conservation of Forest Genetic Resources has attempted to assess the state of knowledge of forest genetic resources and the related research. The apparent level of interest and knowledge in this important area is disturbingly low.

The Forestry Commissions of the FAO offer an opportunity for multi-national initiatives on forest genetic resources through regional working groups. The North American Forest Genetic Resources Working Group focuses primarily on temperate tree species at risk in Mexico, which have closely related species in Canada and the United States. Research that will contribute to development and implementation of gene conservation strategies is conducted on the three species of *Picea* found in Mexico, and several *Pinus* species.

In Canada an initiative is underway to develop a coordinated gene conservation program, beginning with a preliminary assessment of tree and shrub species requiring gene conservation attention across the country. A total of 200 native tree and shrub species were identified as requiring conservation attention in one or more Canadian provinces and territories.

Conservation of genetic resources

Conservation of genetic resources is receiving increasing attention globally, for several reasons. The prediction, and increasingly, the realization of changing conditions including global warming, loss and fragmentation of habitats, and introduction of novel pathogens and pests to all parts of the globe highlight the need to maintain genetic diversity in natural and managed populations. Genetic diversity is a necessary ingredient for any planned response to such threats to global forests. Genetic resources are necessary to counter threats as well as to capitalize on opportunities presented by changing conditions.

In spite of a growing recognition of its importance, efforts to manage and conserve forest genetic resources remain inadequate, with only a few exceptions. Genetic management for the relatively small number of species with present commercial importance for plantation forestry receives attention, but most species, lacking commercial importance, receive little if any attention.

IUFRO Task Force 4: Management and conservation of forest genetic resources

In 1997, FAO asked the IUFRO to provide information on the state of forest genetic resources globally and Task Force 4 was formed with representation drawn from all of the IUFRO Divisions, because of a recognition that forest genetic resource management must extend beyond geneticists. The following terms of reference were given to the Task Force:

1) Gather and synthesize scientific information on:
    a. Scientific knowledge necessary for the conservation of forest genetic resources: management of base and breeding populations, maintenance of representative diversity, including rare populations, case studies on *in* and *ex situ* conservation.
    b. Interaction between human activity and integrity of forest genetic resources: silviculture, forest operations, agroforestry, forest and landscape management, etc.
    c. Effect of environmental factors on the integrity of forest genetic resources: insect pests, diseases, air pollution, climate change.
2) Organize sessions, particularly during the IUFRO Congress 2000
3) Prepare publications on all these items
4) Report to the IUFRO Expanded Board twice a year until August 2000: April and August.

To determine whether there was information available and where there are gaps in knowledge in all the areas listed above, a questionnaire was developed to assess the state of research on forest genetic resources and it was sent to all IUFRO members. The questionnaire included questions on policy, behaviour and society, economy, management and operations, ecology and environment, and biology as they relate to forest genetic resources.

Questionnaire Results:

After a poor first response to the questionnaire sent out in 2000 (only 72 responses), a second attempt in 2002 resulted in only 16 additional responses. The largest number of responses was from Europe and responses from Europe generally indicated a higher level of knowledge on the various questions than did responses from other areas. In general, there was little response on policy and economics questions, and more is known about commercially important species than species of less economic importance. The poor response and the number of empty cells in questionnaires that were returned indicate a need for more research in a number of areas as well as a need for broader recognition of the importance of forest genetic resources.

Results of the questionnaire will be presented in Brisbane, 2005 and published in "*State of the Art Report on Research on Forest Tree Genetic Diversity*". The Task Force is organizing two sessions involving management and conservation of forest genetic resources at the Brisbane Congress, as well. Task Force 4 will likely end at the Congress and the work will be absorbed into working parties including a new one on endangered tree species.

North American Forestry Commission: Genetic Resources Working Group

The FAO has six forestry commissions in different areas of the world. The Forest Genetic Resources Working Group (http://www.fs.fed.us/global/nafc/genetics/aboutus.htm) of the North American Forestry Commission is one of seven working groups constituted of three members, with diverse skills, from each of the three countries: Mexico, the United States and Canada. The mission of the Working Group is to encourage and promote conservation of all forest genetic resources and the focus is primarily in Mexico, where the need seems greatest, on rare and threatened temperate species of the genera: *Pinus*, *Picea*, *Pseudotsuga*, and *Abies*.

The Working Group was established in 1961 under a different name and had its first meeting in 1965. In 1967 members recommended to the North American Forestry Commission "…to establish a program…to conserve endangered germplasm of North American forest trees", and later, in 1969, another recommendation was "…to implement programs for the preservation of endangered arboreal germplasm".

Objectives of the Working Group are to:

1) Collect and disseminate information
2) Coordinate research, conservation, and training
3) Facilitate exchange of genetic resources
4) Encourage tree improvement (as an aid to conservation of forest genetic resources)

Activities

Examples of collection and dissemination of information include the production of a report on the status of temperate North American forest genetic resources at the request of FAO, publication of a guide to the management of forest genetic resources, translated into Spanish, organization and delivery of numerous symposia and training sessions, as well as publication of symposia proceedings and other publications, and organization and participation in study tours. Symposia and Short Courses (ranging in length from 1 day to 4 weeks) offered by the Working Group in the past 12 years include:

- Placerville, California, USA – 1993
- Chapingo, México, México – 1995
- Berkeley, California, USA – 1995
- Sainte-Foy, Québec, Canada – 1996
- Colegio de Postgraduados, México – 1998
- Mérida, Yucatán, México – 2000
- Colegio de Postgraduados, México – 2000
- Xalapa, Veracruz, México – 2002
- Colegio de Postgraduados, México - 2002
- Québec City, Canada – 2003
- Colegio de Postgraduados, México – 2004
- Chapingo, México, México – 2004
- Morelia, Michoacan, México – 2004

The three species of endangered Mexican spruce, *Picea chihuahuana*, *P. mexicana*, and *P. maximartenizii*, were the focus of a series of studies carried out by Tom Ledig and others (Ledig, *et al*. 2004; 2002; 2000; 1997) evaluating genetic diversity and population genetic structure. Ledig *et al.* (1997) found low levels of allozyme diversity and evidence for almost complete selfing in *P. chihuahuana* populations. They recommend under-planting seedlings among the small populations to foster artificial gene flow. Seed collections have been made from populations of all three species over the years.

Genetic studies have also been carried out by members of the working group, in collaboration with Mexican scientists, on the *Pinus ayacahuite-strobiformis-flexilis* complex, Mexican species of *Abies*, and the Douglas-firs: *Pseudotsuga flahaulti, guinieri, macrolepis*, and *rehderi* or *menziezii*. A current ecological and genetics study of *Pinus pinceana* involves scientists and students at two Mexican universities, a PhD student studying in Canada and working group members from all three countries. *P. pinceana* is one of several endangered piñon pine species endemic to Mexico, with a distribution stretching from the northern state of Nuevo Leon, south to Hildalgo, but only in three clusters of small isolated populations. The ultimate objective of the work with this species is to develop a strategy for its conservation.

The working group publicizes threats to valuable, genetically-unique species and populations by writing articles and making presentations to government and university scientists and practitioners. An expedition, undertaken by Jesus Vargas of the working group and others, to Guadalupe and Cedros Islands to evaluate the status of Pinus radiate populations there and make collections, resulted in heightened awareness within Mexico and elsewhere, of the threats to these populations.

A working group member participated in the North American test of criteria and indicators for sustainable forest management in Idaho, 1998 (Woodley, et al. 2000) and in a subsequent test in Chihuahua, 2001. The working group's involvement ensured that genetic diversity indicators were developed and tested as measures of sustainable forestry.

The working group has offered intensive short courses in conservation genetics in Mexico at the Colegio de Postgraduados in Montecillo and at Chapingo University in 1999, 2002 and 2004. The courses, usually offered at the graduate level, ranged from two weeks to four weeks in length. Training for Mexican students and scientists has been facilitated by the working group, and the working group developed and updates a directory of educational and training opportunities in genetics for Mexican students.

The working group facilitates movement of seed for research, restoration and economic development. Genetic analyses of Mexican species, conducted in research facilities in the United States and Canada, assist in conservation efforts in México. It is highly probable that genetic resources from the south will be needed to restore more northerly retreating forests with climatic warming. Storage of seed or germplasm in several locations including locations outside the country is important in certain circumstances. The seed centre at the Chapingo University in Mexico burned, resulting in significant losses, but seed from many of the seed lots was also stored in the United States. Fire destroyed the type locality of *Picea mexicana* at Sierra la Marta but disaster was averted because of movement and storage of seed. Seeds had been collected and

grown in field gene banks in Canada. Seedlings from the gene banks were grown and returned to México to restore the destroyed population.

Tree improvement is a valid component of conservation under some circumstances and the working group has encouraged such activities. For example, the group was instrumental in the creation of the Forest Genetics Centres in Chapingo and Veracruz. The group has also encouraged and members have assisted in developing seed zones and seed production areas in México.

The working group expects to continue working on conservation of forest genetic resources, with a primary focus on temperate Mexican species, by participating in research projects, training students, publishing research papers, practical guides and manuals, encouraging and facilitating exchange of scientific personnel, and organizing and participating in workshops and short courses.

Gene Conservation needs and initiatives in Canada

Country-wide survey
Although there are strong programs for conservation of forest genetic resources, and the research to support it, in some provinces such as British Columbia, Canada lacks a national coordinated program for forest gene conservation. The Canadian Forest Service is a research organization and an initiative is underway to develop and implement a national program to carry out and coordinate research to support conservation of forest genetic resources. As a first step, a country-wide survey was conducted in 2003 to assess the need for gene conservation measures for native forest tree and shrub species (Simpson, *et al*. 2004).

The survey was completed by experts in each provincial and territorial jurisdiction. For each jurisdiction (ten provinces and three territories), they compiled lists of tree and shrub species expected to require gene conservation attention, on the basis of previous information: published or unpublished reports, ranking from Conservation Data Centres, COSEWIC (Committee on the Status of Endangered Wildlife in Canada) reports, and other sources. These lists were sent to the identified experts in each jurisdiction who were asked to review them and provide recommendations on whether additional species should be listed, or if any of the listed species should be removed from the list. They were asked to identify which criteria, if any, from a list of nine potential ones, would apply to each listed species and to identify any species for which information is insufficient to judge whether or not conservation attention is needed.

Just over two-hundred taxa of trees and shrubs were identified as needing gene conservation attention in some part of their Canadian range. Twenty-six additional species were identified as potentially in need of conservation measures, but information is insufficient to determine their current status. In some cases, a species was identified as requiring attention in one province but may be common in other parts of the species range. For example, *Pinus strobus* is rare and restricted to a few small populations in the eastern province of Newfoundland, and populations are threatened by white pine blister rust throughout the species range, but it is still common in the central part of its range. Other species, such as *Juglans cinerea*, were identified as needing attention throughout the species range. Several species of a number of genera require

conservation attention across the country. For example species of *Salix, Betula, Ulmus, Crataegus* and *Amelanchier* were identified in all parts of the country as needing gene conservation measures. Forty-two of the 56 species and varieties of *Salix* were noted to require conservation attention somewhere within their range.

The survey is an aid in setting priorities for gene conservation and for identifying knowledge gaps and research needs. A comprehensive response to gene conservation needs would include basic research on distribution and autecology of lesser-known species, taxonomic clarification, particularly for species prone to hybridization, and an understanding of the levels and structure of genetic diversity among and within populations of many of the listed species.

National Tree Seed Centre

In situ conservation is important for all species requiring gene conservation, but *ex situ* approaches are important as back-up measures. In cases such as *Juglans cinerea*, for which reserves are not likely to be effective, *ex situ* conservation is the only alternative. The National Tree Seed Centre has two facilities, at the Atlantic Forestry Centre in Fredericton, New Brunswick and at the Laurentian Forestry Centre in Quebec City, Quebec; both research centres of the Canadian Forest Service. The primary mission of the Atlantic facility (http://www.atl.cfs.nrcan.gc.ca/seedcentre/seed-center-e.htm) is to conserve genetic resources of native tree and shrub species. The long-term goal is to store representative samples from throughout the natural ranges of all Canadian tree and shrub species and toward that end, the Centre currently has seed in storage from approximately 100 Canadian tree and shrub species. Most material is stored using conventional seed storage techniques, but facilities are also available for preparation and cryogenic storage of material from recalcitrant species such as *Juglans cinerea*.

Provincial activities

British Columbia has a strong gene conservation program with collaborative efforts between the provincial government and the Centre for Forest Gene Conservation at the University of British Columbia (http://genetics.forestry.ubc.ca/cfgc/), headed by Sally Aitken. Work ranges from assessing the status of commercial and non-commercial tree species for current conservation status and basic research to guide conservation strategies, to the hands-on gene conservation work. Work is guided and prioritized by the Forest Genetics Council of British Columbia.

An informal multi-stakeholder group in New Brunswick, the NB Gene Conservation Working Group, has evaluated gene conservation requirements for all tree and shrub species in the province and has initiated research and conservation collections for species of greatest concern (Loo, *et al*. 2004). Gene conservation strategies have been developed for several tree species.

Active gene conservation work is carried out by other organizations across the country, for example the Forest Gene Conservation Association in Ontario, headed by Barb Boysen (http://www.oen.ca/dir/detail.php?id=1264). They have a mandate for conservation of forest genetic resources in southern Ontario and have a strong focus on the provincially rare Carolinian species.

There is a need in Canada, as in other countries, for a coordinated approach to pull together the various initiatives and fill the gaps in provinces and territories where conservation of forest genetic resources does not receive much attention.

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Gene Conservation in a Changing Climate: Counting the Deck Chairs or Manning the Lifeboats?

S.N. Aitken[1], A. Hamann[2], T. Wang[2] and A.D. Yanchuk[3,4]

[1]Associate Professor, [2]Research Associate, [3]Adjunct Professor
Centre for Forest Gene Conservation, Department of Forest Science
University of British Columbia, Vancouver, BC, Canada
[4]Manager, Forest Genetics, Research Branch, BC Ministry of Forests

Many gene conservation programs are anchored by a network of *in situ* reserves for the purpose of maintaining large populations indefinitely over many generations. In these largely unmanaged reserves, populations should be able to adapt to new conditions as long as the rate of environmental change does not exceed a species' genetic capacity for response. Migration between *in situ* reserves, another route for adaptation to climate change, will be hampered by habitat fragmentation and land management practices between conservation areas, and may not be able to keep pace with climate change. Our spatial analyses incorporating climate change predictions from global circulation models with current ecological maps and botanical plot data for around 50 tree species in British Columbia indicate many protected conservation areas will no longer fall within the climatic envelope of species they currently support. Large conservation areas that span a wide range of topography and elevation will have a higher probability of species persistence than smaller or more homogeneous reserves. A comprehensive gene conservation effort will need to: 1) monitor ecotones for early warning signs of species range shifts (e.g., natural regeneration failure); 2) contemplate assisted migration between reserves to help populations track their climatic niches; and 3) back up *in situ* protection with extensive *ex situ* collections, including samples from peripheral and disjunct populations that might be well adapted to larger geographic areas under future climatic conditions than at present.

Are climate change predictions relevant for improved populations in managed forests? Many breeding programs started with locally adapted base populations and selected genotypes for high performance under current range breeding zone climatic conditions. We have predicted the performance of breeding and seed orchard populations of lodgepole pine (*Pinus contorta*) in British Columbia under climate warming scenarios for the next century. Seed from some current breeding zones will become useful over much broad geographic areas in the future, while others will have little utility. Additional genotypes from populations not currently included in improvement programs may become important, supporting the need for a strong gene conservation program. There are considerable opportunities to increase average forest productivity through redesigning improved seed deployment strategies in anticipation of global warming; however, with *status quo* deployment, productivity will likely decrease. In either case, the productive area for this species will shift northward if global warming continues.

Pan-European Initiative on Conservation of Forest Genetic Resources: Implementation and Experiences

Matyas, Cs. – Koskela, J. et al.

The demand for cooperation to promote sustainable forest management and to enhance conservation of biodiversity in general, including forest genetic resources emerged following an alarming decline of health condition of forest ecosystems across Europe in the 1980's. International cooperation has been accelerated by the preparatory process for the UN Conference on Environment and Development in Rio, 1992. Ministerial Conferences on the Protection of Forests in Europe (MCPFE) have been organised regularly since 1990 and served as a major political process pushing forward biodiversity and gene conservation issues. In 1994, the European Forest Genetic Resources Programme (EUFORGEN) was set up as an implementation mechanism of the Strasbourg Resolution (1990) on promoting and coordinating the conservation of forest genetic resources. In the same year, Pan-European Criteria and Indicators for Sustainable Forest Management were also adopted following the Helsinki Resolution (1993) on sustainable forest management. For the first time, sustainability indicators included also genetic aspects.

Up to the present EUFORGEN has been operating separate networks taking into account the differing genetic and ecological background of species groups (conifers, mediterranean oaks, temperate oaks and beech, poplars, noble hardwoods). Nearly all European countries participate in network activities. Financing is provided by the individual contributions of countries. In the past decade progress has been achieved in coordinating and synchronizing national activities, promoting public awareness and in developing common methodologies, however it turned out that priorities and approach in gene conservation cannot be homogenized across the continent. Legal and organizational difficulties demanded a reshuffle of goals and of procedures towards a more practice-oriented approach. In many European countries more resources have been channelled to habitat and species conservation than to the development of national programmes on forest genetic resources. Still less than 30 per cent of the European countries have such programmes, which are necessary for implementing genetic sustainability in practical forestry. A better linkage is therefore necessary to the national forest programmes, which are determining sectoral policy and also allocating resources for implementing.

The Cooperative Forest Genetic Research Program: Breeding Pure Species and Hybrids

D.A. Huber and G.L. Powell[1]

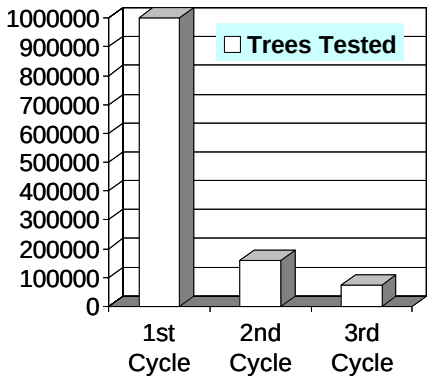
The Cooperative Forest Genetic Research Program (CFGRP) at the University of Florida was established in 1953 and continues today with 9 cooperators (7 private industrial and two state organizations). The mission of the CFGRP is to provide improved varieties of southern pines for use in the lower coastal plain of the southeastern USA. The CFGRP has practiced tree improvement on four southern pine species: slash pine (*P. elliotti* var. *elliottii*), Florida-source loblolly pine (*P. taeda*), longleaf pine (*P. palustris*) and sand pine (*P. clausa*). The active tree improvement programs are in slash and Florida-source loblolly pine, along with slash pine hybrids.

SLASH PINE

The slash pine program is currently in the third cycle of improvement. In every cycle the program has become more efficient in producing genetic gain per unit of time at a lower cost. The primary reasons for this increased efficiency are better mating and field testing designs, assiduous implementation and care of field tests, enhanced analytical techniques, and accelerated breeding techniques. The selection interval has been decreased from 34 years for the first cycle to a projected 11 years for the third cycle. The number of trees tested has decreased from 1,000,000 in the first cycle to a planned 43,000 in the third cycle. The across-site individual-tree heritability for volume at age 5 has been increased from 0.07 in the first cycle to 0.25 in the second cycle (Huber *et al*. 2003). Together these attributes contribute to an efficient breeding program.

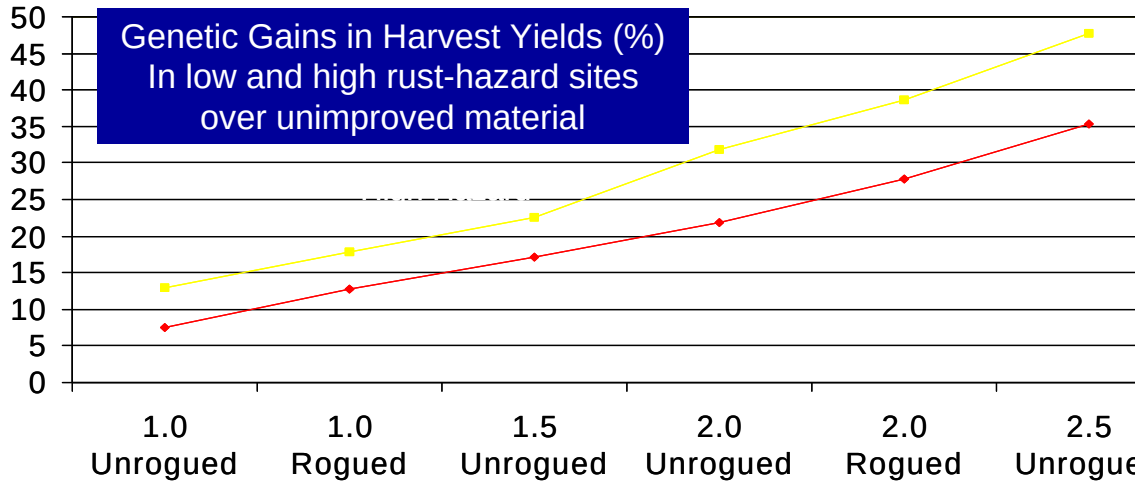
The gains calculated for slash pine orchards during the life of the program illustrate the efficiency increases with relative gains increasing per cycle, decreasing numbers of trees tested, and decreasing length of each cycle (Figure 2).

Figure 1. Total numbers of trees in slash pine progeny tests for each cycle of improvement.



[1] Co-directors of the CFGRP, School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA

Figure 2. Genetic gains in percentage volume at harvest for two target environments across cycles of selection and roguing. The upper line represents volume gains in an environment where the fusiform rust hazard based on unimproved material is 30% infection or greater, accounting for gains in volume growth and rust resistance. The lower line represents volume gains in a low fusiform rust hazard environment (less than 30%), accounting for gains in volume growth only.



FLORIDA-SOURCE LOBLOLLY PINE

The Florida-source loblolly pine program generates interest among the cooperators because of the extremely fast growth rate of the source (Sierra-Lucero *et al*. 2002). This source was never intensely sampled, so in 1999 new selections were made in wild stands. This new selection effort tripled the size of the base population (now 350 individuals) and greatly enhanced the selection coverage of the range of loblolly pine in Florida. Progeny tests have been planted across a wide range of environments to evaluate the new material.

SLASH PINE HYBRIDS

Slash pine hybrids with loblolly pine and Caribbean pine (*P. caribea* var.) were tested across 12 locations as slash pine maternal families. The overall performance of the hybrid families was undesirable; however, many outstanding individuals were produced (Lopez-Upton *et al*. 1999). The slash by loblolly hybrid is of particular interest to the CFGRP as the first step in an introgression program between slash pine and Florida-source loblolly pine. The CFGRP has interest in enhancing the performance of each of these species through introgression of specific alleles from the other. Disease resistance, growth rate and wood properties are among the traits that could be enhanced in each of the species through donor alleles from the other. A trial introgression program using backcross breeding is being initiated and will incorporate the use of molecular markers to track the donor allele and remove unwanted donor DNA from the recipient.

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Cooperative Advanced-Generation Breeding and Testing of Coastal Douglas-fir and Western Hemlock – Strategy And Implementation

K. J.S. Jayawickrama[1], **G.R. Johnson[2]** and T. Ye[1]

FIRST-GENERATION TESTING OF DOUGLAS-FIR

As in many temperate regions of the world, forest tree improvement got underway in the Pacific Northwest of the USA in the 1950s, with a number of companies and agencies starting independent tree improvement programs. Booth-Kelly Lumber Co., Crown Zellerbach Corp., the Industrial Forestry Association, Port Blakely Mill Co., Simpson Timber Co., Timber Service Co., the US Forest Service, and Weyerhaeuser Co. were among the first to select coastal Douglas-fir trees and graft them into clonal orchards. Graft incompatibility, both immediate and delayed, became evident by the early 1960s and dampened enthusiasm for grafted clonal orchards for this species (Silen and Copes 1972). The problem of graft incompatibility was eventually greatly reduced due to work done by Don Copes of the PNW Research Station, but by that time it had played an important role in shaping tree improvement in the region.

The IFA-PNW "Progressive Tree Improvement System" was launched in 1966 due to the failure of grafted clonal orchards (Silen 1966, Silen and Wheat 1979). The emphasis here was on forming local cooperatives to share costs, and on progeny testing large numbers of trees using wind-pollinated seed in small testing zones. Silen and others felt that due to steep variation in environmental factors (e.g. temperature) caused by the mountainous topography of the Pacific Northwest, it was not appropriate to move Douglas-fir seed far from the source. Improved seed was to be delivered from seed orchards established using full-sib crosses made on the parent trees.

The first-generation testing phase ran from 1967 till 1993, during which over 28,000 first-generation Douglas-fir and western hemlock parents were tested in 115 breeding units, with over 3 million progeny test trees planted. The typical measurement schedule was five, 10 and 15 years from seed. Height was usually measured on each occasion, while diameter was usually measured at age 15 and sometimes at age 10 as well. The incidence of ramicorn branches, forks and stem sinuosity was assessed in some of the later testing programs, as information was collected on the inheritance of these traits (e.g. Adams and Howe 1985, Temel and Adams 2000).

ADVANCED-GENERATION BREEDING AND TESTING

Data from the first-generation tests were used to draw several conclusions relevant to advanced-generation breeding and testing; other decisions were based on computer simulation and inferences from other breeding programs (Johnson 1997, 1998a, 1998b; Johnson et al. 1997). It was recognized that full-sib breeding had several advantages over open-pollinated breeding. It appeared that there would be little marginal gain per test beyond six successful progeny tests (in terms of ranking families), that two or three crosses would give a reasonable estimate of a

[1]Northwest Tree Improvement Cooperative, Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752 and [2]Genetics Group, USDA Forest Service – PNW Research Station, 3200 Jefferson Way, Corvallis, OR 97331

parent's GCA, and that final selection around age 12 years would be efficient. An advanced-generation breeding and testing strategy for coastal Douglas-fir was developed between 1996 and 1997.

Implementation of the advanced-generation testing program has been fairly similar to the proposed strategy, although there have been some deviations. First-generation testing programs have been merged in the north-south direction into larger ***testing zones*** to share genetic material. To date, 52 first-generation programs have been consolidated into 8 second-generation breeding programs. The emphasis has been on low to mid-elevation lands (up to 3,000 feet). While there were several first-generation programs above 3,000 feet, it appears that the limited advanced-generation progeny-testing dollars now available should be concentrated on the most productive timberlands.

Breeding population size for a second-generation program is at least 200 selections, but most are greater than 300. Within a breeding population, ***breeding groups*** of 20-30 selections were constructed, each breeding group was from a single first-generation program. This resulted in sublines to manage inbreeding, and multiple populations to maintain locally adapted gene complexes. The rule of thumb in choosing second-cycle selections has been a 1 in 10 among-family selection intensity. Most selections were made on age-15 height; information on DBH, stem form and wood specific gravity were also considered. The top 10 percent of selections within a breeding population were assigned to an ***elite population***.

The breeding population for each new testing zone includes families/selections from the "local" breeding groups that come from the testing zone, and only the highest-ranked selections from breeding groups originating further away from the testing zone. Each selection is used in at least two crosses, with elite selections being used in up to four crosses. Some of the elite crosses are made across first-generation zones. Unimproved checklots have been included in all tests planted after 2001. Two types of tests have been used: family-ranking/selection tests and longer-term stability tests which are thinnable. Some second-generation programs may implement adaptability-screening tests (probably by collecting tissue samples from field tests and evaluating them in laboratory tests).

In total, the Douglas-fir breeding effort will be comprised of over 2,600 crosses (80% have been completed), 95 tests (41 have been established) and about 300,000 test trees planted. Thus the total number of trees will be around 10% of the trees planted in the first generation; this is similar to the reduction of number of test trees in the CFGRP slash pine program (White et al. 2003). Between five and six tests are established per testing zone, with 20 trees per cross per site in single-tree plots.

Trials established to date have contained from 143 to 283 full-sib crosses, planted as containerized seedlings and fenced for protection against browse. When crosses of a given program are planted in two Phases, the separate Phases are linked by at least 10 common linker crosses. The goal is to keep the tests weed-free for three years after planting, and control harmful competitors (such as aggressive hardwoods) until crown closure. Tests will probably be measured twice, when the trees are 15 and 30 feet (4.5 and 9 meters) tall respectively. This may

take only five and 10 years from planting on the most mesic southern sites, in contrast to seven and 13 years on colder, mid-elevation, northern sites.

WESTERN HEMLOCK

Western hemlock is a prolific and dominant species in the coastal forests of the Pacific Northwest. First-generation testing got underway in the 1970s, but interest in this species increased markedly after growing incidence of Swiss Needle Cast disease on Douglas-fir on the Oregon Coast, and the white pine weevil on Sitka spruce from Oregon to Alaska. A single advanced-generation testing zone has been developed for western hemlock, from the middle on the Oregon coast (44° 30') to northern Vancouver Island (51°). The parental selections (no forward selections) come from six first-generation programs, selected on age-10 height. The main population has 150 parents (chosen from over 1,500 parents) crossed in six-parent disconnected diallels forming 342 crosses. The elite population of 30 parents has 166 crosses (Jayawickrama 2003). Over 130,000 test trees were planted between 1997 and 2001 in single-tree plot tests and in family blocks. Age-5 heights for all these trees are to be collected by the end of 2004.

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GENETICS OF MEDITERRANEAN CONIFERS: A BRIEF REPORT ON THREE CONIFER SPECIES IN TURKEY*

Kani ISIK[1], Hikmet OZTURK[2], Fikret ISIK[3], Sadi SIKLAR[2]

[1]Biology Dept, Akdeniz Univ., Antalya, Turkey. kani@akdeniz.edu.tr
[2]Forest Tree Seeds & Tree Breeding Res. Inst., Ankara, Turkey. seed@ortohum.gov.tr
[3]North Carolina State Univ, Forestry Dept., Raleigh, NC, USA. fisik@ncsu.edu
[3]Formerly with Southwest Anatolia Forest Res. Inst., Antalya, Turkey

Abstract. Among the Mediterranean conifers *Pinus brutia* Ten., *Cedrus libani* A. Rich and *Cupressus sempervirens* L. show their primary natural distribution range in Turkey, located within the eastern flank of the Mediterranean basin. ***Pinus brutia*** has been given the highest priority in forest genetics and tree improvement programs due to its high socio-economic values (fast growth, adaptability to diverse habitats, wide uses of its products) in the region. In Turkey, by the year 2003, 78 seed stands have been selected, 47 clonal seed orchards have been established, various provenance and progeny tests have been set up, 49 natural stands have been designated as *in situ* gene conservation forests, and five major breeding zones have been determined. The species has long been recognized as a drought tolerant conifer for planting in dry regions in the Middle East, Australia and New Mexico. It exhibits a clinal variation for several growth, quality and isozyme traits along altitudinal gradients on the Taurus Mountains in southern Turkey. Populations from the central part of the elevational distribution range show better performance and higher variability than the distal populations. High variation in *P. brutia* offers opportunities for tree improvement through selective breeding. ***Cedrus libani*** is a well-known species for its both economic and ethno-botanical values. Sixteen natural stands have been assigned for *in situ* genetic conservation purposes, 23 seed stands have been selected, and 11 clonal seed orchards have been established in Turkey. Studies using various molecular markers indicated that populations from Taurus Mountains were distinctly different from those in Lebanon; and that *C. libani* in general has higher heterozygosity compared to *C. deodora* and *C. atlantica*, but lower heterozygosity relative to *C. brevifolia*. ***Cupressus sempervirens*** has been planted since Phoenician times throughout Mediterranean region. Under the long history of human interference, the gene pool of the species has been extensively modified both by natural and artificial selection. One of the largest natural forest of the species is located in a relatively inaccessible area in Turkey. Among the eastern Mediterranean group, Turkish populations have been found to show the largest total genetic diversity. As an initial measure for *in situ* genetic conservation purposes, one natural stand has been designated as gene conservation forest, and one seed stand has been selected in the same area. Tree breeding activities are being monitored and fine-tuned as new information accumulate from field trials, and from more recent biochemical and molecular genetic markers studies.

Key words: Pinus brutia, Cedrus libani, Cupressus sempervirens, forest genetics, tree breeding, geographic variation, isozyme analysis, genetic markers, drought tolerance.

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Breeding teak in Costa Rica.

Murillo, Olman[1] & Badilla, Yorleny[2]

Abstract

Teak tree improvement programs in development through the Costa Rican breeding cooperative (GENFORES) are described. Clonal, seedling and mixture of both breeding strategies are discussed. Up today 315 plus trees have been selected by GENFORES members. Clonal gardens are still in development, but small scale clonal commercial plantations began in 2004. First progeny tests results (4–year-old) in northern Costa Rica are presented. Relatively low heritabilities and genetic gain (between 11% for commercial volume and 22% for the quality index) were obtained. Narrow sense heritabilities for commercial volume was 0,09, while for quality was 0.21. Selection differential for commercial volume was 24% against the control lots. Selection differential for quality index was 39% against the control material. Specific gravity and hartwood percentage traits are now being considered as selection criteria. Progress in GENFORES-members breeding programs is presented. Ongoing activities and breeding goals are discussed.

Key words: tree improvement, teak, *Tectona grandis*, Costa Rica, clonal forestry

I. Introduction

With the advancement of investment in industrial teak plantations in the region, the inclusion of tree breeding programs becomes mandatory. Since the end of 80's and early in the 90's, the Hojancha Farmers Center (CACH) initiated a small scale breeding program supported by local public universities (Merayo y Murillo, 1990). High quality seed stands as well as a seed bank was properly established, taking the regional market for seed procurement. Some other organizations in the country started their own seed stands and some breeding activities later on, as MACORI, a local private company which developed the first clonal and breeding program in Costa Rica (Víquez, 1998).

At the end of 2001, the first costarican breeding cooperative (named GENFORES) was created, based on clonal forestry as a general orientation (Murillo et al., 2003).

In the last 3 years over 300 plus trees have been selected by GENFORES members in different Seed Zones of the country. About 70% are already in clonal gardens and in the process of field testing (Murillo et al., 2003). A series of progeny tests was also established in the North Pacific Seed Zone of the country in 1999, which is now becoming in small seed orchards (Rojas, 1999; Montero, 2000). A brief discussion on future teak breeding directions are presented.

[1] Escuela de Ingeniería Forestal, Instituto Tecnológico de Costa Rica. Cartago, C.R. omurillo@itcr.ac.cr

[2] Fundación Tecnológica de Costa Rica. ybadilla@costarricense.cr

II. Program advancement by GENFORES members

Table 1 shows the genetic collections up to October 04. The genetic material is now under propagation and clonal testing in the field. Some of the members have established conventional progeny tests as well, following GENFORES design (Rojas, 1999; Montero, 2000; Murillo and Badilla, 1999; Murillo and Badilla 2003a).

Table 1: GENFORES Teak breeding program advancement (october 04)

Members	**Seed Zone**	**Plus Trees**	**Plus Trees already cloned**
EXPOMADERAS	North Zone	58	56
ECOdirecta	North Zone	54	54
CACH	Dry Pacific	40	0
Panamerican Woods	Dry Pacific	66	66
ITCR	North Zone	25	25
Ganadera BASA	North Zone	26	0
BARCA S.A.	Central Pacific	35	18
Total		**314**	**210**

In the first evaluation (9 months-old), significative differences among families were determined in height growth increment and in quality index. Control material for both traits ranked the lowest. Top 50% families grew 21% higher than control material. Meanwhile, quality index increased only 14.5% with respect to control lots. Narrow sense heritabilities for volume and quality were $h^2_{VOL} = 0.247$ y $h^2_{Qlty} = 0.32$ respectively (table 1).

Table 1: 9 months-old variance analysis in a teak progeny test established at 3 locations from Dry Pacific Seed Zone in Costa Rica.

Source of	**Height**				**Quality**		
variation	**D.F.**	**MS**	**F**	**h^2**	**MS**	**F**	**h^2**
Loc	2	127004.19	74.31		1.358	23.35	
Family	28	4672.088	2.73		0.106	1.82	
LocxFamily	56	1709.073	2.40		0.066	1.14	
Block (Loc)	15	9261.480	13.02		0.443	7.61	
Fam x Block (Loc)	420	711.343	1.21		--	--	
Pair (Fam Loc*Block)	1052	588.987	2.15		--	--	
Error term	1467	274.483		**0.247**	0.058		**0.317**

Four year-old progeny test results are shown in tables 2 and 3. Significative differences were found among families for both traits, volume and quality index,

Table 2: Four year-old variance analysis for volume in a teak progeny test established in San Mateo, Alajuela, Costa Rica.

Source of variation	**Degrees of freedom**	**MS**	**F (p> F)**	**Heritability (h^2)**
Block	4	0.00375987	4.96 (0.0010) **	0.093
Family	27	0.00126716	1.67 (0.0341) *	***Genetic gain*[1]**
Block x Fam	108	0.00075836	0.97 (0.558) NS	0.0052 (11.5 %)
Error	627	0.00077892		

Expected genetic gain $G = i * h^2 * s_E$, where $i = 2$

Eventhough expected genetic gains are considerable low, due to heritability values, GENFORES is developing mostly a clonal breeding strategy with higher expected gains.

Table 3: : Four year-old variance analysis for quality in a teak progeny test established in San Mateo, Alajuela, Costa Rica.

Source of variation	**Degrees of freedom**	**MS**	**F (p> F)**	**Heritability (h^2)**
Block	4	4.380114	7.78 (<.0001) ***	0.217
Family	27	1.2576048	2.23 (0.0019) **	***Ganancia* Genética[1]**
Block x Fam	108	0.56274165	1.3 (0.0333) *	0.286 (21.18%)
Error	596	0.43447		

Expected genetic gain $G = i * h^2 * s_E$, where $i = 2$

Recently, specific gravity and hartwood percentage traits have been considered as selection criteria. Figures 1 and 2 shows 4-year-old data from a teak progeny test. Our findings show that the faster the tree grows the higher the hartwood percentage will be (Leandro *et al*., 2003). However, no relation was found between the growth rate and the wood specific gravity.

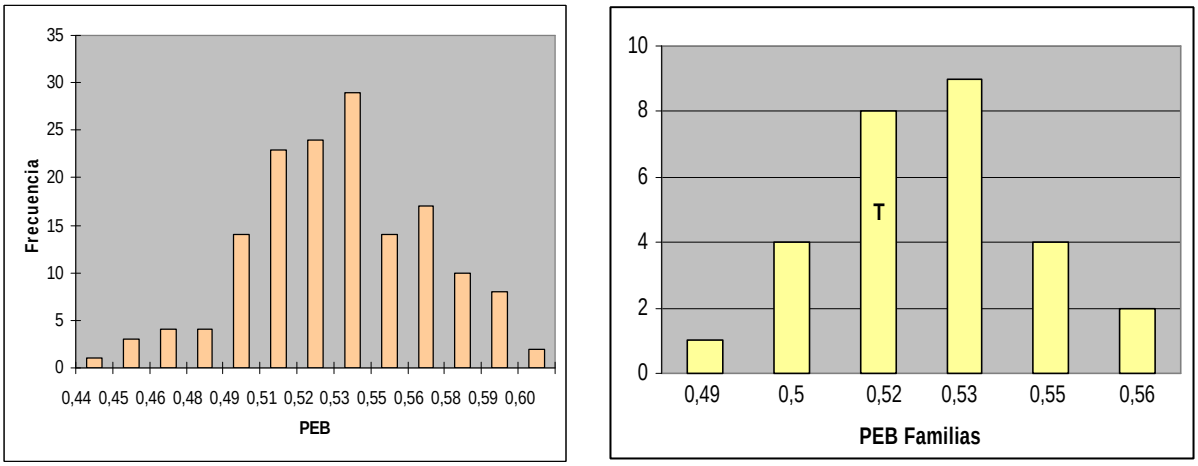

Figure 1: Specific gravity distribution among 153 tress (left) and among 28 families (right) in a 4-year-old teak progeny test at San Mateo, Alajuela. T means control lot.

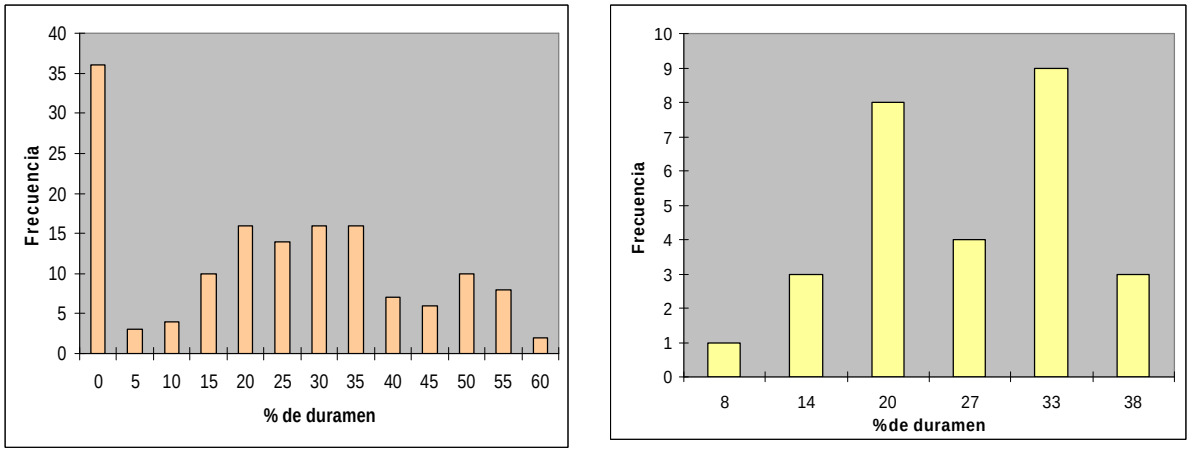

Figure 2: Hartwood percentage distribution among 148 trees (left) and among 28 teak families (right) in a 4-year-old progeny test at San Mateo, Alajuela.

IV Clonal forestry progress

Experience with tissue culture and other propagation options are well described for this tree species (Monteuuis *et al*., 1995). Teak is a tree species easy to propagate vegetatively, however, very sensitive to diseases and environmental conditions. In clonal gardens, an average rooting percentage achieved is around 50%, with some clones with 70 to 75% (Murillo and Badilla, 2003b). Some success has been obtained propagating trees from low branches, but traditionally, stump sprouts are preferred. Fertilization directly to the sprouts has produced higher rooting rates, as well as, larger amount of sprouts in the Pacific dry region of the country. Sand as rooting substrate under micro tunnels within greenhouses, are part of our regular rooting environment. Recent advances are now being obtained through the development of mini clonal

gardens within the greenhouse. A promising future is clear for clonal forestry with teak in tropical conditions.
Due to soil conditions in Costa Rica, breeding is moving strongly to the selection of teak clones adapted to acid and poor soils. In this direction, there is an opportunity to get cheaper lands for planting teak. Selection against diseases is nowdays mandatory. Problems with *Fusarium* and *Nectria* are more and more under careful attention. Possibilities of selecting tolerant material to these phytosanitary problems have not yet started but planned.

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Clonal Progeny Testing and Selection in Forest Tree Improvement Programs

Fikret Isik [1], Bailian Li [2], Barry Goldfarb [2]

[1] Research Assistant Professor, [2] Professors
Department of Forestry, North Carolina State University, Raleigh, NC, 27695, USA

In progeny testing of forest tree improvement programs, family means are estimated with certain precision by sampling environmental variance. However, within family selections are based on phenotypes that are not efficient. Advancement in macro and micro vegetative propagation techniques of forest trees provide an opportunity to improve breeding and testing strategies. In this study, we explored efficiency of cloned progeny testing for within-family selection by summarizing recent empirical studies on *Pinus taeda* L. in the southern U.S.

Cloned progeny testing proved greater efficiency in sampling micro environmental variation than seedling progeny of the same full-sib families. Consistent smaller within-plot variances were reported from rooted cuttings than seedlings of the same full-sib families. Better sampling of environmental variance by clones reflected in higher precision of additive genetic variance estimation with smaller standard errors. Similarly, greater family and within-family heritabilities were estimated from cloned progeny than seedlings. For example, full-sib mean heritability for 6-year volume from clones (~0.62) was about two times greater than heritability (~0.30) from seedlings. The difference between within family heritability for volume from clones (0.70) and seedlings (0.03) was even more dramatic.

Cloned progeny tests and clonal deployment serve an efficient way to capture all genetic variance, particularly genetic variance due to epistatic gene interactions. In *P. taeda*, we found strong indications of epistatic genetic effects on fusiform rust disease resistance (*Cronartium quercuum sp. fusiforme*). Epistatic genetic variance for rust infection was 20% greater than additive genetic variance at age four. Strong non-additive or major gene effects on fusiform rust incidence have been also detected with RAPD markers and by a recent wide scale clonal deployment study.

We found that, even when genetic gains from cloned family and within-family selection were adjusted for the cost (number of trees tested) and for longer testing cycles, cloned progeny tests were superior over seedlings progeny. The major difference in genetic gain between seedling and cloned progeny testing was due to within-family selection. In conclusion, cloned progeny trials should be considered to increase the efficiency of current and future breeding programs of forest trees. A complementary breeding strategy of polymix mating for selection of parents and clonal selection of cloned full-sib family strategy may be preferred.

Comparing Clonal Lines on Experimental Sites (CCLONES)

Brian Baltunis[1], John Davis[2], Tim Martin[2], Eric Jokela[2],
Dudley Huber[3], Tim White[4], Gogce Kayihan[5]

[1]Assistant Program Manager of Forest Biology Research Cooperative (FBRC),
[2]Co-Directors of FBRC, [3]Co-Director of Cooperative Forest Genetics Research Program,
[4]Director of School of Forest Resources and Conservation, [5]Graduate Student
School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA

The Forest Biology Research Cooperative at the University of Florida is an integrated university-industry cooperative. The FBRC's mission is to optimize forest productivity, health, and sustainability of intensively managed loblolly (*Pinus taeda* L.) and slash pine (*Pinus elliotti* Engelm.) ecosystems by investigating the interactions of genetics, pests, silviculture, competition control, nutrition and soils. CCLONES is a series of experiments that are focused on understanding clonal biology by utilizing many families and clones planted across a range of cultural treatments and site types to examine the genetic mechanisms controlling tree-level growth strategies, ecophysiology, nutrition, pest resistance and wood quality. CCLONES consists of three series of field plantings containing elite full-sib families and randomly generated, untested clones within those full-sib families: (1) Series 1 – Lower Coastal Plain (LCP) loblolly pine, (2) Series 2 – slash pine series covering both the LCP and Western Gulf (WG), and (3) – WG loblolly pine series including both WG and Atlantic Coastal Plain (ACP) sources. This report focuses on Series 1.

More than 239,000 stem cuttings from nearly 2,200 clones of loblolly pine from 70 full-sib families were set in five rooting trials. Overall rooting success across the five trials was 43% and significant seasonal effects were observed. Rooted cuttings from these trials were used to establish six field sites across the southeast United States. The field trials are now two years old and traditional growth traits, such as height and diameter, will be measured at all sites. This will enable us to understand the genetic structure underlying productivity in these populations.

In addition non-traditional phenotypic measurements are being assessed on the loblolly pine clones. Disease resistance phenotyping has been completed for ~1,400 clones at the Resistance Screening Center and at the University of Florida. A total of three disease traits were phenotyped: pitch canker lesion length, fusiform rust gall length, and fusiform rust gall score. A number of clones that form short galls have been identified which may be a more durable form of resistance (not race specific). For water deficit phenotyping, foliar stable carbon isotope discrimination analyses will be completed on ~900 clones at two of the field trials. The ^{13}C:^{12}C ratio of foliage gives a time integrated index of the ratio between photosynthesis and stomatal conductance. The genetic control of shoot elongation of loblolly pine clones is also being investigated. Traits such as growth initiation, cessation, and number of stem units are being measured. In summary, CCLONES utilizes hundreds of clones, approximately 15 clones from each of 60 full-sib families at each site, plus seedlings from the same full-sib families, derived from a partial diallel mating design. This makes the experiment a powerful tool for quantifying genetic control of both traditional and non-traditional phenotypic traits.

Improved Family Forestry – the Evolution of Breeding and Deployment Strategies for *Eucalyptus pilularis* in New South Wales, Australia

Smith, H.J.[1], Johnson, I.G.[2] and Henson, M.[1]

Abstract: Breeding populations of *Eucalyptus pilularis* Smith (blackbutt) were established by Forests NSW during 1997/98, using family seedlots from 359 plus trees selected in older commercial plantations in NSW and from high quality natural stands in NSW and Queensland. The progeny were planted in trials over three sites in north coastal NSW and two in south-eastern Queensland, with a total of 40 provenances represented within the breeding populations. Initial selections were made at age 3-3.5 years, based on growth and form. Superior individuals were captured by grafting and established as clonal seed orchards (CSOs) to produce seed for operational deployment. The best performing individual from the best 20 families was also grafted to establish an elite nucleus population split into two sub-lines, to further the breeding and deployment program.

Identification of superior families has lead to development of clones for field-testing. Seedling-based clones have been developed from 'plus tree' family seedlots and 'field selects' from family selection blocks planted at the same time as the progeny trials. These have been supplemented by selections made in young plantations within the Forests NSW estate. The earliest clone trial was assessed for growth and form at age 3 years, and a small number of clones selected for further testing, as well as limited commercial deployment.

Over the past year initial steps have been made to follow a strategy of Improved Family Forestry for deployment of *E. pilularis,* in which young genetically improved seedlings are screened for a range of characteristics that are directly related to performance traits in the field, prior to establishment as mother plants for cuttings propagation. Deployment by Improved Family Forestry is being favoured as it will avoid mother plant maturity issues and still allow exploitation of significant amounts of non-additive genetic variation. The program is being implemented in stages, commencing with family forestry using seedlots from selected 'plus tree' families. Fifteen families were selected from the top 30 families identified in the analysis of the progeny trials and mother plants grown from seed. Propagation of these seedling-based clones by mini-cuttings was more rapid than propagation of partially tested clones. Productivity of the seedling-based mother plants was almost 8 times the productivity of the tested clones. Approximately 10,000 rooted cuttings of *E. pilularis* were deployed during 2003/04 and this will increase to 80,000 during 2004/05, predominantly through family forestry.

Keywords: Improved Family Forestry, *Eucalyptus pilularis,* blackbutt, progeny trial, breeding strategy, deployment strategy

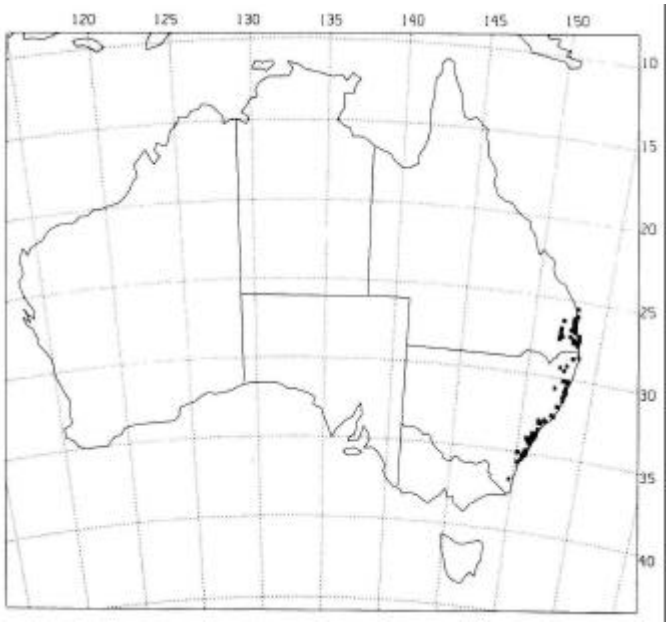
[1] Forests NSW, Tree Improvement Program, PO Box J415, Coffs Harbour Jetty NSW 2450 Australia
[2] NSW Department of Primary Industries, Science and Research, PO Box 100, Beecroft NSW 2119 Australia

INTRODUCTION

Eucalyptus pilularis Smith (blackbutt) is a naturally occurring eucalypt in coastal regions of NSW and south-eastern Queensland (Figure 1). The range of latitude is from south of Bega, NSW (37º 30'S) to Maryborough, Qld (25º 30'S) and includes Fraser Island (Boland *et al*. 1984). The range in altitude is from near sea level to 600m. The species occurs on a wide range of soil types from infertile coastal sandy soils to very fertile basalt soils, and over a rainfall range of 900-1750 mm. It is one of the key species for high quality log production from native forests in these regions and is also an important plantation species within its natural range. Forests NSW, a State Government Trading Enterprise, is responsible for a total forest estate of 2.9 million hectares, of which 49 000 ha are hardwood plantations. Since commencing a hardwood plantation program in the 1940's, approximately 15 000 ha of *E. pilularis* plantations have been established by Forests NSW, predominantly in northern NSW.

In the year 2003/04, approximately 150 800 m^3 of *E. pilularis* logs were harvested from the mid-north coast and north coast regions of NSW. This is over 50% of the large high quality quota logs from native forests and almost 25% of the total log harvest from native forests and plantations. The recent emphasis on the development of the eucalypt plantation program over the past 10 years is aimed at supplementing the supply of high quality logs from native forests for the sawlog market. Regional Forest Agreements (RFAs) developed between the State and Commonwealth governments for the future use and sustainable management of native forests within NSW, has lead to transfer of some north coast State Forests into the National Park estate. This has resulted in loss of areas of native forests for harvesting and the impetus for plantations to supply a large proportion of the log quota to support the industry in the future.

Figure 1. Natural distribution of *Eucalyptus pilularis* within Australia. (From Boland *et al.* 1984)



WOOD PROPERTIES OF *E. PILULARIS*

Eucalyptus pilularis is the principal species of hardwood timber sawn in coastal NSW. It is an extremely versatile high quality timber, being utilised for poles, sleepers, decking, flooring, furniture and building framework (Bootle 1983; Timber Development Association (NSW) Ltd. 2003). The wood properties of *E. pilularis* from both native forests and plantations are shown in Table 1.

Table 1. Summary of wood properties of *Eucalyptus pilularis*.

Property	Native Forest[1]	4 year-old Plantation Forest[2]	29 year-old Plantation Forest[3]
Air Dry Density (kg/m^3)	900		794
Basic Density (kg/m^3)	710	455	
Janka Hardness (Dry) (kN)	8.9	4.0	9.6
Durability Class	2		
Strength Group	S2/SD2	SD6	SD2/SD3*
Joint Group	J2/JD2		
Shrinkage – radial (approx %)	4	2.3	4.2
Shrinkage – tangential (approx %)	7	6.7	10.8
Structural Grades - unseasoned	F14/F17/F22		
Structural Grades - seasoned	F22/F27		
Modulus of rupture (Dry) (MPa)	144	80	138
Modulus of elasticity (Dry) (MPa)	19,000	9,100	17,923

*Borderline case
[1]Bootle (1983). Wood in Australia.
[2]Muneri & Leggate. QFRI report: Wood properties and sawn timber characteristics of fast, plantation grown 4 year old *Eucalyptus pilularis*.
[3]Bill Joe (*pers. comm.*)

With structural grades at greater than F11, a strength group of S2 and durability of Class 2, *E. pilularis* is an ideal timber for construction framework (both internal and external), as it has adequate load carrying capacity and life expectancy, when exposed to the weather and insect and fungal attack. With these properties the timber is also suitable for use as poles and decking. The wood is classed as hard, with a Janka rating of 9.6 (plantation) or 8.9 (native forests regrowth), showing good resistance to indentation and abrasion, making it a good timber for flooring. For these reasons it is a preferred species for industry on the north coast of NSW.

BREEDING PROGRAM

Forests NSW commenced a tree improvement program for *E. pilularis* in 1964, when 11 provenance trials were established on the coast and northern escarpment of NSW (Johnson and Stanton 1993). A pedigreed program was initiated in the mid-1990's, to produce superior genotypes for solid wood production on the north coast of NSW. A breeding plan was derived (Johnson & Nikles 1997) for developing and deploying genetically improved varieties of *E. pilularis* in NSW. From late 1993 to 1995, intensive phenotypic selection of superior trees was

undertaken in high quality natural stands and older commercial plantations within central and north coastal NSW. The NSW south coast sites and sandy coastal sites such as Myall Lakes were not included in the selections due to poor performance in previous provenance trials (Johnson & Stanton 1993). A total of 600 trees were selected, but cost restraints, logistical difficulties and natural factors reduced seed collection to approximately 310 selected trees from 34 provenances (Figure 2).

Breeding populations, as progeny trials, were established during 1997/98 on three sites in coastal northern NSW (Figure 2), ranging from a high quality site to a marginal site for *E. pilularis*, and on two sites in south-eastern Queensland. Seedling progeny from open pollinated family seedlots from 307 of the NSW selected plus trees and 52 plus tree selections from natural stands in Queensland were planted in the trials. Forty provenances were represented within the breeding populations with a maximum of 324 families on one site (Table 2). In addition to the breeding populations, the two main NSW sites at Hannam Vale and Clybucca were planted with:

- Family Selection Blocks (FSB) of the expected best 115 NSW and 10 Qld families, to provide superior trees for clone development and testing;
- Extensive Seedling Seed Orchards (ESSO) from bulked OP seedlots from 100 phenotypically superior NSW selections, planted for early improved seed production; and
- Provenance Resource Stands (PRS) from bulked seedlots or provenances not represented in the breeding populations, to provide genetic diversity and an infusion population for the second generation of the breeding populations.

Figure 2. Provenances of *Eucalyptus pilularis* with families included in the breeding populations (dots) and location of the three NSW progeny trials (crosses).

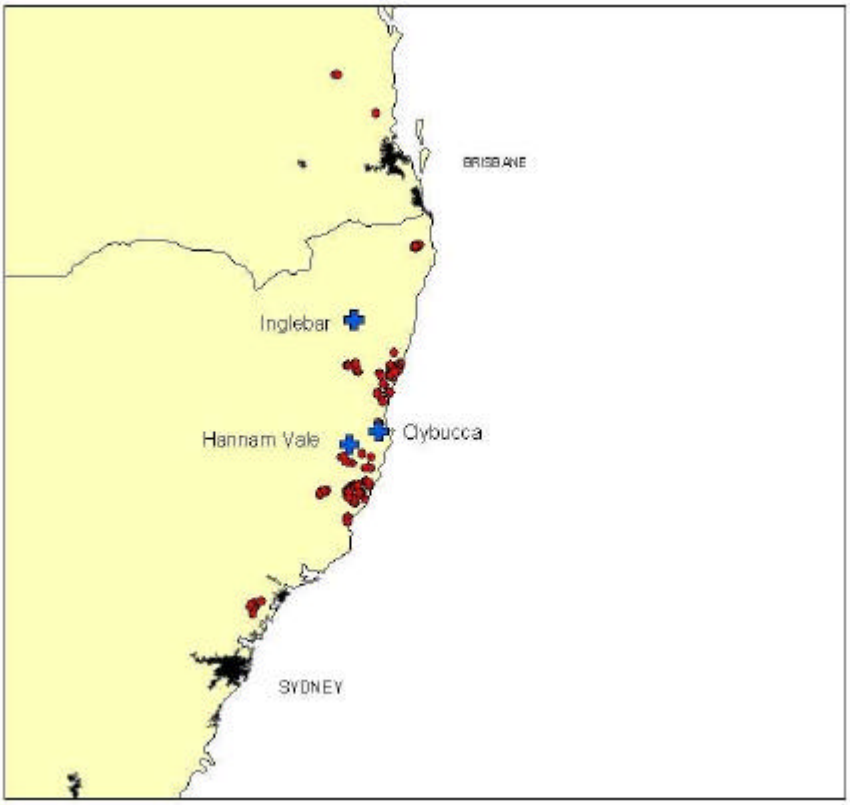


Table 2. Forests NSW *Eucalyptus pilularis* breeding populations site details.

	HANNAM VALE	CLYBUCCA	INGLEBAR
Property	Buttsworth LP	Rolleston LP	Inglebar Station
Latitude/Longitude	31º40' / 152º33'	30º54' / 152º55'	29º31' / 152º36'
Annual rainfall (mm)	1500	1260	1200
Altitude	150-170 m	150-170 m	285-305 m
Soils	Deep Yellow Earths to Yellow Podzolics	Deep Red Earths and Red Podzolics, shallower Yellow Podzolics	Deep Red and Yellow Podzolics
Number replicates	6	4	4
Design	Row-column: 18R x 17C	Row-column: 18R x 18C	ICB, 22 blocks x 14 plots
Plot size	4 tree row	4 tree row	4 tree row
No. of families	306 – 289 NSW plus tree; 12 Qld plus tree; 5 routine	324 – 281 NSW plus tree; 40 Qld plus tree; 3 routine	308 – 270 NSW plus tree; 35 Qld plus tree; 3 routine
Planting date	March 1997	March 1998	February 1998

Initial analysis of variance of individual trials, using the SAS Mixed Procedure, allowed identification of superior plus tree families for each trait. Superior genotypes were selected, based on assessments of volume, stem straightness and branch diameter on the NSW sites at age 3-3.5 years. A later analysis (Jarvis 2003) of the 3-year assessment data using ASREML within and across all five sites (NSW and Qld) was carried out to predict breeding values for parents and progeny. Breeding values for each trait were estimated using Best Linear Unbiased Prediction (BLUP) analysis, and an index constructed to identify the best performing parent trees and progeny for the growth traits. The best performing individual from the best 20 families were captured by grafting to establish an elite nucleus population, split into two sub-lines, and included in a pot-based breeding arboretum to further the breeding and deployment program.

The breeding populations will be reassessed next year at age 8 years. Measurements of growth and form will be coupled with wood structural and appearance quality traits such as stability, hardness, and collapse and growth stress, to further refine the selections for the next generation of breeding and for the deployment population.

GENETIC CONTROL OF GROWTH AND FORM TRAITS

At age 36-42 months, the progeny trials were assessed for height (HT), diameter at breast height over bark (DBHOB), stem straightness, and branch diameter. Mean individual tree volume was highest at Hannam Vale ($0.032m^3$) and lowest at Clybucca ($0.014m^3$). Individual heritability

estimates (h^2) for the growth and form traits for the NSW progeny trials were in line with the normal range of values for growth traits and are shown in Table 3 (Jarvis 2003).

There is a lot of variation within the breeding populations, as shown by the CV values, which can be exploited during selection. Genetic correlations among growth and form were low to negligible on all sites (Jarvis 2003) with the exception of stem straightness/branch diameter on all sites (0.64 to 0.93) and branch diameter/height at Inglebar (0.65). The predicted gains in volume of the best 20 families across the NSW sites, over the routine trees as a group, ranged from 18% (Clybucca) to 27% (Hannam Vale) (Jarvis 2003).

Table 3. Details of assessment of NSW progeny trials and heritabilities for growth and form traits. (From Jarvis 2003)

Site	Age (months)	Trait	n	mean	CV	h^2 (SE)
		DBHOB				
Hannam Vale	38	DBHOB (cm)	5254	10.6	23%	0.20 (0.04)
Clybucca	38	DBHOB (cm)	4654	7.3	30%	0.24 (0.05)
Inglebar	42	DBHOB (cm)	3015	10.7	26%	0.18 (0.05)
		HEIGHT				
Hannam Vale	38	Height (m)	5254	9.6	16%	0.30 (0.06)
Clybucca	38	Height (m)	4654	6.7	24%	0.32 (0.06)
Inglebar	42	Height (m)	3015	8.6	22%	0.17 (0.06)
		BRANCH DIAMETER				
Hannam Vale	38	Branch Diameter (1 worst – 6 best)	5254	4.5	25%	0.08 (0.03)
Clybucca	38	Branch Diameter (1 worst – 6 best)	4654	4.5	22%	0.28 (0.05)
Inglebar	42	Branch Diameter (1 worst – 6 best)	3012	5.0	19%	0.17 (0.06)
		STRAIGHTNESS				
Hannam Vale	38	Straightness (1 worst – 6 best)	5254	3.6	32%	0.34 (0.05)
Clybucca	38	Straightness (1 worst – 6 best)	4654	3.7	30%	0.26 (0.05)
Inglebar	42	Straightness (1 worst – 6 best)	3013	3.8	31%	0.20 (0.06)

Heritability assuming a coefficient of relatedness of 4.0

GENETIC CONTROL OF PEST AND DISEASE TOLERANCE

Eucalyptus pilularis is less affected by major disease and pest problems making it an ideal species, in this respect, for growing in plantations in its natural range where pest and disease pressure can be great. This is in comparison to some species of the Symphyomyrtus Section (for

example *E. grandis* and *E. dunnii*) that are highly susceptible and commonly attacked by a range of insects (Carnegie 2002); this susceptibility often limits the commercial viability of these species.

At 38 months the progeny trial at Clybucca was assessed for severity of *Mycosphaerella* Leaf Disease, caused by *Mycosphaerella cryptica* (Cooke) Hansf. and *Mycosphaerella marksii* Carnegie & Keane, and Target Spot, caused by *Aulographina eucalypti* (Cooke & Mass.) von Ark & Muller, at the same time as growth and form was assessed. These are the most important and damaging leaf fungi in the NSW *E. pilularis* plantations (Stone *et al.* 1998). Significant variation was found, by Carnegie *et al.* (2004), in disease and defoliation among the provenances and families tested. The individual heritability estimates were moderate for *Mycosphaerella* damage (h^2_{OP} = 0.38)[2], and low for defoliation (0.22) and *Aulographina* damage (0.13). The heritability for *Mycosphaerella* damage is lower than that found for infection by *M. nubilosa* by Potts *et al.* (2004) in *E. globulus* (h^2_{OP} = 0.6).

The general trend in the provenances with good family representation, was for *Mycosphaerella* damage to decrease with increasing altitude of the provenance. Genetic (0.27 s.e. 0.14) and phenotypic (0.12) correlations between *Mycosphaerella* damage and defoliation were low but significant. These correlations, along with those between *Mycosphaerella* damage and volume (genetic -0.64 s.e. 0.14; phenotypic -0.25), and defoliation and volume (genetic –0.16 s.e. 0.18; phenotypic -0.21) suggested that leaf damage from *Mycosphaerella* spp. contributed to defoliation in the crown and reduced tree growth (Carnegie *et al.* 2004).

GENETIC CONTROL OF WOOD PROPERTIES

A 55-month-old clonal trial of *E. pilularis* planted near Coffs Harbour underwent preliminary assessment for non-destructive wood quality traits including: spiral grain, pilodyn and acoustic velocity (time of flight). The results (Table 4) suggest that structural wood property traits are under strong genetic control.

Table 4. Details of assessment of NSW clonal trial (Crabtree LP) and heritabilities for growth, form and wood quality traits.

Age (months)	Trait	n	mean	CV	Clonal H^2 (SE)
		DBHOB			
34	DBHOB (cm)	313	9.1	25%	0.413 (0.077)
55	DBHOB (cm)	298	13.5	26%	0.416 (0.077)
		HEIGHT			
34	Height (m)	313	8.0	16%	0.363 (0.080)
55	Height (m)	298	12.8	18%	0.397 (0.080)
		VOLUME			
55	Volume	298	0.072	54%	0.445 (0.075)
		STRAIGHTNESS			
55	Straightness (1 worst – 6 best)	297	3.6	37%	0.222 (0.066)

[2] Coefficient of relatedness of 2.5, and Provenance included in model as fixed affect.

	GRAIN & STRUCTURAL TRAITS				
55	Spiral Grain	295	-0.97		0.177 (0.065)
55	Pilodyn (mm)	295	12.0	14%	0.563 (0.072)
	ACOUSTIC TRAITS				
56	FAKOPP (μs)	292	560.5	6%	0.609 (0.064)

Henson *et al*. (2004) and Dickson *et al.* (2004) have demonstrated strong correlations (0.64-0.65) between acoustic velocity and stiffness in 9-year-old *E. dunnii.* A very strong genetic correlation (0.96) between acoustic velocity and stiffness was reported by Henson *et al.* (2004) in the same study.

It is planned to screen the *E. pilularis* breeding populations for wood property traits during 2005 and 2006.

DEPLOYMENT

Deployment of improved material from the breeding program for *E. pilularis* has had a multifaceted approach and is evolving as more improved material and technologies are available.

1. Deployment of plus tree seed.

The commercial plantations in northern NSW have traditionally been established using bulked wild seed from native forest collections of preferred provenances. After identification of superior families from the breeding populations, a strategy of planting of seedlings from selected open pollinated plus trees seedlots was followed when seed was available. Commercial planting stock produced from this source would have estimated genetic gains of 4.3% (Johnson and Nikles 1997) over the bulked collections.

2. Clonal Seed Orchards

Although clonal seed orchards are expensive to establish, it is estimated that they will produce seed with a genetic gain of 10.2% over unimproved bulk collections (Johnson and Nikles 1997). Although the genetic gains are similar to those from a seedling seed orchard (Johnson and Nikles 1997), they can be achieved in a much faster time frame. Scions taken from mature parts of the tree are physiologically mature and retain some of this maturity after grafting, tending to flower within one or two seasons (Eldridge *et al.* 1993). A more rapid supply of seed of improved *E. pilularis* genotypes for operational plantations can be produced from clonal seed orchards than from seedlings, that may take up to eight years to flower (Johnson and Nikles 1997).

Superior individuals of *E. pilularis* were selected based on the assessments of 3-year growth and form data on the two largest progeny trials in NSW. A total of 53 ortets were selected for the initial seed orchard, including both the original plus trees and progeny trees in the breeding populations. These trees were unrelated, with either parent or one progeny selected from the better performing families. Selected trees were grafted onto related juvenile rootstocks and grafted ramets planted into half of the seed orchard site. Further selections of superior individuals were made after the analysis of data over all five sites and ramets grafted to complete the planting. In total, 96 clones from 29 provenances are represented in the clonal seed orchard. Grafts of another 46 clones from 22 provenances (a subset of the 29) have been grafted for planting in a second seed orchard this season. Multiple planting sites are being established to reduce the risk of loss of seed production and genetic material due to unforeseen circumstances

or extreme weather conditions, such as fire and hail damage. To date, the *E. pilularis* clonal seed orchards have a total area of 3.1 ha. Flower bud development was obvious in the seed orchard within 15 months of planting. Currently, 24 months after planting, 64% of the original ramets are in bud, with flowering expected to commence within the next 2 months.

The assessment of the breeding populations at age 8 years will be an opportunity for roguing the clonal seed orchards to remove inferior clones. Wood quality traits, for which no information has been available to date, will be an important selection criteria to determine which clones remain within the seed orchard.

Although these seed orchards will provide Forests NSW with a source of genetically improved seed, deployment by seed has a number of problems for *E. pilularis*. Development of seed for this species is lengthy, with bud initiation to flowering being 15-18 months (Clemson 1985) and the interval between flowering and mature seed being approximately 12 months (Florence 1964; Gunn 2001). Seed viability is often low, with averages of 200-700 viable seed per 10 grams (Florence 1964), 517 ± 213 viable seeds per 10 g (Gunn 2001) and 693 viable seeds per 10 g (range 280-1350) in the Forests NSW Tree Improvement Seed Centre (G. Greenwood 2004 pers. comm.). Germination rates for commercial seed lots are often as low as 10% (G. Greenwood 2004 pers. comm.) resulting in production problems in the nursery.

In addition, *E. pilularis* seedlings often have shallow root systems and poor root quality, being affected by j-root and root coiling. During the 2003/04 planting season, 82% of the sampled Forests NSW commercial *E. pilularis* seedlings were recorded as having some level of j-root (D. Thomas 2004 pers. comm.). When seedlings are established in plantations, this often leads to lack of stability, resulting in socketing, buttsweep and windthrow. Generally the number of deaths in plantations are low, at <1%, but this may occur in consecutive years. On boggy sites or in years of high winds, the death may be up to 10% (Carnegie 1999).

3. Vegetative propagation, clone development and deployment

Plants derived from vegetative propagules, particularly mini-cuttings, have better root quality than seedlings, with no taproot and stronger lateral roots (D. Thomas 2004 pers. comm.) and may provide better plant stability in the field. Vegetatively propagated plants (clones) may overcome the plant quality issues in *E. pilularis* as well as allow capture of greater genetic gain in the planting stock. The advantages of clonal forestry have been well documented (Eldridge *et al.* 1993) and include uniformity of the crop for management and harvest aspects, matching of clones to sites, the ability to rapidly capture a greater amount of both additive and non-additive genetic variation and uniformity of the wood product harvested.

The identification of better performing families within the *E. pilularis* breeding populations has allowed the development of clones for field-testing. Seedling-based clones have been developed from 40 plus tree families and field select clones from Family Selection Blocks planted at the same time as the progeny trials. These clones have been supplemented by field select clones developed from selections in young plantations within the Forests NSW estate. Testing of these clones has commenced with the field trials shown in Table 5. Three further large clone trials (approx. 300 clones from plus tree seedlings and field selects) are currently being propagated for and are to be established during the next year. The earliest clone trial, comprising of field select

clones, was assessed for growth and form at age 3 years, and five clones selected for further testing and limited commercial deployment. At age 4.5 years, assessment of wood properties, particularly density, stiffness and grain angle, resulted in selection of another five candidate clones for further testing and pilot scale commercial deployment.

Table 5. Forests NSW *Eucalyptus pilularis* clone trials.

Location	Planting date	No. clones	Clone origin
Crabtree LP	Nov 1999	32 + 3 seedling controls	Field selects (young plantations)
Gurranang Plantation	April 2002	162 + 3 seedling controls	Plus tree seedlings and field selects
Halls JV	May 2002	118 + 3 seedling controls	Plus tree seedlings and field selects

4. Family forestry

Family forestry is the mass vegetative propagation of seedlings of selected pedigreed families, without testing and identification of individual clones (Eldridge *et al.* 1993). Genetic gains can be captured more rapidly in the deployment population using this technique than clonal forestry, where thorough screening for rooting and field-testing of clones is required, increasing the length of time to commercial deployment. Significant amounts of non-additive genetic gains can still be captured with family forestry. Although the strict uniformity of tested clones is not achieved, it provides wider genetic diversity in the plants to counteract the site specificity exhibited by clones when planted into the sub-optimal environments of NSW sites that are edaphic and climatically variable.

During the past year Forests NSW has adopted a deployment strategy of family forestry for *E. pilularis* (Henson and Smith 2004). Sources of superior seed are identified and bulked as seedling-based clones using rooted cuttings. The deployment program is being implemented in several stages as improved material becomes available for deployment and screening techniques for young seedlings are developed:

- Stage One: Family Forestry using seed from selected superior plus trees (2003-2006)
- Stage Two: Family Forestry using best bet CP seed from selected parents (2005-2008)
- Stage Three: Family Forestry using tested CP seed (2008-2010)
- Stage Four: Improved Family Forestry using tested CP seed and screening of seedling clone mother plants (2008 -)

The first stage that has been implemented to date is family forestry using seedling-based clones from selected superior plus trees. Fifteen of the best performing families in the *E. pilularis* breeding populations were selected from the 3-year assessment of growth and form traits. Seedlots from the original plus trees were germinated and a total of 1402 seedlings established as mother plants in a flood irrigated hydroponic system. Mini-cuttings were harvested to propagate plants for commercial deployment.

Concurrently, the five partially tested field select clones (Refer Section 3 – Vegetative propagation, clone development and deployment) were established as mother plants in the same hydroponic system to compare performance in both mother plant productivity and rooting of

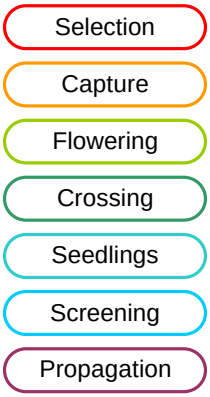
cuttings. Henson and Smith (2004) found that the cuttings from the seedling-based mother plants were more likely to root, with a mean rooting of 84% for the seedling-based clones and 36% for the partially tested clones. The number of harvest cycles achieved during the propagating season was also greater for the seedling-based clones compared to the partially tested clones. Overall, productivity (rooting success coupled with mother plant vigour) of the seedling-based clones was almost 8 times greater than that for the tested clones. Use of seedling-based clones can reduce mother plant maturity issues that result in poor rooting of cuttings, and therefore reduce elimination of genotypes from the program that may have excellent growth or wood traits.

During 2003/04, over 10 ha of plantation were established using plants of *E. pilularis* propagated through family forestry, and it is planned to establish 80 ha during the 2004/05 planting season.

5. Improved family forestry

The final stage of development of the deployment program is Improved Family Forestry. As shown in Figure 3, selected genotypes are captured, generally by grafting, and grown on in a pot-based breeding arboretum. Early flowering of the grafted ramets allows controlled crosses to be made. The seed produced by controlled pollination will be germinated, and seedlings screened for a range of characteristics that are directly related to performance traits in the field.

Figure 3. Diagrammatic representation of the Improved Family Forestry system



The identification of traits that can be used for early screening has commenced, including Specific Leaf Area as a reflection of good growth potential (Montagu *et al*. 2002). The screening technologies are aimed to be efficient, simple and cost effective, so a large number of individuals can be screened prior to inclusion as mother plants. It is hoped that these screening techniques will improve gain through the elimination of inferior genotypes, ensuring that only those seedlings that meet defined standards are established as mother plants for cuttings propagation. In parallel, the group is also improving selection techniques for trees in the field, for qualitative traits such as disease and insect tolerance using Crown Damage Index (Stone *et al*. 2003) and wood quality (Henson *et al*. 2004), for inclusion of genetic material into the program.

Collection of DNA samples from all original selected ortets and other selected genotypes in the improvement program is currently underway. This may eventually allow the use of screening of potential for specific identified traits and enable the Tree Improvement Team to react quickly

and efficiently to changes in market demands or biological risks to plantations, by incorporation of new genotypes into the deployment population.

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Using the Crown Damage Index (CDI) as a Selection Tool for Insect Resistance in *Eucalyptus grandis* in Northern New South Wales, Australia

Henson, M.[1], Smith, H.[1], Boyton, S.[1], Carnegie, A.[2], Stone, C.[2] and Henery, M.[3]

Abstract: *Eucalyptus grandis* is a successful plantation species outside its natural range, however in northern NSW, Australia, it is prone to a wide range of insect pests. Progeny trials of *E. grandis* were established in northern NSW by Forests NSW in April 1999 and February 2000. They included 215 families including 2nd generation material and non-improved material from 41 provenances. One of the trials was subjected to flooding followed by severe drought, resulting in the trees becoming stressed and subsequently being heavily attacked by defoliating insects. This trial was assessed for insect damage using the Crown Damage Index. Leaf insect damage was found to be under medium to strong genetic control ($h^2 = 0.53$). A relationship between provenance latitude and insect attack was found ($R^2 = 0.48$), the lower latitude provenance having the least insect damage. Three "susceptible" and three "resistant" families were selected for bioassay studies *ex-situ* with chrysomelid leaf beetles. A range of bioassay traits were quantified including: larval mortality, larval growth rate, and larval relative consumption. Clear differences were found between the families and are discussed in this paper.

Keywords: *Eucalyptus grandis*, Flooded gum, Crown Damage Index.

INTRODUCTION

Eucalyptus grandis W. Hill ex Maiden is an indigenous species to northern NSW and Queensland, Australia. It is widely planted outside of Australia, where it is a very successful plantation species in countries such as South Africa and Argentina. However, when grown in plantations within its natural range, the species is problematic; it is extremely site specific and susceptible to a wide range of defoliating and stem boring insects.

Forests NSW is a State Government Trading Enterprise that is responsible for managing 49 000 ha of eucalypt plantations within a total estate of 2.9 million hectares. The pre-1994 eucalypt plantation estate comprises 8 000 ha of *E. grandis*, the majority of which was established for pulp production, but later managed for solid wood products. Currently the species is not favoured as a plantation species in the region owing to insect problems and its problematic solid wood properties. Of the 27 000 ha of post-1994 eucalypt plantation, only 2 500 ha is *E. grandis.* Forests NSW has a long history of tree improvement with *E. grandis* and is focusing on the improvement of growth, insect resistance and wood quality of the pure and hybrid genotypes.

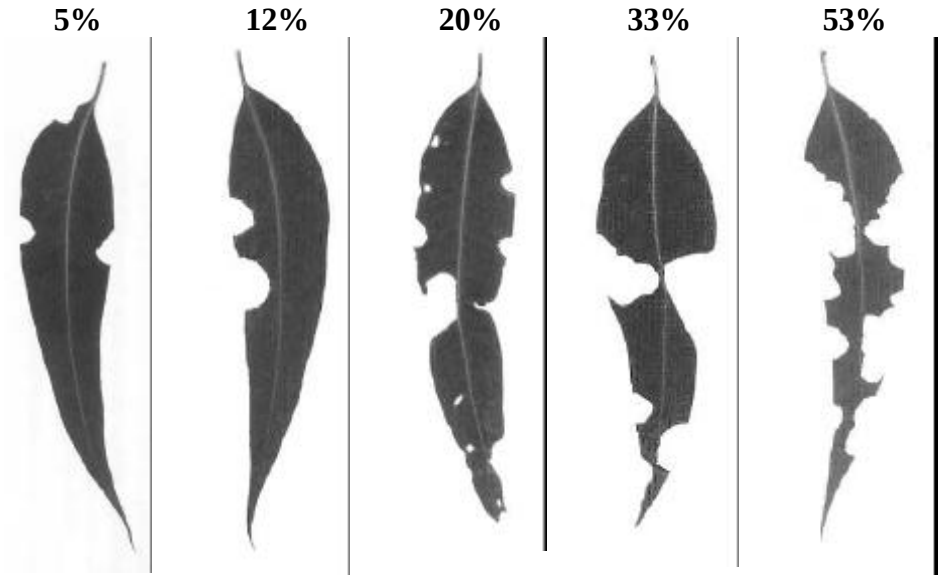
Some of the more economically important defoliating insects that occur in *E. grandis* plantations in northern NSW include; chrysomelid leaf beetles (*Chrysophtharta ssp.* & *Paropsis ssp.*) and

[1] Forests NSW, PO Box J19, Coffs Harbour Jetty, NSW, 2450, Australia
[2] DPI Science and Research, PO Box 100, Beecroft, NSW, 2119, Australia
[3] School of Botany and Zoology, Australian National University, Canberra, ACT 0200 Australia
Contact e-mail: michaelhe@sf.nsw.gov.au

eucalypt sawflies (*Perga ssp.* & *Pergagrapta ssp.*) (Carnegie 2002). The species is also highly susceptible to Christmas beetles (*Anoplognathus ssp.*) (Simpson *et al.* 1997), and the Giant Wood Moth (*Endoxyla cinerea*) with the stem damage often being exacerbated by cockatoos feeding on the larvae within the stem (Monteith 2000). Susceptibility of *E. grandis* to insect pests often results in plantations low in productivity and with poor wood quality. The species does offer the potential for fast growth rates but only if pests and diseases are managed and kept below economic thresholds (Simpson *et al.* 1997).

The Crown Damage Index (CDI) was developed to assess the extent and severity of damage to young eucalypt plantations in the pre-canopy closure phase (Stone and Coops 2004). The index is derived from a visual estimate of damage on a leaf basis, multiplied by the level of incidence throughout the canopy of the tree, for three types of damage: defoliation, necrosis and discolouration, caused by insects, fungi or abiotic agents (Stone *et al.* 2003a). The CDI provides a standardised, repeatable and statistically valid measure of pest and disease damage so that quantifiable comparisons can be made and statistical analysis can be undertaken irrespective of the cause of damage, the site or host (Mohammed *et al.* 2004).

Figure 1. An example of CDI scoring defoliation as a percentage of total leaf area (Stone *et al.* 2003a)



MATERIALS AND METHODS

Forests NSW *Eucalyptus grandis* tree improvement program commenced in 1972 with the establishment of a progeny trial at Pine Creek near Coffs Harbour. This trial was subsequently destroyed by fire but a second trial consisting of 103 open pollinated families was established at Wedding Bells in 1974. The trial was converted into a seed orchard and has subsequently provided seed to the *E. grandis* breeding program through out the world.

In 1999 and 2000 a second series of *E. grandis* progeny trials (Table 1) were established consisting of a total of 215 open pollinated families; 55 second generation or plus tree selects from plantation or provenance trials (Table 2); and a further 160 progeny from 41 provenances covering the native range of the species (Table 4 / Figure 2).

Table 1. Information on 1999/2000 series of E*ucalyptus. grandis* progeny trials

	HILLS PURCHASE	CRABTREE PURCHASE
Latitude / Longitude	28º 37'S 153º 03'E	30º 09'S 153º 06'E
Altitude (above sea level)	230-250 m	85 m
Soil Type	Prairie soils (Northcote Gn3.93) Clay-loam surface Medium clay to 100 cm Fine sandy clay-loam below	Dark loams over red-clay loams (Northcote Gn2.14) to Krasnozems (Gn4.14) or Red Podzolics (Dr2.21) in places
Spacing	4.0m x 2.0m	4.0m x 2.0m
Number replicates	5	5
Design	Row Column 15R x 14C	Row Column 14R x 15C
Plot size	5 tree row	5 tree row
No. families	204 Families	204 Families
Planting date	April 1999	February 2000

Figure 2. Provenances of *Eucalyptus grandis* with families included in the breeding populations (dots) and location of the two progeny trials (crosses).

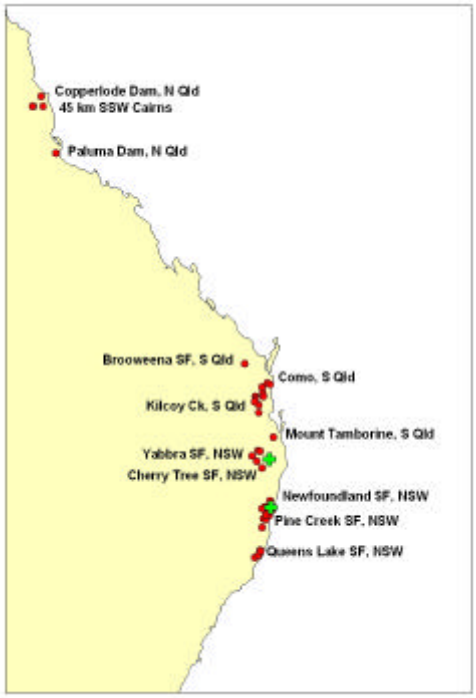


Table 2. Sources of improved families included *Eucalyptus grandis* 1999 and 2000 progeny trials

Source	State	Latitude	Longitude	Altitude	Type	Families	
						Halls	Crabtrees
Lannercost (Shell Trial)	QLD	18º 16' S	146º 16' E	6	Provenance Trial	9	9
Murray Upper (Shell Trial)	QLD				Provenance Trial	1	1
Toolara (Shell Trial)	QLD	26º 00' S	152º 47' E	40	Provenance Trial	12	12
Weeding Bells	NSW	30º 09' S	153º 06' E	105	BSO	18	17
Wagga Effluent Trial	NSW	35º 07' S	147º 22' E	230	Irrigated Plantation		5
Dubbo Effluent Trial	NSW	32º 00' S	141º 15' E	275	Irrigated Plantation		3
Koorlong	VIC	34º 16' S	142º 05' E	30	Irrigated Plantation		6
Koorlong Trial	VIC	34º 16' S	142º 05' E	30	Provenance Trial		1
South Africa CP Elite	RSA				Seed (Bulk)	(2)	(1)
Total						40	53

All trees in the progeny trial at Hills was assessed at 41 months for growth and form traits. The trial was also assessed for defoliation caused by insects using the Crown Damage Index (1-unaffected to 8-severe defoliation).

Table 3. Scoring System used for CDI in *E. grandis* Progeny Trials

Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Crown Damage	0-3%	3-6%	6-12%	12-25%	25-50%	50-75%	75-95%	>95%

The progeny trial at Crabtrees established in 2000 was subjected to flooding followed by severe drought resulting in the trees becoming stressed and subsequently being heavily attacked by defoliating insects. Owing to the significant insect attack and poor growth, only defoliation was assessed at this site at 26 months using the same CDI method as Hills. Only 3 of the 5 tree in each plot were assessed for CDI at both Hills and Crabtrees.

Table 4. Provenances included *Eucalyptus grandis* 1999 and 2000 progeny trials

Provenance	State	Latitude	Longitude	Altitude	Families	
					Hills	Crabtrees
Copperlode Dam	QLD	16º 59' S	145º 40' E	428	17	5
45 km SSW Cairns	QLD	17º 18' S	145º 44' E	680		2
Atherton	QLD	17º 18' S	145º 25' E	1100		2
Wongabel	QLD	17º 18' S	145º 24' E	1000		2
Paluma Dam	QLD	18º 47' S	146º 10' E	900	5	4
Brooweena SF	QLD	25º 33' S	152º 16' E	100	1	1
Como	QLD	26º 10' S	152º 59' E	40	5	3
Kin Kin	QLD	26º 12' S	153º 10' E	40	4	4
Woondum	QLD	26º 18' S	152º 49' E	80	4	4
Belli	QLD	26º 29' S	152º 50' E	100	4	4
Borumba Ra	QLD	26º 35' S	152º 36' E	500	5	5
Mapleton	QLD	26º 36' S	152º 52' E	300	5	5
Conondale Range	QLD	26º 40' S	152º 36' E	560	4	5
Kilcoy Ck	QLD	26º 45' S	152º 35' E	400	5	4
Bellthorpe	QLD	26º 52' S	152º 43' E	300	10	10
Mount Mee	QLD	27º 08' S	152º 43' E	200	4	2
Mount Tamborine	QLD	27º 55' S	153º 11' E	500	5	5
Mt Lindsay	QLD	28º 21' S	152º 45' E	340	5	5
Mt Lindsay SF	NSW	28º 20' S	152º 41' E	500	2	2
Yabbra	NSW	28º 30' S	152º 30' E			
Richmond Ra	NSW	28º 41' S	152º 39' E	520	5	5
Cherry Tree SF	NSW	28º 54' S	152º 49' E	480	5	4
Newfoundland SF	NSW	29º 56' S	153º 05' E	83	3	3
Bagawa SF	NSW	30º 07' S	152º 54' E	440	5	5
Coffs Harbour	NSW	30º 09' S	152º 97' E	211	6	6
15km N Coffs Harbour	NSW	30º 10' S	153º 07' E	100	3	2
Wedding Bells	NSW	30º 10' S	153º 07' E	100	5	5
Lower Bucca SF	NSW	30º 11' S	153º 03' E	180	1	1
Wild Cattle Creek	NSW	30º 12' S	152º 49' E	619	4	4
Near Coffs Harbour	NSW	30º 14' S	152º 58' E	412	9	8
Orara East SF	NSW	30º 14' S	153º 03' E	135	3	3
Orara West SF	NSW	30º 14' S	152º 56' E	610	1	1
Tuckers Nob SF	NSW	30º 23' S	152º 57' E	68	4	4
Pine Creek SF	NSW	30º 26' S	152º 79' E	25	2	2
Gladstone SF	NSW	30º 31' S	152º 52' E	45	4	4
Newry SF	NSW	30º 32' S	152º 57' E	27	7	7
Ingalba SF	NSW	30º 48' S	152º 49' E	60	1	2
Burrawan SF	NSW	31º 32' S	152º 45' E	40	1	1
Queens Lake SF	NSW	31º 35' S	152º 47' E	34	4	4
Middle Brother SF	NSW	31º 42' S	152º 42' E	90	2	2
Lansdowne SF	NSW	31º 46' S	152º 35' E	58	4	4
Total					**164**	**151**

The trial data was analysed using an individual tree model in ASREML. Provenance and Replicate were treated as fixed effects and Tree, Row, Column and Plot as random effects. Heritability was calculated using a coefficient of relatedness of 4, assuming no inbreeding. Genetic correlations between traits and for the same trait across sites were also estimated using individual tree models in ASREML.

RESULTS AND DISCUSSION

Individual heritability estimates (h^2) for the growth and form traits at the Hills progeny trials were in line with the normal range of values for growth traits and are shown in Table 5. Growth at the site was fair with a mean height of 6.4 m at 49 months.

Table 5. Summary of Results from 3 year assessment of *E. grandis* progeny trials

Age	Trait	n	Mean	CV	h^2 (SE)
	HILLS				
41 months	DBH (cm)	4704	6.60	40%	0.134 (0.036)
41 months	Height (m)	4704	6.49	30%	0.177 (0.048)
41 months	Volume (dm^3)	4704	12.82	85%	0.121 (0.037)
41 months	Straightness (1 poor - 6 best)	4689	3.89	30%	0.187 (0.045)
41 months	Branching (1 poor - 6 best)	4689	2.87	40%	0.168 (0.039)
36 months	CDI (1 unaffected - 8 severe)	3218	3.10	37%	0.096 (0.058)
	CRABTREES				
26 months	CDI (1 unaffected - 8 severe)	3169	6.28	11%	0.526 (0.092)

Heritability for CDI (defoliation) at Crabtrees was high (0.53) compared to a low estimate of heritability (0.10) for the same trait at Hills where defoliation was much lighter with a mean CDI of 3.1 (6-12%) compared to 6.3 (50-75%) at Crabtrees.

There were significant differences among provenances and sources ($P < 0.001$) for DBH, Height, Volume, Straightness and Branching. Results for provenance and sources are presented in Table 6 and Table 7.

Table 6. Provenance Results for 3 Year Assessment of *Eucalyptus grandis* Progeny Trials

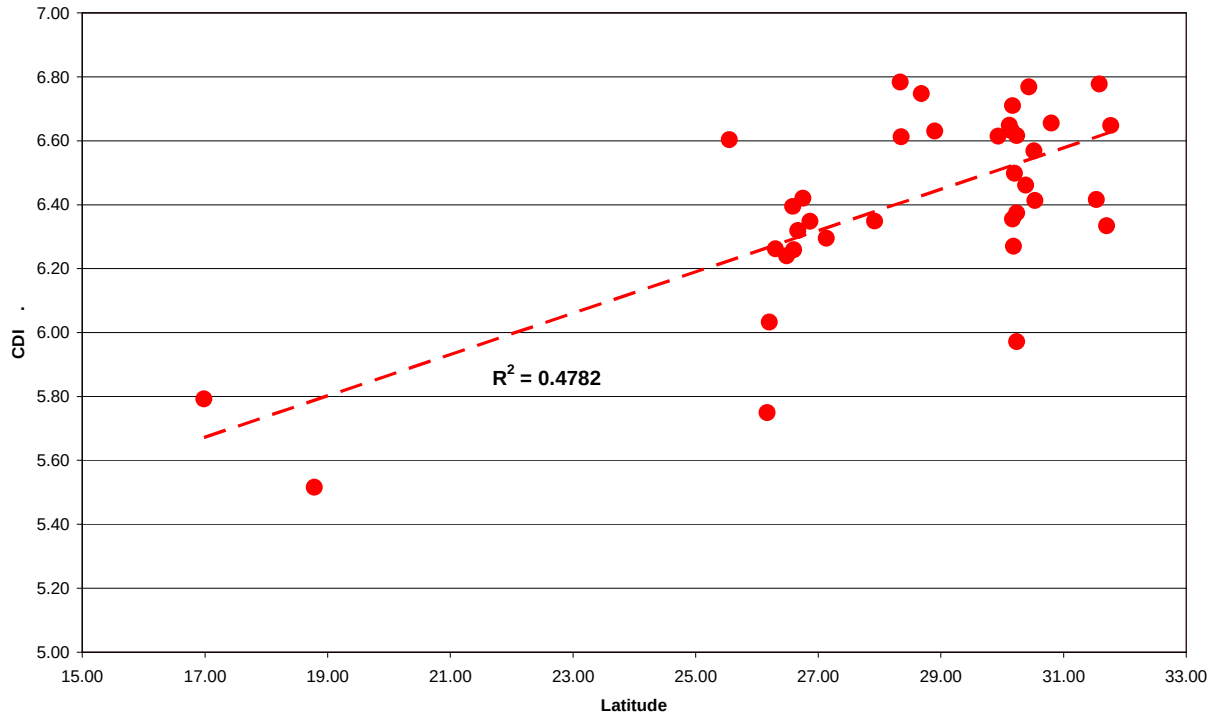
Origin	State	Crabtrees CDI 26 m	CDI 36 m	Hills Height 41 m	Hills DBH 41 m	Hills Volume 41 m	Hills Form 41 m
Copperlode Dam	QLD	5.79	2.10	6.52	6.61	13.24	3.61
45 km SSW Cairns	QLD	6.45					
Atherton	QLD	5.10					
Wongabel	QLD	5.63					
Paluma Dam	QLD	5.52	2.19	6.21	6.21	11.61	3.10
Brooweena SF	QLD	6.60	2.45	5.44	5.47	8.24	3.35
Como	QLD	5.75	2.54	6.60	6.60	12.74	3.66
Kin Kin	QLD	6.03	2.67	6.06	5.91	10.20	3.36
Woondum	QLD	6.26	2.41	6.28	6.56	12.50	3.70
Belli	QLD	6.24	2.63	6.77	6.81	13.08	3.76
Borumba Ra	QLD	6.39	2.96	6.11	6.28	11.00	3.42
Mapleton	QLD	6.26	2.44	6.22	6.41	11.72	3.56
Conondale Range	QLD	6.32	2.61	6.73	6.68	12.24	3.81
Kilcoy Ck	QLD	6.42	2.90	6.57	6.53	11.86	3.53
Bellthorpe	QLD	6.35	2.46	6.79	6.69	13.06	3.58
Mount Mee	QLD	6.30	2.73	5.91	5.77	8.82	3.37
Mount Tamborine	QLD	6.35	2.52	7.25	7.13	15.31	3.84
Mt Lindsay	QLD	6.61	2.76	6.51	6.24	11.79	3.51
Mt Lindsay SF	NSW	6.78	2.84	5.88	5.93	9.34	3.54
Yabbra	NSW		2.80	6.69	6.79	15.21	4.01
Richmond Ra	NSW	6.75	2.69	7.09	6.62	13.96	3.80
Cherry Tree SF	NSW	6.63	2.73	5.99	5.93	10.44	3.42
Newfoundland SF	NSW	6.61	3.16	5.62	5.34	8.16	3.09
Bagawa SF	NSW	6.65	3.02	6.52	6.08	10.68	3.82
Coffs Harbour	NSW	6.63	2.79	6.73	6.79	12.91	3.56
15 km N Coffs Har	NSW	6.35	2.46	6.97	6.80	14.10	3.65
Wedding Bells	NSW	6.71	2.68	6.60	6.39	11.54	3.46
Lower Bucca SF	NSW	6.27	2.42	7.32	6.77	12.84	3.91
Wild Cattle Creek	NSW	6.50	2.70	6.38	6.33	12.19	3.67
Near Coffs Har.	NSW	6.62	2.62	6.25	6.04	10.63	3.54
Orara East SF	NSW	6.37	2.48	6.93	6.79	13.22	3.41
Orara West SF	NSW	5.97	2.32	6.95	6.76	12.83	3.95
Tuckers Nob SF	NSW	6.46	2.29	7.67	7.80	18.66	4.00
Pine Creek SF	NSW	6.77	2.58	6.97	6.80	13.49	3.75
Gladstone SF	NSW	6.57	3.00	6.81	6.72	13.26	3.82
Newry SF	NSW	6.41	2.36	7.34	7.21	16.09	3.78
Ingalba SF	NSW	6.66	2.73	6.84	6.85	12.56	3.47
Burrawan SF	NSW	6.42	2.36	7.01	6.64	11.62	3.81
Queens Lake SF	NSW	6.78	2.82	6.29	6.06	10.35	3.33
Middle Brother SF	NSW	6.33	2.57	7.27	7.13	14.47	3.98
Lansdowne SF	NSW	6.65	2.73	6.57	6.59	11.65	3.57

Table 7. Source Results for 3 Year Assessment of *Eucalyptus grandis* Progeny Trials

Source	State	Crabtrees CDI 26 m	Hills CDI 36 m	HT 41 m	DBH 41 m	Vol 41 m	Form 41 m
Weeding Bells	**NSW**	6.54	2.45	7.29	7.13	15.29	3.81
Wagga Effluent Trial	**NSW**	6.27					
Dubbo Effluent Trial	**NSW**	6.47					
Koorlong	**VIC**	6.61					
Koorlong	**VIC**	6.27					
Lannercost (Shell Trial)	**QLD**	6.00	2.20	6.53	6.57	13.37	3.33
Murray Upper (Shell Trial)	**QLD**	5.81	1.56	8.03	8.36	22.45	4.06
Toolara (Shell Trial)	**QLD**	6.16	2.31	7.24	7.16	15.42	3.94
South Africa	**RSA**		2.53	6.94	7.09	14.38	3.70

Significant differences ($P < 0.001$) among provenances/sources were found at both sites for CDI (insect defoliation). A correlation of 0.69 between provenance latitude and insect defoliation was found at Crabtrees (Figure 3), the correlation was weaker (0.40) at Hills.

Figure 3. Estimated CDI for Provenance against Latitude



One possible reason for this relationship is the fact that the southern provenances of *E. grandis* may be exposed to less insect pressure as the cold winters and frost often limit insect population numbers. The northern provenances may have adaptive mechanisms to combat insect defoliation that are being expressed in this trial. However it is important to note that at Crabtrees all

treatments were severely defoliated by insects even though genetic differences between treatments were significant.

A genetic correlation of -0.73 (s.e. 0.15) was found between volume and insect defoliation at Hills, a negative genetic correlation was also found between stem straightness and insect defoliation at the same site (-0.47 s.e. 0.24).

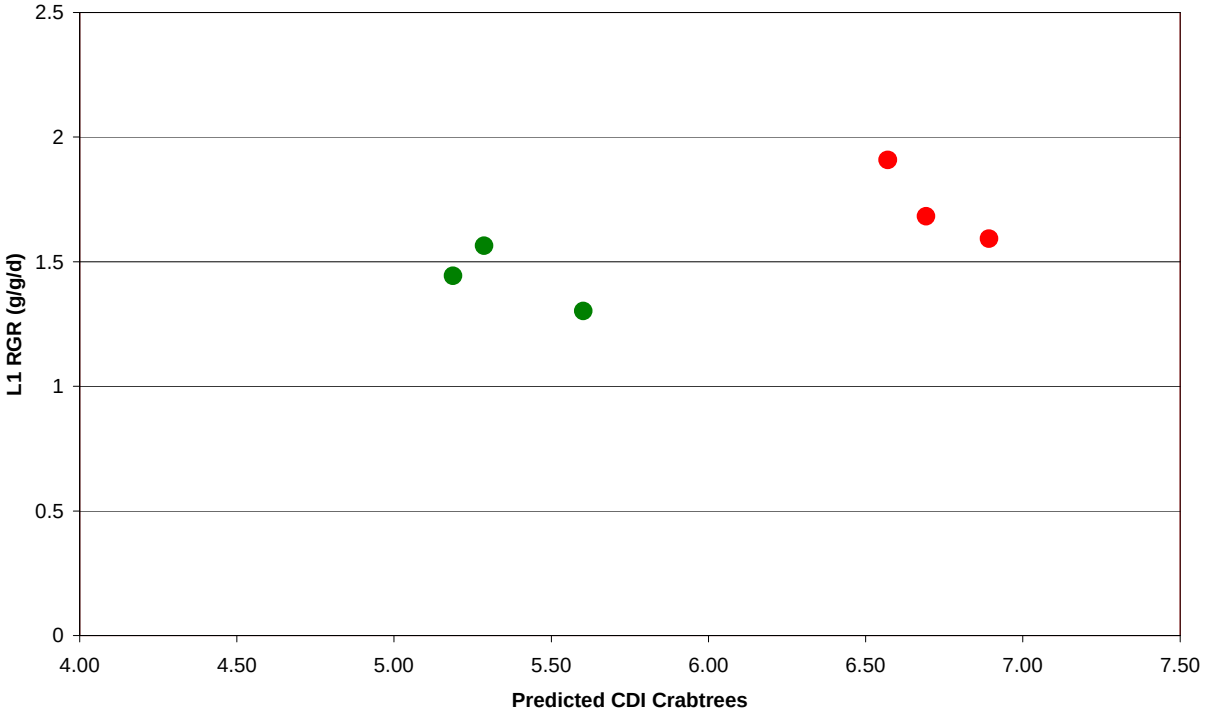
A genetic correlation of 0.64 (s.e. 0.17) was estimated for CDI (defoliation) between sites demonstrating that the family performance is relatively stable between sites. A correlation of 0.66 was found for Provenance CDI between Hills and Crabtrees.

Bioassay

From the results of the CDI assessment at Crabtrees three defoliation susceptible (high predicted CDI) families were selected along with 3 "resistant" families (low predicted CDI) for bioassay studies. Seedling of each family were grown in pots, five trees per family of sufficient size were selected at random for bioassays which consisted of measuring growth and development parameters for cohorts of *Paropsis atomaria* larvae feeding on the leaves. Each assay consisted of five replicated cohorts of 20 newly hatched first instar *P. atomaria* larvae placed on separate cut branchlets suspended in plastic bottles of water. The branchlets were cut in such a way to ensure that a full array of leaves at different stages of expansion were available for the larvae to consume. Relative consumption rate (RCR) and relative growth rate (RGR) were calculated and larval mortality was also recorded.

Relative Growth Rate was found to be significantly lower in trees from resistant families during the first instar. However this effect was transient as it was not manifest in any later instars. This effect also seemed primarily due to a significant lengthening of first instar development times in some of the trees assayed from the three resistant families. All other parameters including mortality and RCR were found to be similar across all families in the subsequent instars. Predicted means of proportional mortality were higher in first instar larvae developing on resistant family leaf material although this effect was not statistically significant.

Figure 4: Mean Larval (instar 1) Relative Growth Rate of *Paropsis atomaria* on selected families



CONCLUSIONS

The results of the trials clearly demonstrate that there is genetic control of insect defoliation in *Eucalyptus grandis*, the genetic patterns of susceptibility seem to be stable between trial sites. However all genotypes suffered severe defoliation (mean > 50%) at the worst affect site, Crabtrees, where the trees had been stressed by flooding and subsequent drought.

The giant wood moth (*Endoxyla cinerea*) is a major problem in *E. grandis* plantations in northern NSW, although not assessed in these trials, results from 3 year old clonal trials (Forests NSW, unpublished data) suggest that there is little or no genetic variation in resistance to this stem boring pest.

The exact mechanism of the "resitance" could not be identified through the limited bioassay studies. Although significant differences between resistant and susceptible families were found in the Relative Growth Rate of the 1st instar larvae of *P. atomaria*, the difference was not manifest in any later instars. The limited effects of the resistance on any other parameters or life stages measured leads to the proposal of several theories. It suggests that an antifeedant may not be the primary factor involved in reducing RGR and more specifically development time in first instars unless the toxin is somehow instar specific. Another possibility is that some physical property/s of the resistant leaves may inhibit first instar larval growth but this effect is lost when the larvae grow large enough to overcome this inhibitory factor. One of the significant differences between early and latter instars is the capacity of the insects chewing mouthparts to

cope with adverse conditions. i.e. tougher cell walls, difficult to digest or masticate epidermal or cuticular compounds.

One important question that needs to be answered is "what is the cost (on growth) of insect 'resistance'?" Anecdotal evidence suggests that genetic material of *E. grandis* from breeding programs in South Africa is more susceptible to insect defoliation than local material when planted in Australia (Lee *pers comm*). Has breeding for growth traits in the absence of insect pressure in South Africa reduced insect resistance? The strong negative correlation (-0.73) between volume and insect defoliation found in this study may support this hypothesis. Forests NSW has established two clonal trials in 2003 where the clones have been planted with and without systemic insecticides, it is hoped that results from these trials will help estimate the cost of defoliating insect resistance in *E. grandis*.

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Propagation via Somatic Embryos – Why or Why Not?

Sara von Arnold

Professor, Cell Biology of Forest Trees, Department of Plant Biology and Forest Genetics, SLU, PO Box 7080, S-750 07 Uppsala, Sweden

The possibility of vegetative propagation creates significant advantages both for breeding and for propagation of genetically improved material. Today the potential to use somatic embryos for clonal propagation is an attractive option for economically important tree species, and especially for the conifers, which are difficult to propagate via cuttings. Another advantage with somatic embryos is that they can be cryopreserved. The whole procedure of conifer plant regeneration through somatic embryogenesis is comprised of a sequence of steps including establishment and proliferation of embryogenic cultures, maturation, partial desiccation and germination of somatic embryos, and finally *ex vitro* acclimatization of somatic embryo plants. *Ex vitro* growth of somatic embryo plants are under a cumulative influence of a number of previously applied treatments. Consequently, before the method to propagate plants via somatic embryos is applied on a large scale, it is of utmost importance to identify those treatments given during the *in vitro* phase that have a negative influence on the subsequent growth of the plants.

Norway spruce can today efficiently be propagated via somatic embryos from seeds of all tested families. However, several factors have to be considered in order to obtain high quality somatic embryo plants. These factors include (1) synchronization of early somatic embryo development by stimulation of programmed cell death, (2) short maturation treatment without osmotic stress, (3) a two phase germination treatment, first on solidified medium and then in liquid medium and (4) selection of somatic embryoplants with lateral roots at *ex vitro* transfer.

In general, pine species are more difficult to propagate via somatic embryos than spruces. This is also the case for Scots pine. There are several reasons for this. Initiation frequency of embryogenic cultures is low, varying from 0.2 to 11 % depending on collection time and family. Embryogenic cultures proliferate by embryo formation through meristematic activity in the secondary suspensor, alternating with cleavage polyembryogeny. Multiplication rate is very high and proliferation is difficult to stop. The current procedure of somatic embryogenesis gives rise to increased genetic instability in some families as evidenced from the analysis of four nuclear microsatellite loci.

Possibilities and limitations of propagation via somatic embryos will be discussed.

Commercial Implementation of Multi-Varietal Forestry Using Conifer Somatic Embryogenesis

Y.S. Park

Research Scientist,
Natural Resources Canada, Canadian Forest Service – Atlantic Forestry Centre
PO Box 4000, Fredericton, New Brunswick E3B 5P7 CANADA
ypark@nrcan.gc.ca

The use of genetically tested clones in plantation forestry offers many advantages, including: (1) obtaining much a larger genetic gain than is possible with conventional tree breeding, (2) flexibility to rapidly deploy suitable clones with changing breeding goals and environment, and (3) ability to manage genetic gain and diversity in plantation forestry. Despite these advantages, clonal forestry in conifers has rarely been practised because of the general lack of an efficient vegetative propagation system that can mass produce the same tested clonal lines repeatedly over time. Owing to recent refinement in somatic embryogenesis (SE) technology, the implementation of commercial high-value clonal forestry with several conifer species has begun in New Brunswick, Canada.

The implementation of clonal forestry takes four phases: (1) development of an efficient cloning and cryogenic storage technique; (2) development of superior clonal lines; (3) large-scale mechanized production in a greenhouse; and (4) prudent deployment of tested clonal lines. For each phase, however, there are several technical issues to consider which are discussed in this presentation.

For several conifers, SE is an efficient cloning technique and is sufficiently refined. It has been demonstrated that SE initiation and plant conversion rates are high and SE-derived clonal lines are genetically stable and show no sign of abnormality in the field at age 12. In conjunction with cryopreservation, it is the key technology that makes the practice of clonal forestry possible. Furthermore, SE is the enabling technology for deploying all conifer biotechnology products. The development of superior clonal lines involves the establishment of long-term genetic tests of clonal lines developed by SE, which should be closely aligned with a multi-generation breeding program. The development of semi-mechanized tree propagation by SE is in progress. However, alternatively, the conventional rooting of cuttings from tested donor plants retrieved from cryopreservation may also be used. The deployment of clones in clonal forestry requires careful consideration to balance genetic gain and diversity in the plantation. This involves choosing an appropriate number of clones to deploy, as well as an appropriate configuration for clonal plantations. In New Brunswick, it is proposed to use a mixture of clones and seedlings (MOCAS).

Improvements in Pine Somatic Embryogenesis Tissue Multiplication, Embryo Production and Embryo Harvest for Large-Scale Production And Deployment

M. Kamal Chowdhury, **Michael R. Becwar**[1], Narender S. Nehra, Mary J. Cook, John J. Clark, Jerrin M. Victor, Tim J. Stout, Leshaun M. Eadie and Jessica S. Sage
[1]Science Fellow, ArborGen, PO Box 840001, Summerville SC 29484 USA
mrbecwa@arborgen.com

Somatic embryogenesis (SE) provides an effective method for mass propagation and clonal deployment of genetically improved conifers. This approach involves: 1) genotype capture via SE, 2) cryogenic storage of SE genotypes, 3) concomitant clonal field tests to identify genotypes with traits that impart significant genetic gain over the seedling population, and 4) mass propagation of selected genotypes via SE. Increasing the number of genotypes capable of producing high numbers of somatic embryos has several positive impacts on implementation of clonal forestry in loblolly pine. First, it increases the probability that genotypes selected with desirable traits in clonal field tests will have sufficient embryo production capacity for large-scale deployment directly via SE. Second, it decreases the probability that directional selection might occur if a limited number of genotypes serve as the base population for selection of clones for deployment. Here we describe improvements made at ArborGen in loblolly pine SE, including very rapid multiplication of SE tissue for use in large-scale embryo production, with additional improvements in embryo production, germination and conversion. The combined effect of these improvements ensures that more genotypes qualify for large-scale production and deployment directly via SE.

Manufactured Seed – Low cost Delivery of Somatic Embryos to Nurseries

W.C. Carlson

Weyerhaeuser Company, US

Cost is often the major barrier to implementation of new technology. Manufactured seed and automated processing of somatic embryos offer a low cost method of introducing clonal technology into forest nurseries.

Developing a Commercial Somatic Embryogenesis (SE) Production Platform for Conifers.

S.M. Attree, P. Denchev, L. Kong, I. Lobatcheva, R. Folk, and B. Lawrence**.**

CellFor Inc., PO Box 133, Brentwood Bay, BC V8M 1R3, Canada.

Abstract: CellFor Inc., is a technology company serving the Forest Industry and private land owners. The majority of CellFor's employees are located on Vancouver Island, at our research and production facility. CellFor is the first company to commercialize the conifer somatic embryogenesis (SE) production process. CellFor produced about 2 million bare root somatic seedlings in 2004, and production volumes are expected to double or triple each year for the next few years. This creates significant challenges for CellFor's scientists and staff to identify the problems and bottlenecks associated with producing such volumes. At the same time, costs of production are required to decrease significantly. While some costs can be simply reduced by "economies of scale", there are nonetheless significant challenges to be overcome to reduce direct costs. Plant tissue culture has traditionally been a labor intensive process. Published methods for producing conifer seedlings describe inefficient methods for bulking up tissue, transferring embryos and germinating seedlings *in vitro*, prior to transferring them to the nursery environment. To be efficient it is essential to reduce the labor component of the production process using automation. However, improvements to the overall efficiencies throughout the process, and reducing or eliminating various sources of variability, are also key components. This paper provides an overview of CellFor and the challenges faced, and provides and insight of some of the processes developed to meet these challenges.

Keywords: Somatic embryogenesis, loblolly pine, *Pinus taeda.*

INTRODUCTION AND DISCUSSION

SE of conifers exploits the breeding inefficiencies in conventional seed production systems. As a result, SE can provide more rapid deployment of maximum gain compared to seed orchards. Somatic embryogenesis also provides for greater uniformity, both in tree form as well as wood quality. Somatic embryogenesis is also a platform for the production of transgenic conifers, although CellFor does not do any genetic modification.

The SE process can be broken into two parts, firstly the selection of elite trees from clonal field trials, followed by the multiplication of the elite trees for plantation establishment. The process begins by collecting full-sib seed from selected elite parents to establish numerous embryogenic cultures which are placed in cryogenic storage while the field trails are conducted. Data from the field trials are used to select the most desirable genotypes, which are then removed from cryogenic storage and mass propagated in tissue culture, then grown into seedlings. CellFor is testing field trials of southern pines at multiple sites, some of which are in their fifth year of field testing.

The key requirements of a commercial SE production system are that it should be capable of producing embryos and seedlings in high volume, the embryos should be storable in some way to allow year round somatic embryo production, the process should be cost effective and efficient, the product should be high quality, and the process needs to be reliable and predictable. Traditional SE systems in the literature typically describe somatic embryos with little or no storage ability, the process is very labor intensive. For example, the embryos would require frequent hand transfer to fresh culture medium during bulk-up and maturation, and the mature somatic embryos would be hand transferred to germination trays for *in vitro* germination, then again hand transplanted at the nursery for soil establishment and seedling growth. The high labor input associated with these processes is one of the greatest challenges for large scale production of somatic seedlings of conifers.

The system developed at CellFor scales-up and automates processes and also avoids the comparatively long *in vitro* germination step. For example, in the past at CellFor bulk-up of the embryogenic tissue was done on solidified medium within Petri plates. However, due to the high labor input, this method was feasible for producing only about 0.5 kg of tissue per week. In order to produce tens of millions of somatic seedlings it is necessary to produce many kg of embryogenic tissue per week. Therefore, CellFor has developed methods for producing sufficient tissue using bioreactors ranging is size from 2 to 20 liters. Now many kg of tissue can be produced per week for relatively low labor cost. The culture medium in the bioreactors consists of sugars, salts, and plant growth regulators specifically formulated to stimulate the embryogenic proliferation of the tissue, which consists of immature embryos (Fig. 1). In order to stimulate the production of mature embryos the embryogenic tissue needs to be plated onto fresh medium formulated for that purpose. This is another potentially labor intensive step in the SE production process, in order to prepare the large quantities of culture medium required, and also to manually transfer the tissue to the fresh medium. CellFor has developed automated equipment to complete this process. The equipment consists of a media maker/sterilizer which subsequently pours the media into dishes then dispenses the embryogenic tissue onto the media. The unit can produce sufficient SE maturation cultures to yield 40-60 million seedlings per year, staffed with just 1-2 full time equivalents (FTE's). In the absence of automation, this combined process would require 20 FTE's to achieve the same productivity.

Maturation cultures yielding mature somatic embryos are produced on a daily basis throughout the year. However, seedling production needs to fit within the conventional narrow nursery delivery window for zygotic seedlings. This requires the somatic embryos produced year round need to be stored in some way until required for germination in the Spring. CellFor has developed methods to bulk desiccate somatic embryos (Fig. 2). Desiccation enables long term storage of the mature embryos in a quiescent state similar to mature conifer seed. The desiccated embryos are then placed in frozen storage which promotes long term storage stability of the mature embryos. In the absence of an induced quiescent step, conifer somatic embryos tend to turn green and commence germination leading to lack of synchrony and uniformity. Quiescence of desiccated embryos during long-term storage thus promotes subsequent germination synchrony and uniformity. Desiccation and frozen storage is also simple and inexpensive to perform.

Fig. 1. Immature loblolly pine somatic embryos following bulk-up culture in a bioreactor

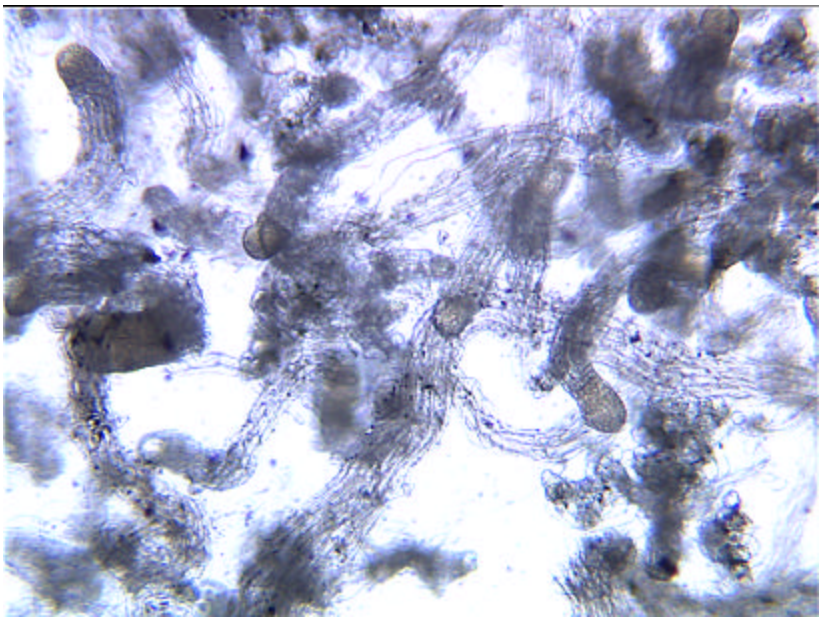

Fig. 2. Bulk desiccated loblolly pine somatic embryos.

A quality control (QC) department is in place at CellFor. The function of the QC department is to assist CellFor in monitoring the production process, to analyze trends and make production projections, and to carry out data mining to provide better tools to control the production process. Thus, QC staff carry out routine assessment of:

- Germinant yield per gram of tissue after desiccation, and monitor conversion frequency for every production line.
- Spot checks of SE moisture content before and after desiccation.
- Genetic fidelity tests after cryopreservation and at the nursery.
- Elemental analysis of chemicals and media
- Screening of tissue and media for the presence of microbial contaminants.

- Environmental monitoring (temperature, light, humidity) in culture rooms and glasshouses.

The data are used for predicting production targets for tissue production, mature embryo counts in inventory, germinant counts, and seedling conversion frequency (shoot and root establishment), in order to accurately deliver the desired seedling volumes. Variability exists in any production process, and SE production is no exception. Variability exists in two types, that within the normal range and that outside the normal range. Variability within the normal range cannot be controlled and is regarded as "noise" By analyzing data collected from the QC tests we hope to understand and control any variability that exists outside the normal range. Variability outside the normal range or control limits (i.e., $\pm$ 3 standard errors) suggests that additional outside factors are influencing the result. Identifying this type of variability allows CellFor identify the factor, which might be a medium error, or equipment breakdown, and take appropriate action. Thus, monitoring many parameters of embryo development gives us a good understanding of what we are producing, and what key factors control the efficiency of the production process.

In the past CellFor used *in vitro* techniques to germinate embryos. This required that mature embryos be hand transferred to containers containing germination medium. The germination cultures were cultured for 8-10 weeks in a temperature and light controlled germination room, prior to hand transplanting the germinated seedlings with root and shoot to soil for acclimation at the greenhouse. This was a very labor intensive step requiring numerous handling steps, and needed to be completed within an 8-10 week period. The germination room at CellFor could hold 1-2 million *in vitro* seedlings. Increasing volumes beyond this required building additional expensive germination rooms, which would only be used for a few weeks per year. To overcome this germination room bottleneck CellFor developed methods to give the desiccated embryos just a short rehydration and pre-germination treatment of about a week. They are then transferred directly to non-sterile *ex vitro* conditions, initially under high humidity, to complete germination and undergo conversion to seedlings. During the greenhouse step, the seedlings are grown in miniplug containers each of about 1 square foot and containing 400 cavities. The miniplugs allow the greenhouse nursery space to be maximized during the acclimation period, so reducing overhead costs. The miniplugs also give high survival of seedlings and allow for easy consolidation of empty cavities, which is important for subsequent transplantation to the bare root beds.

Transfer to the bare root beds from the miniplug trays is achieved using an automated transplanter. The seedlings are transplanted into the beds at a rate of about 70,000 per hour, and are arranged in conventional rows consisting of 8 seedlings wide (Fig. 3). The seedlings are lifted at the end of the year using standard tractors and lifting equipment used for lifting conventional seedlings. The seedlings are of excellent quality and show good root collar diameter, a well developed tap root, and extensive mycorrhizal development. The seedlings are packaged in conventional bags and labeled with their specific varietal identification number, before being shipped to the plantation site. CellFor seedlings have been shipped to a number of operational plantation sites, the largest of which is currently around 300 acres.

Fig. 3. Bare root bed containing approximately 1.3 million CellFor somatic seedlings.

CONCLUSION

CellFor has developed processes that enables production volumes in the order of tens of millions of seedlings to be achieved. Mature somatic embryos produced by our process are extremely desiccation tolerant allowing year round production and storage. Automation methods and equipment are reducing labor requirements, so helping to reduce costs. The somatic seedlings in the bare root nursery are generated from elite parents and have been further selected based upon field performance. The seedlings show normal healthy growth in the nursery, and so are of excellent quality. Finally, the QC function developed at CellFor allows a more reliable and predictable process to be put in place. Since 2000, CellFor's production volumes for loblolly pine have increased from tens of thousands, through hundreds of thousands, to several millions. At the same time direct costs of production per seedling have fallen several fold. This has largely been accomplished by automation improvements which have led to large improvements in lab productivity. Thus, during 2000 CellFor only produced tens of thousands of seedlings for every FTE working in the production lab. This number is currently hundreds of thousands of seedlings for every FTE. Moving forward, CellFor intends to produce tens of million, and ultimately hundreds of millions of seedlings per year. This will be achieved by further refinements of the processes described here and within the next 12 months should exceed a productivity of over 1 million delivered bare root seedlings per lab FTE.

Reconstructing A Genetic History For *Pinus taeda*

C.G. Williams[1] S. L. LaDeau[1], S. Anderson [1,2], M. A. Al-Rababah[3], J. L. Hamrick[4]

[1/] Duke University, Nicholas School of the Environment & Earth Sciences, Durham NC, USA; [2/] Forest History Society; [3/] Jordan University of Science and Technology, Irbid, Jordan and [4/] University of Georgia, Department of Botany, Athens GA, USA

Combining phylogeography with population dynamics on a forest landscape scale opens some interesting questions about how selective forces, geological events and recent settlement history shape present-day population structure. *Pinus taeda* is ideal as a case study because of its continuous distribution throughout the southern United States. We have begun to piece together the genetic history of *Pinus taeda* along various spatial and temporal scales. DNA analysis of population expansion and contractions during Holocene climate change, presettlement forests and old-field succession show present-day patterns of genetic variation in *Pinus taeda* have been shaped by climate change, human disturbance, slow onset of reproduction and an outcrossing mating system.

Defining Pollen Structure as the Probability of Paternal Identity

P.E. Smouse[1,2] and J.J. Robledo-Arnuncio[1,2,3]

The pollen structure of forest tree populations is a matter of growing interest, particularly in seed orchards, where - for production purposes - we would prefer most pollination to be from within the orchard, or in small population isolates, where - for conservation purposes - we would prefer most pollen from external sources. In either case, an ability to assess the *pollen structure* of the population is as useful as an estimate of the rate of pollen immigration. Our objectives here were to: (1) develop estimation methods for the probability of paternal identity (PPI), using a set of direct paternity designations of individual seedlings, and (2) illustrate the new methods with a set of microsatellite data from a small relict population of *Pinus sylvestris* from Coca, Spain, inferring the degree of pollen structure for individual mothers and for the population as a whole.

The Theory

Imagine a set of G seed parents (mothers); the g^{th} mother has a sample size of n offspring. There are K potential pollen donors (fathers). Denote the number of offspring for the g^{th} mother who have the k^{th} (father) as x_{gk}. The fraction of offspring for the g^{th} sibship, fathered by the k^{th} father is $p_{gk} = (x_{gk} \div n)$. The obvious estimate of PPI is provided by $q_{gg} = \Sigma_k p^2_{gk}$, the probability of randomly drawing the same father twice for a pair of offspring from the g^{th} mother. Here, we introduce an alternative estimator, $r_{gg} = (nq_{gg}–1) \div (n–1)$, equal to the observed rate of paternal matching for two offspring, drawn without replacement from among the n offspring of the g^{th} mother. The estimate q_{gg} is biased, and bias (β) increases as the number of offspring per mother (n) becomes small, and is largest when there are many fathers with evenly distributed paternity (small PPI). The paternal matching estimate (r_{gg}) is unbiased, and is preferable on that basis. It develops that the variance of q_{gg} is smaller than that of r_{gg}, and on that basis, we should prefer q_{gg}. Combining squared bias (β^2) and variance (σ^2) into the mean squared error (MSE = $\beta^2 + \sigma^2$), we discover that r_{gg} is the superior estimator when PPI < 0.40 and that q_{gg} is superior for PPI ≥ 0.40. The literature (see Smouse and Sork, 2004) suggests that PPI < 0.40 in most cases.

We can also measure PPI for the g^{th} and h^{th} sibships, r_{gh}, the rate of paternal sharing between mothers g and h. We can construct a G x G matrix (**R**) of paternal sharing, listing the within-mother values down the diagonal and between- mother values off the diagonal, and can translate the entries of **R** into an estimate of PPI for the population, i.e., $R_0 = (n–1)[\, r_0 + G\bar{r}_{gh} \,] \div (Gn –1)$, with r_0 and $\bar{r}_{gh}$ being the average within-mother and between-mother paternal matching rates, respectively. R_0 represents uneven male reproductive contributions for the entire population.

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[1] Professor and Postdoctoral Associate, respectively, Department of Ecology, Evolution and Natural Resources, Rutgers University, New Brunswick, NJ 08901 USA

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Pinus sylvestris

Pinus sylvestris is a monoecious, wind-pollinated, mostly outcrossing conifer. The species reaches the southern limit of its distribution in Spain. There is a small population at Coca, an isolated remnant of a more widespread population that grew in the Northern Meseta Plains. Holocene climatic warming and recent human-mediated lowering of the water table have reduced the population to its present size of 36 adults. The adults are scattered through a larger, continuous population of *Pinus pinaster* woodland, over approximately 15 ha (~ 2.4 trees/ha), with average inter-tree spacing of 182 m. The closest conspecific population is 30 km away.

Robledo-Arnuncio and Gil (2004) have used four chloroplast and two nuclear microsatellite loci to determine the paternal genotype of 813 open-pollinated seeds collected from 34 out of 36 trees in the stand (22-24 seeds per tree), with two trees contributing no seed. The expected paternal exclusion probability for the six-marker set was 0.996, but each of the potential fathers was uniquely identifiable, and it was possible to assign paternity categorically to exactly one of the 36 resident fathers for 778 of the 813 offspring (95.7%). Those same 36 resident males were categorically excluded as the fathers of the remaining 35 offspring (4.3%), assignable to at least 30 different paternal donor genotypes, all external to Coca.

The PPI estimate for the average mother is $r_0 = 0.317$, translating into an average single-mother estimate of the effective number of pollen donors, $N_{ep} = 3.15$; mating within the population is far from panmictic. The PPI-value for the entire sample of offspring from the 34 mothers is much smaller, $R_0 = 0.0425$, indicating that the pollen pool for the population is more diverse than that for any particular mother. There is, of course, paternal sharing among mothers ($\bar{r}_{gh} = 0.035$). Allowing for the overlap, the effective number of pollen donors for the entire population is $N_{\bullet p} \sim 23.5$, about (2/3) of the total adult tally. The local fathers contribute unequally.

Discussion

For convenience of illustration, we suppressed 35 offspring that were not compatible with any of the resident males. To include them in the characterization, we increased the number of potential fathers from $K = 36$ to $K = 66$, and repeated the analysis above. Comparing the results, we obtain $r_0 = 0.317$ and $R_0 = 0.0425$ (ignoring the immigrants) and $r_0 = 0.294$ and $R_0 = 0.0393$ (allowing for immigrants). Here, inference is affected little by a low rate of pollen inflow, but this is an atypically low rate for a forest tree population, and we more usually find a substantial fraction of offspring that cannot be assigned to any of the identifiable males within the stand (see Smouse and Sork, 2004). In general, ignoring the progeny sired by external males will bias the results.

For many studies, especially with mendelian nuclear genes, paternal designation is inherently non-categorical. There are two options: (a) assign the offspring to that male genotype with the greatest likelihood of having produced the offspring, contingent on that likelihood being much higher than those of all other genotypes, or (b) assign paternity proportionally to all of the credible candidates, in proportion to their relative likelihood values. Strategy (a) has the advantage that we can choose the most likely candidate, but it leads to biased estimates of the x_{gk}'s, which will bias the PPI estimates in turn. If the threshold value is sufficiently stringent, the

bias is tolerable for any given offspring, but virtually every offspring yields such an outcome, and the cumulative impact can be non-trivial. Strategy (b) is less biased, but requires more bookkeeping and computation. It is possible to convert the resulting non-integer x_{gk}-values into estimates of PPI, but available algorithms will need further refinement. When pollen immigration is substantial, the estimate becomes as much 'adjustment' as it is 'result'.

In those situations where accurate paternal inference is not feasible, either because the genetic battery provides insufficient resolution or because the spatial scale of pollination is too large to allow genetic assay of the majority of fathers, many of the unassigned offspring have probably been sired by external males. Ignoring the outside fathers leads to serious biases in PPI estimates. In such situations, we must rely on one of three indirect methods of estimation: (*i*) TWOGENER analysis (Smouse et al., 2001), (*ii*) MLTR analysis (Ritland, 2002), or (*iii*) Relationship Metric analysis (Hardy et al., 2004). Each method has its own strengths and weaknesses, but all three lead to estimates that are empirically comparable to that gained from the PPI method.

Conclusions

We have developed estimates of the probability of paternal identity (PPI) for the case where the vast majority of male parentage can be specified unambiguously. We show that the obvious estimate can be severely biased under usual sampling conditions, and develop an alternative that is unbiased and that has smaller MSE for the frequent cases in which paternal contributions are not severely skewed (PPI < 0.4). The pattern of paternal identity can be captured in a G x G matrix of paternal matching fractions, $\mathbf{R} = \{r_{gh}\}$, and translated into statements about the average within-mother and total population rates of PPI, as well as a statement of the rate of inter-mother paternal matching, providing a detailed picture of the 'pollen structure' of the population.

The ability to assess contemporary pollen immigration and internal 'structure' is important for both seed orchard and conservation studies of plant species. Increasingly powerful molecular assay methods have improved our genetic resolution sufficiently to place compelling biparental designation almost within reach. We require new statistical methods, such as those described here, to take advantage of the emerging data and to translate the molecular assays into both accurate and precise inference on the mating patterns of our forest tree populations.

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Importance of Timing in Pollen Competition in Northern Scots Pine

S. Varis[1], A. Pakkanen[1], A. Santanen[1], T. Häggvik[1], A. Pappinen[2], and P. Pulkkinen[1]

Darwin (1871) defined sexual selection as a sexual competition among the members of one sex, usually male, to mate with individuals of another sex, usually female. There also has to be preferences of one sex, usually female, for particular mates. Sexual selection in plants has been under study and there have been results for and against it (reviewed in Skogsmyr & Lankinen, 2002). In plants sexual selection is thought to be pollen based.

We are investigating the mechanism and the consequences of post-pollination selection in northern populations of Scots pine (*Pinus sylvestris* L.). Our aim is to analyze the significance of pollen (based) selection during pollination and fertilization, and female selection at the polyembryonic stage in different environmental conditions. The results will be combined with those from studies of adaptive variation and prepollination selection in northern pines. Our major hypothesis is that the origin of pollen is connected to the seed siring success resulting genetically different offspring in different environmental conditions. In cases when the amount of gene flow is considerable the male gamete selection during the generative cycle and the possible consequences of different selection mechanisms for adaptation may provide an effective mechanism with which the population can rapidly react to changing environmental conditions such as global warming.

Gene flow is possible between populations by pollen migration. It requires medium or long distance pollination and transported pollen has to have competition ability against local pollen during the pollination and/or fertilization phases. To make pollen competition possible pollen from more than one donor must be deposited on pollen chamber of female flower.

Scots pine is a wind pollinated and monoecious plant and windblown pollen can drift hundreds of kilometers due to wing-like structures in pollen. Pollen chamber has room for 1-5 pollen grains (Sarvas 1962). Usually more than one egg cell develops in female gametophyte (most often two). Female flowering in Scots pine often begins 0.5 to 1 day before male flowering on the same tree individual and flowering starts earlier in southern latitudes. Thus pollen from south can be in leading position already in the beginning of the pollen competition. Sarvas even says that there does not exist other selection in Scots pine than that based on timing of the pollen in female flower. In this study we wanted to test the hypothesis that first pollen grain in the female flower is the winner of the pollen competition (First in first served -theory).

[1] Post Graduate, Forest Breeding Officer, Researcher, Graduate Student and Senior Researcher, respectively. Finnish Forest Research Institute
[2] Researcher, University of Helsinki

MATERIALS AND METHODS

We performed artificial pollinations where pollen from one genotype originated from southern or northern Finland were injected two hours before pollen originated from northern or southern Finland. Pollen were collected from seed orchards: southern pollen was collected from Korpilahti lat. 62°N (1150 d.d.) and northern pollen from Rovaniemi lat. 66°N (900d.d.). We used 3 pollen genotypes from south and 3 from north in these crosses. Pollen genotypes were chosen based on approximately equal viability in germination tests. Seven mother genotypes were used and they were originated from southern Finland lat. 62°N. We used grafts which were outdoors in southern Finland at lat. 60.5°N. The female flowers were capped with pollination bags before they came receptive and pollination were done using a syringe. There were 1-5 flowers in each bag. The crosses were performed on May 2001.

Paternity analysis from maturated seeds were done with 2-3 different microsatellite loci. Primers were labeled with an infrared dye and the PCR reaction products were separated on a 6% acrylamide gel using semiautomatic gel electrophoresis. Electrophoretic patterns were analyzed with help of a special computer program.

RESULTS AND DISCUSSION

We analyzed 346 seeds from crosses where southern pollen was injected first. Total numbers of seeds sired by southern pollen was 192 and by northern pollen 154 and thus the ratio is 55:45 which is significantly different from expected 50:50 ratio ($p<0.05$). From crosses where northern pollen was injected first we analyzed 357 seeds. Numbers of seeds sired by northern pollen was 263 and by southern pollen 94 and ratio 74:26 is significantly different from expected 50:50 ratio ($p=0.000$).

First in pollen is most often but not always first served. Thus timing of pollen in flower is not the only selection mechanism in Scots pine like Sarvas wrote. Interesting question is why northern pollen is capable to sire so much more seeds when injected first than southern one when it is injected first? Is there differences in the size of pollen grains between southern and northern pollen and that affects the pollen competition? In north pollen has to be adapted for shorter growing season and maybe northern pollen just grows its pollen tube faster than southern pollen.

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The International *Eucalyptus* Genome Consortium (I*Eu*GC): Opportunities and resources for collaborative genome research in *Eucalyptus*

A.A. Myburg[1], I*Eu*GC members

[1]Senior Lecturer, Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

ABSTRACT

Fast-growing eucalypt tree species and hybrids constitute the most widely planted exotic hardwoods on earth. Despite the commercial importance of *Eucalyptus* tree species and great potential for application of tree biotechnology in eucalypt breeding programs, the international *Eucalyptus* genome research community is poorly organised. Most existing resources for genomic research (cDNA libraries, microarrays etc) in *Eucalyptus* were developed through private ventures and are not available to the international community. At a recent Eucalypt Genome Meeting hosted by the CRC for Sustainable Production Forestry (Hobart, Australia) in collaboration with the University of Tasmania, Southern Cross University and the Australian Genome Research Facility, an international group of eucalypt researchers agreed to form the International *Eucalyptus* Genome Consortium (I*Eu*GC, www.ieugc.up.ac.za). Important short-term objectives of this consortium were to pursue funding (approximately U$ 25 million) for the sequencing of the genome of *E. globulus,* and to create a platform for collaborative *Eucalyptus* genome research. The availability of the *Eucalyptus* genome sequence was envisioned to have a profound impact on research in the following areas: The evolution of woody plant genomes, the conservation and management of internationally valuable eucalypt genetic resources, the impact of environmental changes on native populations of *Eucalyptus*, the genetic control of complex developmental processes such as wood formation, disease resistance and abiotic stress tolerance, and the development of novel, genome-directed breeding technologies for superior *Eucalyptus* plantations. Scientists attending the first meeting of the I*Eu*GC agreed on the following principles: all genomic resources generated through the consortium should be fully available in the public domain; funding and in-kind contributions will be actively sought by consortium members from public and private sources, and, as a non-competitive effort, training, scientific exchange, and capacity building will be important objectives of the I*Eu*GC.

BACKGROUND

Most commercial crops such as maize, wheat, rice, and even poplar have benefited tremendously from public genome research. Public resources for these crops have allowed researchers at universities and other public institutions to establish large collaborative research efforts and leverage even larger public investment in their favourite crops. More importantly, the availability of public genome resources has attracted some of the brightest young researchers to these crops, ensuring the long-term stability and vibrancy of their research communities. Despite the considerable commercial importance of *Eucalyptus* plantation forestry and a small number of well-organised national programmes (e.g. Genolyptus, Brazil), the international *Eucalyptus* research community is poorly organised and has not yet enjoyed the full benefits of public genome research. This has forced privately supported *Eucalyptus* research groups to do much of

the basic, descriptive research that could be performed in the public domain, using resources that would be much better spent on applied research.

The International *Eucalyptus* Genome Consortium was established to provide a forum for scientific exchange and collaborative research that will lead to the creation of a series of public genome resources and a vibrant research community able to utilize these resources. The first I*Eu*GC meeting in Hobart, Australia was followed up by a business meeting concurrent with the recent IUFRO *Eucalyptus* Breeding and Silviculture conference ("*Eucalyptus* in a changing world", 11-15 October 2004, Aveiro, Portugal). At this meeting it was announced that the current fund-raising effort lead by the CRC for Sustainable Production Forestry (Hobart, Australia) and the University of Tasmania was not successful. It was suggested that the *Eucalyptus* Genome Consortium be hosted within a working party of IUFRO, and that members collaborate to pursue international funding for the establishment of shared resources such as a BAC library and physical map of the *Eucalyptus* genome, *Eucalyptus* microarrays and a large set of shared microsatellite markers.

More recently, it has come to light that Kazusa DNA Research Institute (Kisarazu, Chiba, Japan) has started a major genome sequencing effort in *E. camaldulensis*. This effort was initiated by researchers in Oji Paper more than 12 months ago, but is largely supported by public funding. The fully annotated genome sequence (5x coverage) will therefore be available in a public database, which is scheduled to be released in April 2006 (T. Hibino, pers. commun.). This exciting development makes it even more urgent for the eucalypt research community to get organised and establish shared resources that can be used to extrapolate genome information from *E. camaldulensis* to other eucalypt species and hybrids.

For more information on the International *Eucalyptus* Genome Consortium, please visit www.ieugc.up.ac.za.

An Integrated Approach for Restoring Butternut to Eastern North American Forests

S. E. Schlarbaum[1], R. L. Anderson[2], M. E. Ostry[3], S. L. Brosi[1], L. M. Thompson[1], S. L. Clark[1], F. T. van Manen[4], P. C. Spaine[5], C. Young[6], S. A. Anagnostakis[7], and E. A. Brantley[8]

INTRODUCTION

Butternut (*Juglans cinerea* L.) is a medium-sized hardwood species native to the eastern forests of North America and closely related to black walnut (*Juglans nigra* L.). Butternut populations have been dramatically declining because of an exotic fungal pathogen (*Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, and Kuntz) causing butternut canker disease. Because healthy trees have been found in close proximity to dead or dying trees, researchers speculate that natural resistance to the disease may exist, offering hope for restoration efforts. A successful restoration program for butternut will require genetically diverse germplasm, an understanding of site requirements, the ability to transfer resistance, and an understanding of the role of environmental conditions in disease development. We are addressing restoration objectives using on an integrated approach. We have developed a geographic information system (GIS) model to locate putative resistant trees more efficiently and determine restoration habitat. Field plantings have been established to determine genetic differences in resistance and to select resistant genotypes for breeding. Dendroecology is being used to understand conditions that promote canker development and refine management practices in butternut restoration efforts.

MATERIALS AND METHODS

Habitat Modeling

We used GIS technology to develop a series of multivariate models to predict the occurrence of butternut trees in the southern Blue Ridge Mountains (SBRM; van Manen et al. 2002) and Mammoth Cave National Park (MACA) in Kentucky. Habitat characteristics of 134 known butternut locations in Great Smoky Mountains National Park and 24 locations in MACA were used in combination with a suite of topographic and land use data layers to calculate an index of habitat suitability based on Mahalanobis distance (D^2). Test plots were created for both SBRM (n = 130) and MACA (n = 125) and field researchers noted presence or absence of the species in

[1]James R. Cox Professor, Graduate Research Assistant, Research Associate, Postdoctoral Research Associate, respectively, Department of Forestry Wildlife and Fisheries, University of Tennessee, Knoxville, TN 37966, USA; [2]Consulting Forest Pathologist, 7899 Country Rd. 3950, West Plains, MO 65775, USA; [3]Research Plant Pathologist, North Central Research Station, USDA Forest Service, 1561 Lindig Ave. St. Paul, MN 55108, USA; [4]Research Ecologist, Southern Appalachian Field Branch, U. S. Geological Survey, Knoxville, TN 37966, USA; [5]Research Plant Pathologist, Southern Research Station, USDA Forest Service, 320 Green Street, Athens, GA 30602, USA; [6]Resistance Screening Center Manager, Forest Health Protection, USDA Forest Service, 1579 Brevard Road, Asheville, NC 28806; [7]Agricultural Scientist, Connecticut Experiment Station, Box 110, New Haven, CT 06504, USA; [8]Instructor of Forestry, Pennsylvania State University – Mont Alto, One Campus Drive, Mont Alto, PN 17237, USA

each of those plots. To assess model validity in SBRM, we used a logistic regression to determine the relationship between butternut occurrence and the D^2 values of the pixels corresponding to the test plots. Cumulative frequency distributions were constructed to test the MACA model. D^2 values of all butternut locations in MACA were compared with those of 125 random locations (null model) to determine a D^2 cutoff value. A bootstrapping technique was used to calculate D^2 for each butternut location and determine the percentage of trees that fell at or below the cutoff D^2 value, with a low percentage indicating poor model performance. Finally, because butternut is a semi-intolerant species, we extended the utility of the MACA model by delineating potential butternut restoration sites using a mid-infrared-corrected normalized difference vegetation index layer as a filter, which helped identify low canopy closure areas.

Resistance Screening Tests

In 1995, two butternut open-pollinated progeny tests were established, using 36 open-pollinated families. One test was planted in an open field, and the other test was established under the canopy of trees with butternut canker disease to expose seedlings to heavy disease pressure. These tests were periodically evaluated for survival, growth and disease resistance as indicated by canker development. In 2002, another series of butternut seedlings were established under butternut trees for resistance screening at multiple locations using 41 open-pollinated families from southern Appalachian provenances. Over two-thousand butternut seedlings were planted at twelve locations, ten in Tennessee and adjacent states (Virginia, North Carolina, Kentucky), one in a western section of the range (Missouri), and one in a northern section of the range (Connecticut). The seedlings were planted in an incomplete block within complete block, design using single-tree family plots. The seedlings are being evaluated at the beginning and end of the dormant season for differences in cankering, survival, and growth.

Dendrochronology

Two areas in the southeastern United States that contain surviving butternut populations were sampled for dendrolecological studies. Butternuts were found growing in the Clinch Ranger District, Jefferson National Forest, which was clearcut in the early 1970s, and a private forest holding near Smithville, TN that has not been logged in recent years. Increment cores were obtained from live butternuts at both sites. At the Clinch site only, cross-sections from the base of dead butternut trees were removed (n = 5), and cores were taken from competing species located in plots positioned around groups of live butternut trees. Tree-rings from cores and cross-sections were examined under a stereomicroscope and butternut tree chronologies were created for each stand. Cross-sections were cross-dated with live butternut tree chronologies. Weather data from weather stations near the Clinch site were averaged and correlated with the butternut chronology.

RESULTS AND DISCUSSION

Habitat Modeling

The model for the SBRM correctly predicted 16 of the 130 test plots would contain butternut. A logistic regression indicated a significant negative association between D^2 and presence of butternut (parameter estimate = -0.162, P = 0.007). Four of the 125 MACA test plots contained butternut and the cumulative frequency distributions showed that the most favorable habitats were defined by D^2 values =20.5. Eighty-three percent of the butternut locations were below the

D^2 value of 20.5, representing only 20.0% of the study area. Areas with the most favorable conditions for potential butternut establishment or restoration were limited to 152.1 ha. The results of these studies provide a useful reference on the amount and location of favorable butternut habitat in SBRM and MACA and can be used to identify priority areas for future butternut restoration. Populations on the fringe of the natural range and putatively resistant genotypes will be grafted and integrated into a breeding/conservation program. Habitat modeling has been successful in locating restoration sites and additional trees to provide material for resistance testing, breeding, and eventual reintroduction.

Resistance Screening Tests

The 1995 tests grown under a canopy of diseased trees showed significant family differences in disease development across family of origin. Individual trees that were found to be free or nearly free from disease were selected for incorporation into a breeding program. In contrast, the planting in an open field did not have enough disease pressure to have family differences. The 2002 tests did not show significant differences among families in cankering at age two, indicating that the plantings are still too young to have meaningful infection or that the disease pressure was relatively minor during the first two years of growth. The plantings had 4 percent mortality after the first growing season, attributed to transplanting shock. In 2003, three planting locations were lost due to fire and possible root rot disease. Mean survival on was 75 percent and varied according to planting site (51 to 99%), provenance (36 to 83%), and family (21 to 100%). In the remaining plantings, mean height was 100 cm and varied significantly according to planting sites (45 to 126 cm), provenances (71–104 cm), and genetic families (27-149 cm). The surviving plantings will continue to be monitored for growth and disease.

Dendrochronology

Butternuts were capable of living for at least 70 years at the Smithville site. None of the dead trees from the Clinch lived longer than 31 years before death, far before achieving maximum longevity. Age and diameter structure revealed that butternuts were the oldest and largest trees at the Clinch district. Butternut recruitment decreased over time with low recruitment 10 years after stand initiation. Butternut tree-ring widths were correlated with total precipitation (R = 0.41). Results show that butternuts are potentially a pioneer species, which regenerates immediately following a regeneration cut. Annual growth of butternuts is dependent on amount of rainfall during the growing season. Our results provide some evidence that the butternut canker and competition from other species is leading to premature death of butternut trees in these stands.

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Application of Genomics in Conservation of Tropical Forest Species

Wickneswari R. and Choong C.Y. [1]

In many countries in tropical Asia and the Pacific, both the rapid loss and degradation of forests and associated genetic resources are posing a serious problem. Asia and the Pacific region occupies about seventy percent of the world production of tropical industrial roundwood besides harbouring a rich and diverse array of plants, animals and microorganisms. The timber species are mostly composed of useful indigenous species from natural stands. Several steps have been undertaken to alleviate the problem such as embarking on reforestation programs and designating forest reserves and national parks in which felling is limited or prohibited. Some efforts have been made by national and international research organisations, individually and collectively, in the basic studies of genetic conservation such as reproductive biology and genetic variation of important tree species, ecology of tropical rainforests, and management systems for natural forests. Whilst usage of molecular markers provides a better understanding of the genetic structure and mating systems of populations, there exists a knowledge gap between these genetic markers and the expression of adaptive traits among individuals, families and populations. In this paper, we explore and report some examples of DNA-based systems for gene expression analysis for the understanding of gene expression in different tissues and response to biotic and abiotic stresses which can provide a direct link between genotype and phenotype. Genetic mapping of quantitative trait loci (QTL) especially loci which are involved in response to biotic and abiotic stresses will enable rapid assessment of ecosystem integrity and sustainable management of ecosystems.

Keywords: genomics, EST analysis, genetic diversity, tropical forest species, conservation.

INTRODUCTION

In many countries in tropical Asia and the Pacific, both the rapid loss and degradation of forests and associated genetic resources are posing a serious problem. Asia and the Pacific region account for about seventy percent of the world production of tropical industrial roundwood and harbour a rich and diverse array of plants, animals and microorganisms. The majority of timber species harvested are indigenous species from natural stands. According to FAO, 40% of tropical forests have been lost, with tropical Asia and the Pacific recording the highest harvesting rate.

Several steps have been taken to alleviate the above problem including reforestation programs and designating forest reserves and national parks in which felling is limited or prohibited. Some efforts have been made by national and international research organisations, individually and collectively, in basic studies of genetic conservation such as reproductive biology, genetic

[1] Associate Professor and Lecturer respectively, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia. Email: wicki@pkrisc.cc.ukm.my

variation of important tree species, ecology of tropical rainforests and the development of management systems for natural forests.

In spite of these efforts and warnings, the extent and quality of populations of indigenous commercial species in the tropical production forests (which now occupy a large part of the remaining tropical rainforests) are still diminishing. The timber of these species, especially members of the Dipterocarpaceae and Leguminosae, is particularly important for timber production. The decrease and degradation of their genetic resources will make it difficult to secure the breeding populations and planting stocks for sustained production of the valuable timber. Harvesting practices that do not result in the retention of superior phenotypes as seed trees after a logging operation may further aggravate the loss of superior genotypes of valuable timber species.

Maintenance of genetic diversity in forest tree populations that are undergoing population changes due to natural or human-induced events is seen to be instrumental for adaptability and continued evolution (Muller-Starck 1985; Gregorius *et al*. 1985; Ledig 1988; Namkoong 1991; Muller-Starck *et al*. 1992; Bush and Smouse 1992). Whilst usage of molecular markers provides a better understanding of the genetic structure and mating systems of populations, there exists a knowledge gap between these genetic markers and the expression of adaptive traits among individuals, families and populations. In this paper, we explore and report some examples of DNA-based systems for gene expression analysis for the understanding of gene expression in different tissues and response to biotic and abiotic stresses which can provide a direct link between genotype and phenotype. Genetic mapping of quantitative trait loci (QTL) especially loci which are involved in response to biotic and abiotic stresses will enable rapid assessment of ecosystem integrity and sustainable management of ecosystems.

GENETIC DIVERSITY OF TROPICAL FOREST SPECIES

Traditionally, genetic variation of tree species has been understood through assessment of survival and growth performance parameters in common garden-type trials such as species, provenance and progeny tests. Notwithstanding the long term benefits of such activities in terms of availability of plant materials for continuous assessments and evaluation of genotype x environment interactions, these exercises can be time-consuming, labour intensive and costly. Molecular markers offer quick detection of genetic variation and characterisation of genotypes. These markers include isozymes, Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Directed Amplification of Minisatellite DNA (DAMD), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs). Some of these markers can be effectively used in early selection for traits such as hybridity, though for other quantitative traits such as disease resistance and wood quality, associations need to be demonstrated with data from well designed and replicated field trials.

A number of provenance trials and comprehensive studies on genetic structure of natural populations of commercial tropical timber species have been conducted in the past two decades. The large scale provenance trials include *Acacia mangium* (Harwood and Williams 1992), *A. auriculiformis* (Kamis Awang 1995) and *Tectona grandis* (Wyatt-Smith 1961). Results from these studies have been utilised to some extent for the production of genetically improved

planting materials for plantation establishment in Indonesia, India, Malaysia, Thailand and Vietnam. In the last decade or so molecular markers have been employed to understand the genetic structure of natural populations of *A. mangium* (Moran *et al.* 1989; Butcher *et al.* 1998), *A. auriculiformis* (Wickneswari and Norwati 1993), *Pinus merkusii* (Szmidt *et al*. 1996), *Dalbergia cochinchinensis* (Soonhuae *et al*. 1995), *P. kesiya* (Boyle *et al*. 1990), *Pterocarpus macrocarpus* (Liengsiri *et al*. 1995), *T. grandis* (Kertadikara and Prat 1995; Chantragoon and Schmidt 2000), *Shorea leprosula* (Lee *et al.* 2000a), *S. macrophylla* (Kanzaki *et al*. 1996), *Stemonoporus oblongifolius* (Murawski and Bawa 1994), *Dryobalanops aromatica* (Lee *et al*. 2000b; Lim *et al.* 2002) and *Hopea odorata* (Wickneswari *et al.* 1995). The genetic diversity parameters assessed in some of these species are summarized in Table 1.

Table 1. Genetic diversity of some tropical forest tree species.

Species	**No. of populations**	**Genetic marker used**	**H_e**	**G_{st}/F_{st}**	**t_m**	**References**
A. mangium	11	isozymes	0.017	0.311	high	Moran *et al* 1989
A. mangium	10	RFLPs	0.131	0.331	-	Butcher *et al*. 1998
A. auriculiformis	18	isozymes	0.081	0.270	0.67-0.95	Wickneswari and Norwati 1993; Wickneswari and Norwati 1995
P. merkusii	11	isozymes	0.058	0.104	-	Szmidt *et al*. 1996
P. kesiya		isozymes			0.68-0.97	Boyle *et al*. 1990; Boyle *et al*. 1991
P. macrocarpus		isozymes			-	Liengsiri et al. 1995
T. grandis	8	isozymes	0.362	0.190	-	Kertadikara and Prat 1995
S. leprosula	8	isozymes	0.369	0.117	0.55-1.00	Lee *et al.* 2000a; Lee *et al*. 2000c
S. oblongifolius	4	isozymes	0.282	0.163	0.84	Murawski and Bawa 1994
D. aromatica	10	isozymes	0.459	0.036	0.55-0.92	Lee *et al*. 2000b; Lee 2000
D. aromatica	5	SSRs	0.709	0.067	-	Lim *et al*. 2002
H. odorata	6	isozymes	0.190	-	-	Wickneswari *et al.* 1995

H_e = Expected heterozygosity, G_{st}/F_{st} = Genetic differentiation, t_m = multilocus outcrossing rate

A survey of genetic diversity studies in Asia reveals three major shortcomings:

1. Species choice. Since most species used for such research are selected on the basis of combining economic value with ecological interest, the total number of species studied is quite low, reflecting the limited number of species used for commercial planting. Many of the species used in commercial planting represent a small number of families. For example, *A. auriculiformis*, *A. mangium*, *P. macrocarpus* and *D. cochinchinensis* are all members of the legume family. Patterns of genetic variation observed for species from

the same family might be expected to be more similar than for species from different families, so extrapolation to other species may be difficult. *Pinus kesiya*, being a conifer, is likely to be representative of conifers in general, but not necessarily representative of co-distributed species.

2. Geographic sample distribution. The examples of Dipterocarpaceae given in Table 1 represent studies of rather limited portions of the species' natural distribution. The main reason for this has been the difficulty, at least until very recently, of acquiring samples from outside the country in which the research is being conducted. Countries of mainland Southeast Asia suffered extensive unrest and poor political relations during the last few decades of the 20th century, making cooperation in research very difficult.

3. Pollen and seed dispersal. The relative contribution of pollen and seed dispersal to gene flow is not well understood in most tropical tree species. Hence, the potential of species to maintain genetic diversity cannot be predicted in disturbed forests. This information is important for determining the size of buffer zones and conservation areas.

Recently the first of these shortcomings has begun to be addressed with broad-based studies in which diversity of species were selected, as opposed to only economically valuable species. For example, in a project funded by CIFOR and IPGRI, research conducted in two forest reserves in Peninsular Malaysia (Pasoh and Serting Tambahan), focused on eight species: *Shorea leprosula, Scaphium macropodum, Parkia speciosa, Daemonorops verticillaris, Garcinia malaccensis, Calophyllum ferrugineum* var. *oblongifolium, Labisia pumila* and *Tectaria singaporeana*. Of these, only two are commercially logged tree species (*Shorea leprosula* and *Scaphium macropodum*), and one other species is valued for its seeds (*P. speciosa*). Loss of genetic diversity immediately after logging was higher in commercial timber species of low abundance (9.4% loss in heterozygosity and 25.0% loss in alleles for *Shorea leprosula*) than that in species of high abundance (5.0% loss in heterozygosity and 7.7% loss in alleles for *Scaphium macropodum*) in the ridge forest management unit indicating the need to examine regeneration status for timber species of low abundance before permitting logging of these species (Wickneswari *et al*. 2000). Genetic diversity in regenerated stands is generally maintained after a single low intensity logging event. Species' vulnerability to the threat of genetic erosion posed by selective logging is highly correlated with its abundance in a particular forest management unit. Tree density for the species can be a useful indicator in reflecting the risk of genetic erosion rather the overall disturbance level based on reduction in basal area of all trees.

At two sites in Thailand (Huay Kha Khaeng Wildlife Sanctuary and Sakaerat Biosphere Reserve), six species were studied: *Dipterocarpus obtusifolius, Shorea siamensis, Pterocarpus macrocarpus, Sindora siamensis, Mytragina brunosis,* and *Cycad siamensis*. Again, these represent a mixture of commercial (*Shorea siamensis* and *Pterocarpus macrocarpus*) and non-commercial species, and include a non-tree species (*Cycad siamensis*). Differences in the pattern of genetic diversity along disturbance gradients were related to the species reproductive ecology, and, to a lesser extent, the uses made of the species by local communities (Chaisurisri *et al*. 1997, Changtragoon 1997). For example, the two dipterocarp species differ in their reproductive ecology, and economic use. *Shorea siamensis* is cut by local people for construction timber and is predominantly pollinated by a species of weak-flying bee, while *D. obtusifolius* is not

harvested, and is pollinated by butterflies and moths. Ghazoul (1997) and Ghazoul *et al*. (1998) found that reproductive success of *S. siamensis* was dramatically affected beyond a critical distance of separation among individuals, representing a threshold of disturbance, which was not true for *D. obtusifolius*. The impact on reproductive success in *S. siamensis* was reflected also in reduced genetic diversity.

In India a number of tree species from which non-timber forest products (NTFPs) are harvested were studied at two sites, together with others that are not used. A contrast between two NTFP and one non-NTFP species in the same genus (*Terminalia bellerica*, *T. chebula*, and *T. crenulata* respectively) clearly showed the impacts of NTFP harvesting, in terms of reproductive success, recruitment and genetic diversity (Uma Shaanker *et al*. 1996a; Uma Shaanker *et al*. 1996b).

Greater cooperation and funding are required to address the second shortcoming whilst the third shortcoming has been addressed for some tropical species e.g. *A. auriculiformis* (Wickneswari and Norwati 1995), *D. aromatica* (Lee 2000), *S. leprosula* (Lee *et al*. 2000; Nagamitsu *et al*. 2001) and *Neobalanocarpus hemii* (Konuma et al. 2000). The major constraints in carrying out these studies on pollen and seed dispersal of especially in the Dipterocarpaceae are availability of seeds (Sasaki *et al*. 1979) and recalcitrant nature of the seeds.

GENOMICS AND GENETIC DIVERSITY

The beginning of 21st century has seen the rapid race in sequencing entire genomes of plants, animals and microorganisms. *Arabidopsis* was the first plant genome to be completely sequenced (in 2000). This was soon followed by the rice genome in 2002. With the final sequencing of human genome in 2003, we have entered the age of genomics and post genomics. The complete sequence of plant genomes has profound implications on plant biology, improvement including exploitation of plant products and conservation.

Studies of genetic diversity can have several very distinct and different goals. Genetic diversity studies can be linked to the management goal of identifying high yielding genetic resources for use in production forestry – usually in the context of plantation forestry. At the same time, genetic diversity studies are essential for the design and monitoring of genetic conservation programmes, which apply to natural forests. However, genetic diversity studies are hampered by two main weaknesses:

1. Critical levels of genetic diversity and adaptive potential of forest trees. There is a general lack of understanding on structure of genetic variation and on the evolution of adaptability of affected populations. Though genetic diversity of a number of tropical tree species has been determined, the critical levels of the genetic parameters that render a population more susceptible to extinction are not known.

2. Relationships between ecological or demographic factors and genetic processes. Research on ecological or demographic and genetic processes are generally carried out independently. The maintenance of genetic diversity of forest tree populations undergoing population changes is important to maintaining adaptability and evolutionary

potential. Improving our understanding of the relationships between demographic and genetic parameters may allow us to better predict the ecological effects that changes in the genetic system would cause.

Sequencing entire the plant genome is one of the approaches in forest genomics to study genetic diversity for conservation. This method is fairly efficient for mass gene discovery. The completely sequenced genome may also enable the understanding of gene location and distribution, gene organization and function. *Arabidopsis thalian*, the first plant genome to be completely sequenced has a relatively small genome size (125Mb) that requires much less effort and cost for sequencing entire genomes compared to gymnosperms like pines with a genome size 200 times much larger (Table 2). Temperate forest species (poplars and eucalypts) and tropical forest species (dipterocarps and acacia) seem to have moderate genome size (300-700Mb). With the success in human genome (3000Mb) and rice genome (430Mb) projects, sequencing entire genomes for these important forest species looks feasible. However, the cost and effort are still too much to be justified for forest tree species, which assume lower priority than crop species which are important for food security.

Even though there is a vast difference in genome size for forest tree species (Table 2), the number of genes in a genome is rather constant and conserved across plant species. The excessive nucleotide bases in the genomes turn out to be largely non-coding regions with long and short repeated sequences. Allelic differences in these non-coding sequences quite often utilized in genetic diversity studies hardly contribute to information on adaptive genetic diversity of populations or species. Therefore, the study of adaptive genetic diversity is relied on gene discovery. An alternative to entire genome sequencing for gene discovery is EST (Expressed Sequence Tag) analysis. ESTs are partial protein-coding DNA sequences derived from complementary DNA (cDNA). EST sequences are usually screened for genes through BLAST (Basic Local Alignment Search Tool) analysis against public gene/EST databases. The comprehensive genomic database of *Arabidopsis*, rice and several forest tree species (*Pinus radiata, P. taeda, Picea abies, Eucalyptus* and *Populus*) are good sources for gene identification and comparative genomics in tropical forest tree species.

Table 2: Genome size for some tropical and temperate plant species

Scientific name	**Common name**	**Haploid chromosome number**	**Nucleotide base pairs (Mb)**
Arabidopsis thaliana	buckweed	5	125
Elaeis guineensis	oil palm	16	1,000-1,700
Oryza sativa	rice	12	400
Populus	poplars	19	500
Pinus	pines	12	20,000-30,000
Eucalyptus	eucalypts	11	340-580
Acacia mangium x Acacia auriculiformis	*Acacia* hybrid	13	650
Calamus	rattans	13	1,700-2,100
Shorea		7	570

The information on forest genomics of tropical forest tree species is meagre compared to temperate forest tree species. However, the recent years have seen some efforts being put into forest genomics of tropical tree species (*Acacia* and *Shorea*). In our laboratory, for instance, 6000 ESTs of *Acacia* hybrid (*Acacia mangium x Acacia auriculiformis*) have been generated from the inner bark tissue. The number of genes being identified is overwhelming. The Blast results revealed substantial number of genes involve in xylem and cambium formation in the EST database of *Acacia* hybrid (Table 3). These genes might serve as good candidates for developing molecular markers in adaptive genetic diversity studies for wood and fibre formation traits, or to a lesser extent, pathogen resistance trait.

ESTs provide good sources for genetic variation study through the development of ESTPs (Expressed Sequence Tag Polymorphisms) and EST-SSRs (Expressed Sequence Tag-Simple Sequence Repeats). In ESTPs, detection of allelic polymorphism that leads to different phenotypes is achieved by single nucleotide polymorphisms (SNPs). Tremendous ESTPs have been developed in loblolly pine (Brown *et al*. 2001). On the other hand, the allelic polymorphism of EST-SSRs is detected by presence of SSRs in the ESTs. Though SSRs are prominent in non-coding DNA regions, recent EST surveys show that the occurrence of SSRs in ESTs is moderately high (da Silva 2001). The level of polymorphism of EST-SSRs is lower than that of the genomic SSRs (Gupta *et al*. 2003). However, EST-SSRs are more superior than genomic SSRs as they can detect adaptive genetic diversity. EST databases of many plant species are available in GeneBank, and they can be readily exploited for cost-effective, rapid and efficient strategy of developing EST-SSRs (Qureshi et al. 2004). Both the ESTPs and EST-SSRs use polymerase chain reaction (PCR) approach where specific primer pairs are designed to amplify genomic DNA. The polymorphisms in ESTPs and EST-SSRs being directly inferred from functional genes, offer tremendous potential for assessment of adaptive genetic diversity and QTL (Quantitative Trait Loci) mapping.

Table 3: Genes involved in xylem and cambium formation identified in the EST database of *Acacia* hybrid

Gene	Number of ESTs
Caffeate O-methyltransferase	1
Caffeoyl-Coenzyme A O-methyltransferase	2
Cellulose synthase	1
Cinnamate 4-hydroxylase	4
Cinnamoyl coenzyme A reductase	1
Cinnamyl alcohol dehydrogenase	6
Diphenol oxidase laccase	1
Expansin	2
Extensin	1
Glycine-rich protein	1
Hydoxyproline-rich protein	5
Peroxidase	8
Phenylalanine ammonia lyase	3
Polygalacturonase	2
Proline-rich protein	5
Xyloglucan endo-1,4-β-D-glucanase	6
Xyloglucan endotransglysosylase	4
β-glucosidase	1

Another application of ESTs is to develop DNA microarrarys. DNA microarrays are DNA chips containing thousands of DNA sequences representing the many genes in an organism placed on miniature solid supports and used as hybridization substrate to quantify the expression of genes (Schena *et al*. 1996). Each of these DNA sequences represents unique genes or ESTs. Adaptive response to different environmental stresses and treatments can be studied for many genes simultaneously. It is important to understand the expression patterns of group of genes and their interactions. DNA microarray studies are now under way in most of the forest species to characterize global patterns of gene expression during xylem and fibre differentiations, and in response to different biotic and abiotic stresses.

CONCLUSIONS

DNA-based systems for gene expression analysis for the understanding of gene expression in different tissues and response to biotic and abiotic stresses can provide a direct link between genotype and phenotype. Genetic mapping of quantitative trait loci (QTL) especially loci which are involved in response to biotic and abiotic stresses will enable rapid assessment of ecosystem integrity and sustainable management of ecosystems.

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Gene Flow between European (*Populus tremula*) and Hybrid Aspen (*P. tremula* x *P. tremuloides*)

L Suvanto[1] and P. Pulkkinen[2]

Aspens are dioecious tree species and they can reproduce both sexually and asexually. Both pollen and seeds are dispersed by wind. The seedling survival is however very weak (Latva-Karjanmaa *et al.* 2003) and thus the main form of reproduction is asexual through root suckers. Hybrid aspen has been planted in Finland already from 1950's first as a material for match and lately for paper industry. However, hybrid aspen is a foreign species hybrid for Finnish nature and the genetic and ecological consequences of hybrid aspen plantations have not been studied. European aspen is very important species for boreal forest biodiversity. There are more than 200 species that are living or dependent on dead or alive aspen wood and many of these species are threatened (Siitonen 1999). Some of these species may not be able to live on hybrid aspen or on a cross between hybrid and European aspen.

The global warming seems to be an inevitable phenomenon. Hybrid aspen begins its growth earlier in the spring and continues it longer in the fall than European aspen (Yu *et al.* 2001), which has partly restricted the hybrid aspen plantations to relate only Southern Finland. If the summers will get longer and winters warmer, hybrid aspen can be cultivated further north than at the moment. In addition to this, the crosses between European and hybrid aspen can be stronger than saplings of hybrid aspen and their growth faster than that of European aspen. All these factors enable increased gene flow between European and hybrid aspen.

The impact of foreign gene flow is dependent on which species is the mother and which the father. In a case, where European aspen is the father and hybrid aspen the mother, the gene flow has impact only in the vicinity of hybrid aspen stand. In the vice versa situation, where hybrid aspens are the fathers and European aspens the mothers, gene flow has a broader impact, because pollen can travel much further than seeds.

In order to study the gene flow between European and hybrid aspen, Finnish Forest Research Institute has started a project, which clarifies the impact and mechanisms of gene flow between aspens. In this paper, we report some results from crossing experiments done in 2003 and 2004 between European and hybrid aspen.

MATERIALS AND METHODS

The crosses were made using three European and three hybrid aspen mothers, which were crossed with three European and three hybrid aspen fathers in all possible combinations. We also did competition crosses using pollen mixtures of two fathers. The branches of mother and father

[1] Researcher; Department of Biological and Environmental Sciences, P.O.B. 65, FI-00014 University of Helsinki, Finland and Vantaa Research Centre, Finnish Forest Research Institute, P.O.B. 18, FI-01301 Vantaa, Finland

[2] Senior Researcher, Haapastensyrjä Breeding Station, Finnish Forest Research Institute, Karkkilantie 247, FI-12600 Läyliäinen, Finland

trees were collected in March-April 2003 and 2004. We collected ten two-meter-long branches from each individual. The branches were kept in a greenhouse and the pollen from father trees was collected into paper bags, dried and vacuumed. The mothers were pollinated with 6 cm^3 of pollen twice in order to ensure a successful pollination. After pollination the female flowers were covered with paper bags.

In 2004 the crosses were done in two different temperatures in order to study the effect of global warming to seed production. The so-called natural temperature (mimicking the temperature in the nature) at the time of flowering was 11.9 ^oC and the warmer temperature 13.7 ^oC. After pollination half of the branches were kept in the same conditions, while other half were transferred into a warmer temperature. The prevailing temperatures at the time of seed ripening were thus 13.1 and 17.1 ^oC for "natural" temperature and 15.7 and 17.4 ^oC for "warm" temperature, respectively.

We counted the amount of seeds both in number and weight as well as their germinability and the number of flowers, no of seeds / flower and no of germinated seeds / flower. The results from competition crosses were analyzed using nine microsatellite loci that were originally developed for *P. tremuloides* (Dayanandan *et al.* 1998, Rahman *et al.* 2000).

RESULTS AND DISCUSSION

The results of the crosses show clearly that if the mother and father were from different species, the crosses were more successful. Most seeds were produced, when the mother was hybrid aspen and the father was European aspen. If a European aspen mother was crossed with hybrid aspen father, the seeds were 14% more viable than if the father was European aspen. The effect was even more drastic, when hybrid aspen mothers were crossed with European aspen fathers. In this case the seeds were 75% more viable than with hybrid pollen.

The results are best described by the amount of germinated seeds / flower, because this measure is not dependent on the size of the branches, amount of flowers nor the amount of seeds. As can be seen from Fig 1., the best yield was achieved, when European aspen mothers were pollinated by hybrid aspen fathers. The results can be explained by heterosis, the better fitness of hybrids compared to parent species.

The warming temperature seemed to favor hybrid aspen compared to European aspen. Hybrid aspen mothers produced more seeds than European aspen, when the temperature got warmer. In European aspen the seed crop even diminished with warming temperature. Also the mothers (despite of the species) pollinated by hybrid aspen fathers produced more seeds than mothers pollinated by European aspen. However, constant temperature was best for all father-mother combinations.

In competition situation, father of a foreign species was more successful fertilizer of the mother than father of the same species as the mother. Hybrid aspen father won European aspen father in 57 % of the trials, where the mother was European aspen. European aspen fathers were more successful than hybrid aspen fathers to fertilize hybrid aspen mothers in 52% of the cases. The better success of foreign species indicates that sexual selection could be functioning in the

mating of aspen. If this is true, it would be very interesting to investigate the mechanisms behind the selection.

We conclude that gene flow between European and hybrid aspen can be quite extensive. The species cross well and hybrid aspen can be an even better pollinator of European aspen than its own species. Also hybrid aspen seems to do better in warming climate, which should be taken into account when planning the future plantations of hybrid aspen.

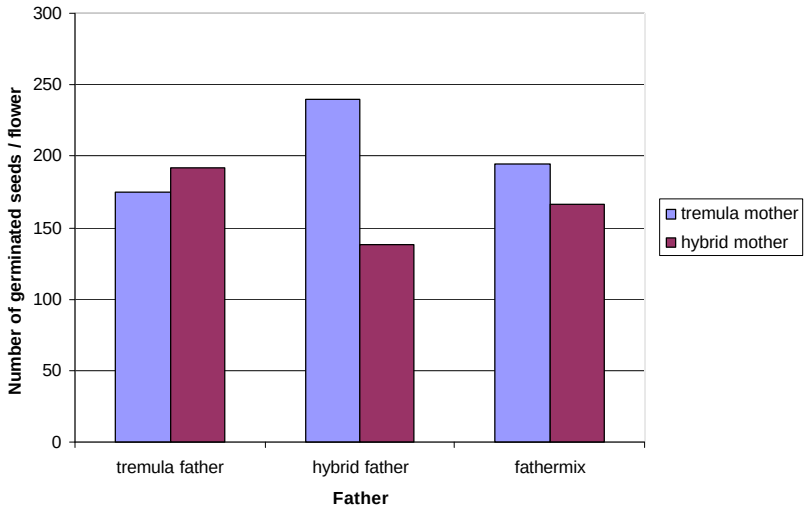

Figure 1. The number of germinated seeds / flower in different mother-father combinations.

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GENFORES, A Costa Rican Tree Improvement And Gene Conservation Cooperative.

Murillo, Olman[1], Obando, German[2], Badilla, Yorleny[2] and Araya, Emanuel[1].

Abstract:

Since early 2001 is working the first Costa Rican tree improvement and gene conservation cooperative (named GENFORES). Its mission is the promotion of a forest genetic resources exploration, conservation, rational utilization and breeding. Its member organizations provide economical and technical support for its operational development and partly, applied research funding. Each member organization develops its own breeding and gene conservation program, according to its needs and goals. The school of Forestry, at the Technological Institute of Costa Rica (TEC), provides I & D, technical direction of GENFORES, develops technology transfer activities, technical training and promotes cooperation with other research units. Today, GENFORES is integrated by 12 members, 35% are regional ONG's. Teak (*Tectona grandis*) program is the most advanced with more than 315 plus trees selected and in development in clonal gardens. *Gmelina arborea* reached more than 90 plus trees. The native tree species programs comprehends over 300 plus trees already selected, for 5 commercial native tree species, and increasing rapidly. Clonal garden designs and its management are discussed. A general breeding strategy, based on clonal forestry, is described.

Keywords: tree improvement, breeding, Costa Rica, cooperative, gene conservation.

I. Forest plantations background of the country and basis for a breeding program

Low productivity and quality of costarican forest plantations has been well documented in the country since early 90's (Murillo, 1992; Murillo *et al.*, 1996; Torres *et al.*, 1995; Rojas y Murillo, 2000). Under such low profitability, it is very difficult that plantations could compete as an investment possibility, not only at international level, but locally. Probably the best way to increase its attraction profile could be through an active research and development program. The first change we have to accomplished will be, from the old tree reforestation concept, to the new concept of farming wood products. International competence, specially Chilean wood products, forced the country toward better organization systems and to the establishment of the costarican tree improvement cooperative (named GENFORES) was created in 2001 (Gutiérrez *et al*, 2003). Technological Institute of Costa Rica (TEC) has the academical responsability in GENFORES, I & D, and the maintenance and management of its genetic resources.

Farming wood products is becoming an important business activity in Costa Rica. It has attracted in the last years a number of recognized local and international companies to this new economical activity for the country. This conception implies a radical change in relation to the traditional planting trees model. The trees are more and more being planted in the same way as a

[1] Escuela de Ing. Forestal, Instituto Tecnológico de Costa Rica. FAX 591-4182. omurillo@itcr.ac.cr
[2] Fundación para el Desarrollo de la Cordillera Volcánica Central. gobando@fundecor.co.cr

sugarcane crop, or banana crop, etc. Best soil conditions are carefully searched when selecting or buying new farms for plantations. Site preparation and soil management is mandatory and more and more under applied research programs.
Under these new concepts, only the best possible genetic material is being planted. Usually seedlings or cuttings coming from plus trees are planted on commercial scale. Plus trees are selected based on its quality index (60%) and volume (40%), at this first generation stage we are. Wood quality traits, like wood specific gravity and hartwood percentage, are recently incorporated. GENFORES breeding strategy is mostly intensive silviculture and clonal forestry oriented. An important clonal garden and greenhouse management technology have been developed through its 12 members in the last 3 years.

II. A little about forestry in Costa Rica

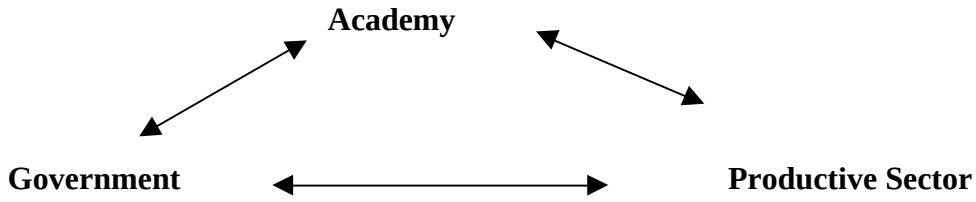
Costa Rica is a small and very mountainous tropical country (50 900 km^2) with something more than 4 million people. Protected area comprises a little more than 26% of the whole country. Deforestation rates have been controlled severely in the last years, with numbers below 5 000 ha/year nowdays. Costa Rica follows an incentive system for planting trees, which has been modified several times after 25 years of development. Around 170 000 ha have planted, mostly in the 90's with *Tectona grandis* and *Gmelina arborea*, and about 50% belongs to small scale forestry. The highest planting rate achieved was 16 000 ha/year and dropped in the last 6 years until 6 to 7 000 ha/year. The local industry and the wood products exports require the consumption of around 8 to 10 000 ha/year. Natural forests are more restricted each year and provide today about 25% of the wood markets with a clear lower participation tendency. This overview shows that the country is dangerously running toward a negative wood stock, and becoming more a wood consumer and importer in the last 6 years.

III. How was created GENFORES?

Due to the country's external pressure, and the need of improving investment conditions of forest plantations, both promoted the initiation of breeding programs in the private sector. Two tree breeding and research programs were at TEC initiated four years ago. One program for high elevation tree species (above 1000 m.o.s.l), and a second program for low humid lands in the north and caribbean region of the country. These two programs were from the beginning jointly developed with FUNDECOR (local costarican foundation for forest development). A considerable research on breeding and vegetative propagation on a number of native and exotic tree species was developed. Trees species with a high industrial value were selected: *Cupressus lusitanica*, *Vochysia guatemalensis*, *Ulmus mexicana*, *Alnus acuminata*, *Hieronyma alchorneoides*, *Terminalia amazonia*, *Tectona grandis* and *Gmelina arborea* mainly (Sánchez y Murillo, 2000; Badilla, 2001; Del Valle, 2001; Murillo *et al.*, 2001).
However, all these information and experience needed to be transfered to the main forest companies. Who is going to use the research results?. Good scientifical publications do not ensure utilization of the produced information!. Who are in our research goals ?. In the other hand, are we producing the right information for the productive sector?. Therefore both ways are important, the information must flows in both directions!.

productive sector ⟷ researcher

When we reach an active two-ways information flow, then we reached the vinculation level. At this stage is when validation level is now working, better sound socio-economical technological packages are produced, and finally, a better understanding of reality and people's needs. However, the ideal model implies the Government as another keystone of the system:



Different from other similar tree breeding cooperatives, GENFORES is not only interested in the participation of large plantation companies, but also in the incorporation of small and medium well organized forestry NGOs. In Costa Rica, these NGOs comprehend around 50% of the planted area.

One of the challengest experiences we have had in the development process of GENFORES, is related to the step from academical reseach to operational breeding forestry. At TEC facilities was necessary the establishment of larger research units, in order to be able to simulate operational breeding in a larger working scale. This resulted in a better technology transfer and training process of GENFORES's members technical staff. Information about breeding production costs and other related data was then obtained. Clonal garden development and management was one of the key areas requested. Most of the information was jointly generated at each GENFORES member itself.
A high services demand from GENFORES was rapidly generated in the costarican forestry sector. The questions at the moment were:
1) How to properly respond to the increasing demand from the forestry sector?.
2) How to keep up with research and development of new information without affecting the development of GENFORES?.
The answer to these two important questions produced a GENFORES organization model and some basic restrictions to new members:

a) GENFORES members must plant no less than 50 ha/year in average,

b) Members should have some planted area where to get potentially new genetic material. Also accepted those members with some access to important genetic material for the rest of GENFORES.

c) Members must be aware and open to the exchange of genetic material on reciprocal basis.

d) Technical visits to research and development facilities from other GENFORES member must be allowed.

e) All members must contribute to the development and economical maintenance of GENFORES. The monthly payment is now on US $ 250.

IV. GENFORES Constitution

GENFORES was then created under the following mission: the promotion and development of **1)** exploration, **2)** conservation, **3)** rational utilization, and **4)** breeding of forest genetic resources in the region.

GENFORES objectives are the following:

a) To reduce costs and increase productivity and quality of GENFORES members plantations.

b) To reduce research costs, training and technology transfer to its members.

c) To improve a teaching process closer to the forestry sector reality in the Forest Engineering School at Technological Institute of Costa Rica.

d) To contribute to the knowledge, rational utilization and gene conservation of forest genetic resources in the country.

e) To contribute to the improvement of investment attraction into forestry sector.

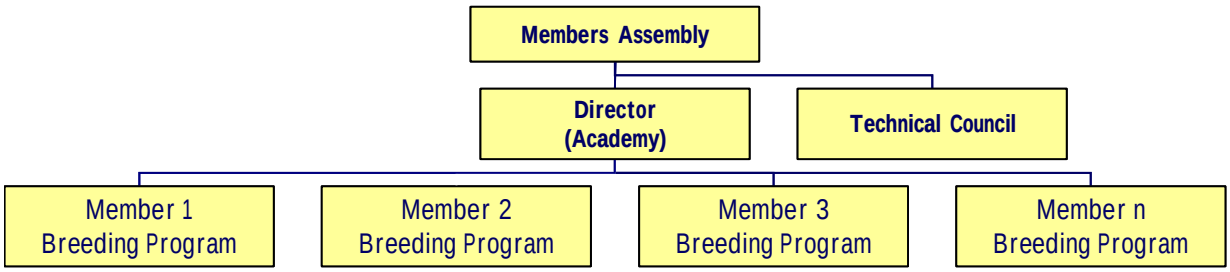


Figure 1. GENFORES flowchart.

Table 1: GENFORES members until november, 2004

Organization/company	Planted Total Area (ha)	Tree species	Seed Zone
Instituto Tecnológico de Costa Rica	3	Natives	High elevation zones (> 1000 m) and North Zone
FUNDECOR	3 000	Natives and exotics	Sarapiquí and Cordillera Volcánica Central
EXPOMADERAS	1 100	Teak	San Carlos and Los Chiles
ECOdirecta	1 000	Teak and natives	San Carlos and Los Chiles
BARCA S.A.	515	Teak and natives	Parrita and Puntarenas, Pacific South
CACH	2 200	Teak and melina	Hojancha and Nicoya Península
Coopeagri	500	Natives , teak, melina and pines	Pérez Zeledón and South Zone
Plywood Costarricense	200	Natives and pines	Sarapiquí and Siquirres
Ganadera Basa S.A.	2000	Teak and Acacia mangium	North zone and Central Pacific
Panamerican Woods	3000	Teak	Dry Pacific, Nicoya Península
Compañía Nacional de Fuerza y Luz	200	Natives and *Cupressus lusitanica*	High elevation zones (> 1000 m) and Central Valley
EARTH (Regional Agricultural School for the Humid Tropics)	600	Natives and melina	Caribbean

V What is the contribution from TEC to the development of GENFORES?

The Technological Institute of CR (TEC), through its Forest Engineering School, provides the technical staff trained in tree improvement and forest genetics. Besides, TEC has 3 research centers involved with the development of GENFORES: 1) Forestry and Wood Technology Research Center; 2) Biotechnology Research Center; and 3) Humid Tropical Agronomical Research Center.

Into these 3 research centers there are nurseries, greenhouses, clonal gardens, seed orchards, seed labs, molecular genetic lab and tissue culture facilities.

With the economical support from GENFORES members and TEC, the technical staff of GENFORES is now based on 3 forest engineers, 1 biotechnologist, 1 lab technician and 2 field workers.

Until now at the TEC's Research Division, there are 4 formal research projects directly related to GENFORES, which provide funds for the basic research.

VI Breeding Progress ans strategy in GENFORES

Up to now, GENFORES has created an important breeding population for teak with some more than 300 plus trees selected (table 2). These collections belong to each of GENFORES

Table 2: Teak breeding program advancement in GENFORES (october 04)

Members	Seed Zone	Plus Trees	Plus Trees already cloned
EXPOMADERAS	North Zone	58	56
ECOdirecta	North Zone	54	54
CACH	Dry Pacific	40	0
Panamerican Woods	Dry Pacific	66	66
ITCR	North Zone	25	25
Ganadera BASA	North Zone	26	0
BARCA S.A.	Central Pacific	35	18
Total		**314**	**210**

members. A sublining approach, with 20 to 24 plus trees per line has been suggested. In a practical view, subline's number of entries will be rounded up within each organization, in order to fit with entire heterotic groups per organization. With collaborative genetic material exchange among GENFORES organizations, sublines will finally fit to complete all lines.

Table 3: Melina breeding program advancement in GENFORES (october 04)

Organization	Number of plus trees
CACH	24 all cloned
Coopeagri	29 all cloned
Plywood Costarricense	36
Total	**89 plus trees, 53 cloned**

Melina program has been reassumed since a rapid destruction of its genetic resources in the country. The pallet industry in Costa Rica has been very aggressive, and has severely exploited most of plantations in the country in the last 6 years. New selections have been made through GENFORES members in the last 2 years (table 3). We hope to reach at least 300 entries in 2 more years of field work. In the caribbean region of Costa Rica, still remains a large genetic collection of *Gmelina*'s provenances introduced by the banana companies late in the 60's (Lega, 1988). A sublining breeding approach will followafter once a large enough collection will be reached.

Native tree species programs are very important in the country. Costa Rica has been very aggressive in this field, and a number of research programs are running since the end of the 80's. At least 5 native tree species are reaching a high competition potential for international markets. The local industry is targeting furniture and housing wood markets for our native woods. Plus tree selections flows slowly since the plantations are still at small and medium scale. However, new collections from native populations are often made and placed in the field for provenance/progeny testing. Table 4 shows an overview of the tree species and its advance up to this year. Basic research on its reproductive biology and vegetative propagation has been very important in the last decade. Clonal gardens and seed orchards for *Hyeronima alchorneoides*, *Vochysia guatemalensis* and *Terminalia amazonia*, are already in place at Technological Institute of Costa Rica. In the near future a clonal breeding approach will be followed. Gene conservation issues are in this case mandatory and new collections with this goal will be also in the next years conducted.

Table 4: Native tree species breeding program advancement (october 04)

Organization	Plus trees
FUNDECOR / TEC	**65 *Alnus acuminata* plus trees.** **Seed Orchard rogued.** **45 *Hieronyma alchorneoides* plus trees, 19 cloned.** **Seed orchard rogued.** **90 *vochysia guatemalensis* plus trees, 65 cloned.** **23 *Dipteryx panamensis* plus trees** **9 *Terminalia amazonia.* (6 cloned).** **Seed orchard rogued.** **4 *Vochysia ferruginea*.**
(Coopeagri)	**25 *Terminalia amazonia*** **3 seed stands**
BARCA S.A.	**9 *Terminalia amazonia***
Plywood Costarricense	**46 *Schizolobium parahybum***
Total	302 plus trees, 90 cloned, 3 seed orchards; 3 seed stands.

Agroforestry based seed orchards have been proposed for several of our members, since most of them are farmers' organizations planting coffee or sugarcane at a large scale. Ideally, 8 x 8 m or 10 x 10m are spacings that do not interfere with the large crop areas they have available. Therefore, with the clonal development, agroforestry systems could now be very competitive for wood production and completely fitted into our breeding programs. A very promising future in this direction is expected in the next years in the country. Coffee and sugarcane farmers' organizations are very well organized and share a long solidarity tradition in the country.

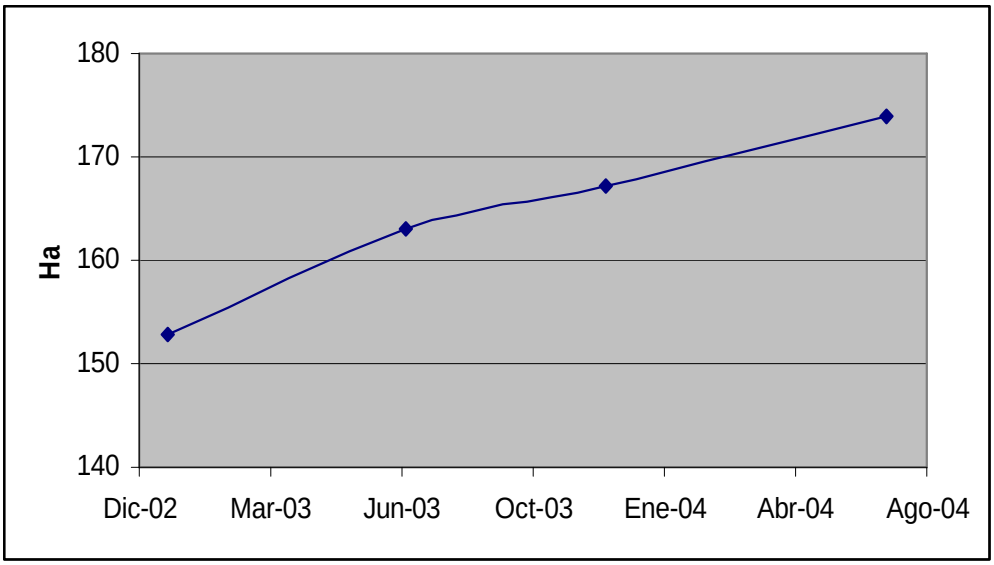


Figure 2: Seed sources developed through GENFORES (ha). Includes seed orchards, improved seed stands and commercial clonal gardens.

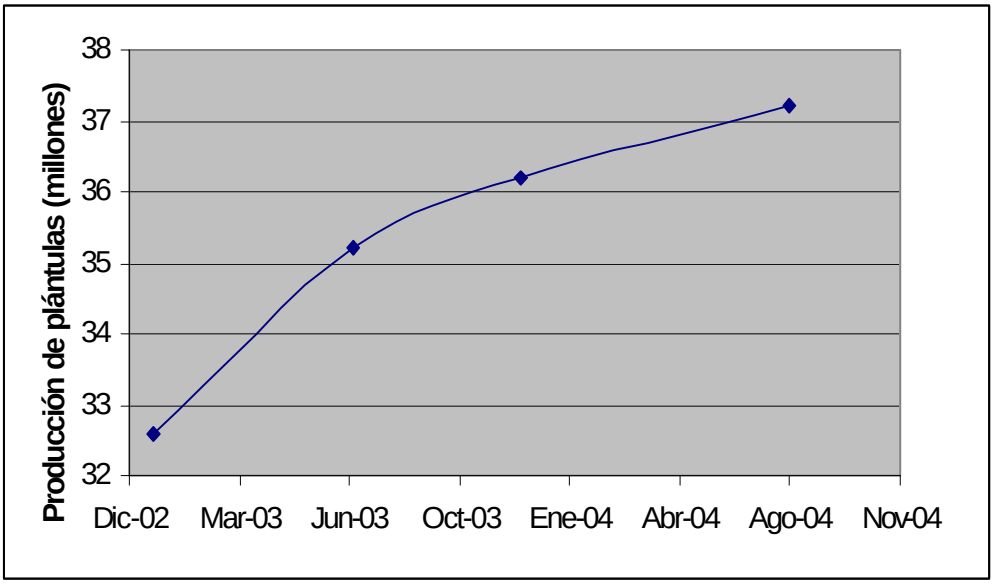


Figure 3: Seedling and cuttings production capacity in all seed sources developed by GENFORES (in millions).

VII Final arguments

Figures 1 and 2 shows GENFORES general progress in the last 2 years. A seed zonification is almost defined for the country and will be the basis for all future breeding work. New seed sources will be established in all those priority Seed Zones of the country. The national plantation programs are now pressing for selected and certified seed, and GENFORES is being considered as key organization for the accomplishment of such goals. For small countries as

Costa Rica, a breeding cooperative concept must works closely with the national forestry policies. Small scale forestry is about 50% of our reality and tree breeding approaches must be fitted into this field.

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Conservation and Restoration Efforts on the US Southern National Forests

Barbara S. Crane

Regional Geneticist, USDA Forest Service
Southern Regional Office, Atlanta, GA, USA

The USDA Forest Service National Forest System land base in the Southern Region (Region 8) encompasses 13 million acres managed within 17 Administrative Units. These national forests are scattered and fragmented across 13 southern states and Puerto Rico. Over the past 150 years, population growth, agricultural expansion and forest health issues have eroded the diversity of the southern forests. Region 8 leads the nation in population growth and accommodates the 2nd largest census. Over the past century more than twenty pests and diseases have been imported, resulting in serious negative impacts on the southern forests. Environmental awareness has grown and become more involved in public land decisions. The southern US has become the "wood basket" of the country, supplying approximately 70% of the nation's forest products needs. These combined factors have put tremendous pressure on the natural resources, and have subsequently influenced the direction of the National Forest System.

In response to dynamic times and changes, the National Forest System recently revised the Forest Plans that direct balanced and diverse ecosystem management. Allied goals target restoration, biodiversity, improved wildlife habitat for endangered, threatened and sensitive species, and forest management. In concert with the Forest Plans' objectives the Genetic Resource Management Program (GRMP) has focused on perpetuating genetic & biological diversity of tree species. Current efforts target conservation of five conifer and four hardwood tree species. Because ecosystem maintenance and restoration on National Forest lands is a long term commitment, the GRMP continues to manage seed orchards of priority species used in restoration efforts. The GRMP promotes genetic diversity and conservation, and species improvement, rather than tree improvement. Further, the GRMP adheres to the National Genetic Resources Strategic Plan, and is engaged in several university, research and private partnerships to facilitate conservation and restoration efforts.

Contrasting Patterns of Genetic Diversity in the Six Iberian Pine Species

A. Soto[1], J.J. Robledo-Arnuncio[2, 3],
S.C. González-Martínez[1], P.E. Smouse[3], A. Gómez[4], and R. Alía[1]

The Iberian Peninsula is considered a long-term glacial refugium for many European plant taxa, whose populations in the Mediterranean area are of considerable interest to genetic conservation programs and breeding. Six different native pine species presently coexist in the Iberian Peninsula (*Pinus uncinata* Ram., *P. sylvestris* L., *P. nigra* Arn., *P. pinaster* Ait., *P. halepensis* Mill., and *P. pinea* L.), showing a wide range of ecological adaptations and population structures. *Pinus uncinata*, *P. sylvestris* and *P. nigra* are mountain pines, tolerant of low temperatures. The first is a timberline species, showing an especially fragmented distribution over mountain ridges and peaks. *P. pinaster* grows from sea level to 2,000 m, with wide climatic and edaphic ranges. *Pinus halepensis* and *P. pinea* are low- to medium-elevation species, intolerant of very low temperatures. Distinct adaptive characteristics have probably determined contrasting demographic and evolutionary histories throughout Holocene glacial cycles, leading to their present-day genetic structure. A comparative study of neutral genetic diversity can provide useful information to infer the relationships between the biology of particular species and the evolutionary factors, such as drift or migration, operating on those species.

MATERIAL AND METHODS

We have investigated neutral genetic variation within and among 127 populations (mean 22 trees/population and 21 populations/species; total N = 2,785 individuals) of the six native Iberian pine species, covering most of their distribution in the region. For this purpose, we assessed the chloroplast haplotype of each tree using a common set of five chloroplast microsatellite markers (Pt15169, Pt30204, Pt36480, Pt71936 and Pt87268; Vendramin *et al*. 1996), following the protocols described in Gómez *et al*. (2003).

We characterized the overall species genetic diversity of each taxon by computing the effective number of alleles per locus, $na_e = (n\text{-}1)/(n\Sigma p_i^2\text{-}1)$, and the effective number of haplotypes, $nh_e = (n\text{-}1)/(n\Sigma q_i^2\text{-}1)$, where n is the number of analyzed individuals, and p_i and q_i are, respectively, the allele and haplotype frequencies of the target species. Differences among species in the average na_e and nh_e across loci were tested by bootstrapping individuals within species 1,000 times. We also estimated the average population diversity of each species by calculating the mean unbiased haplotypic diversity (H_e; Nei, 1987) across its populations. The level of genetic structure was assessed for each species by computing F_{ST} statistics, following an AMOVA approach (Excoffier *et al*. 1992). In order to test the statistical significance of the differences in F_{ST} between the i-th and j-th species, we obtained the 95% confidence interval for $[(F_{ST})_i - (F_{ST})_j]$ by bootstrapping over populations within species 1,000 times. We applied a Bonferroni correction for multiple

[1]Unidad de Genética Forestal, CIFOR-INIA, Madrid, Spain
[2]Unidad de Anatomía, Fisiología y Genética Forestal, ETSI Montes-UPM, Madrid, Spain
[3]Dept. Ecology, Evolution & Natural Resources, Rutgers University, New Brunswick, NJ, USA
[4]Dpt. de Investigación en Desarrollo Rural, IMIA, Madrid, Spain

comparisons of means whenever a statistic was compared across the six species (15 pairwise comparisons), using an experiment-wise significance level of 0.05.

RESULTS AND DISCUSION

There was significant variation among the species in all genetic diversity parameters (Table 1). The mean effective number of alleles per locus ($\overline{na}_e$) ranged from 1.13 in *P. halepensis* to 2.88 in *P. sylvestris*, and was substantially and significantly higher for mountain pines (*P. uncinata*, *P. sylvestris*, *P. nigra*; $\overline{na}_e$ = 2.77 - 2.88), among which differences were not significant, than for the more thermophilic taxa (*P. pinea*, *P. halepensis*; $\overline{na}_e$ = 1.19 and 1.13), which also formed an homogeneous group. *Pinus pinaster* showed an intermediate value of 2.08. A similar trend was observed for the effective number of haplotypes (nh_e) and the mean population haplotypic diversity ($\overline{H}_e$), with the mountain pines consistently showing significantly higher levels of genetic diversity (somewhat lower for *P. uncinata*) than *P. pinea* and *P. halepensis* (Table 1). Differences in overall species haplotypic diversity (nh_e) were especially striking between the homogeneous group formed by *P. sylvestris* and *P. nigra* (nh_e = 89.99 and 71.65) and the two lowland species, *P. pinea* and *P. halepensis* (nh_e = 2.19 and 1.63). On the other hand, among-population variation (SD) in H_e was substantially higher for *P. pinea* and *P. halepensis* than for the other species, suggesting more heterogeneous demographic histories across populations for the more thermophilic pines.

Strongly significant population structure was found for *P. pinea* (F_{ST} = 0.468) and *P. halepensis* (F_{ST} = 0.350), while *P. sylvestris* and *P. nigra* showed low, though still significant, levels of population differentiation (F_{ST} = 0.045 and 0.115, respectively), with *P. pinaster* having an intermediate value of 0.259. F_{ST} estimates were not significantly different between the mountain pines *P. sylvestris* and *P. nigra*, and were substantially lower for these two species than for typically Mediterranean pines (*P. pinaster, P. halepensis, P. pinea*), although the difference with *P. halepensis* was not significant (Table 1). The timberline pine *P. uncinata* showed a strong genetic structure (F_{ST} = 0.352), although this value was not significantly different from those of any of the other species, due to its smaller number of populations (hence larger variance).

Table 1. Genetic diversity variation among Iberian *Pinus* species. *P* is the number of populations analyzed. Variables are the mean effective number of alleles per locus ($\overline{na}_e$), the effective number of haplotypes (nh_e), the mean population haplotypic diversity ($\overline{H}_e$), and the genetic structuring (F_{ST}). Standard deviation (SD) is shown between brackets. All F_{ST} values are significantly different from zero ($p < 0.001$). Means were compared using non-parametric procedures (see Methods). Means with at least one common letter are not significantly different at the experiment-wise (Bonferroni) $p = 0.05$ level.

Species	***P***	$\overline{na}_e$ (SD)	nh_e (SD)	$\overline{H}_e$ (SD)	F_{ST} (SD)
P. uncinata	5	2.77 (0.12) **A**	30.82 (5.53) **A**	0.888 (0.102) **AB**	0.352 (0.136) **AB**
P. sylvestris	29	2.88 (0.04) **A**	89.99 (7.13) **B**	0.973 (0.024) **A**	0.045 (0.013) **A**
P. nigra	14	2.84 (0.07) **A**	71.65 (8.15) **B**	0.974 (0.019) **A**	0.115 (0.022) **A**
P. pinaster	51	2.08 (0.03) **B**	24.79 (2.09) **A**	0.892 (0.075) **B**	0.259 (0.033) **B**
P. pinea	15	1.19 (0.02) **C**	2.19 (0.14) **C**	0.372 (0.267) **C**	0.468 (0.069) **B**
P. halepensis	14	1.13 (0.01) **C**	1.63 (0.09) **D**	0.259 (0.232) **C**	0.350 (0.235) **AB**
Average		2.15	36.85	0.694	0.265

The observed genetic structure for the six Iberian pine species is consistent with the hypothesis that adaptation to cold temperatures, together with the possibility of downhill migrations into inland Iberian plateaus during cold stages, would have allowed a somewhat stable demography, resulting in the long-term maintenance of relatively high within-population and low among-population genetic diversity of (now) highly fragmented montane species (Robledo-Arnuncio *et al*. 2005). By contrast, more thermophilic species would have suffered severe range contractions into small coastal refugia, which may have led to repeated bottlenecks and subsequent population divergence and loss of diversity. These demographic dynamics are consistent with the available macrofossil record in the Iberian Peninsula (Franco-Múgica *et al*. 2001), and suggest that the degree of long-term habitat stability within southern mountain regions would be highly variable among different tree species.

The results of this study, besides providing new insights into the population and evolutionary history of the genus *Pinus* in the Mediterranean region, will be a useful *neutral-variation contrast* to parallel investigations on the variation at quantitative traits and at nucleotide sequences from candidate genes, which are presently being performed on the same species and populations.

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Ex situ conservation strategy for butternut (*Juglans cinerea*)

T. Beardmore, K. Forbes, J. Loo and D. Simpson
Natural Resources Canada, Canadian Forest Service-Atlantic Region, Hugh John Flemming Forestry Centre, PO 4000, Fredericton, New Brunswick, E3B 5P7

Butternut trees are native to North America, predominantly occurring in forests ranging from eastern Ontario to New Brunswick, throughout the New England States, south to Georgia, and west to Missouri. Butternut (*Juglans cinerea* L.) survival is threatened in North America by the fungus *Sirococcus clavigignenti-juglandacearum*. Since the first report of butternut canker in 1967, infected butternut has been found throughout most of its range (Ostry, 1994). In Canada, the first report of canker was in Quebec, 1990 (Innes and Rainville 1996), then in Ontario, 1991 (Davis et al. 1992) and in New Brunswick, 1997 (Harrison, Hurley, and Ostry 1998). To date, there is no control for this fungal disease and the presence of resisitant trees has not bee documented (Ostry et al. 2003).

Butternut canker infects all sizes and age classes of trees on all sites and infection can occur through buds, leaf scars and various wounds (Ostry 1994). The fungus is proposed to be spread by rainsplashed spores and birds and insects and usually starts on small branches and twigs in the crown. The canker is highly aggressive and has spread rapidly since its first report in 1967 (Ostry 1998). It has recently been found on two other hosts, black walnut (*J. nigra*) and heartnut (*J. ailantifolia* var. *cordiformis*), but infection on these species has been limited (Ostry 1997; Ostry and Katovich 1997). To date, a control for the disease does not exist. Overall butternut mortality as a result of this disease exceeds 77% in American forests (Ostry et al. 1994) while in Canada mortality has been estimated to be 80% in Ontario (Fleguel, 1996).

The nuts are considered to be desiccation-tolerant and liquid nitrogen sensitive (Wang et al. 1993). Liquid nitrogen is used for cryogenic storage, where germplasm is stored at -196^{o}C. Nuts have been successfully stored for several years at temperatures of 1 to 4^{o}C, and high relative humidities (i.e., 80 –90%) (Brinkman 1974). Conventional methods for storing the nuts include placing the nuts in a) sealed containers; b) invigoration tubes (which allow airflow over the seeds) (Figure 1); c) out-door pits in the ground. Non-conventional methods for storing butternut axes (root shoot axis, with the majority of the cotyledon material removed, referred to herein as axes) seeds include cryopreservation (Beardmore and Vong, 1998) (Figure 1). Nuts can be stored for 2 to 3 years using conventional methods and it is not known how long butternut can be cryopreserved.

Butternuts produce a fruit, which is a nut surrounded by a fleshy husk (Figure 2a). The nut consists of a hard pericarp, which surrounds the embryo (Figure 2b). The embryo is dormant. Dormancy is broken by stratifying nuts at 5 to10^{o}C under high relative humidities for 90 to 120 days. Nuts are stored first for 12 months in invigoration tubes at 5^{o}C, after which the axes are isolated from the nuts (Figure 2c). The axes are surfaced sterilized, placed in cryo-vials and are then pre-treated by gradually decreasing the temperature from 20^{o}C to 0^{o}C (at a rate of –5^{o}C/min), from 0 to –40^{o}C (at a rate of –0.33^{o}C/min) and at –40^{o}C axes were immersed in liquid nitrogen and placed in liquid nitrogen cooled freezers at approximately –196^{o}C. After the appropriate duration axes are removed from cryogenic storage and thawed in a water bath (40^{o}C

for 5 min.), transferred to Woody Plant media and then placed in a growth cabinet (26^{o}C, 12-h photoperiod).

Butternut axes from 3 trees germinated at 83, 97 and 99% after 12 months of storage (Table 1). After 24 hrs of cryopreservation axes germination declined but increasing the duration of cryopreservation to 1.5 and 6 years did not result in a further decline in germination. These results suggest that the duration of cryopreservation does not affect axes viability.

These results suggest that the cryopreservation of axes may be an appropriate *ex situ* conservation method for butternut. There are limitations to this technique in that it is costly compared to conventional seed storage methods (approximately 25¢/axes/yr), one must have a reliable supply of liquid nitrogen and it is time consuming with regard to preparing the axes for cryopreservation. However, this technique may be appropriate for conserving material that is valuable (e.g., from unique populations or from putativitve resistant trees).

Table 1. Effect of the duration of cryopreservation on axes isolated from 12 month-stored nuts.

Duration of exposure to –196^{o}C	% germination		
	tree-1	tree-2	tree-3
control	83a	97a	99a
24 hrs	68b	77b	83b
1.5 years	65b	72b	80b
6 years	63b	75b	80b

Values are the mean of 3 replicates with 15 axes/replicates. Statistically significant differences determined by Duncan Waller's comparison of the means (p # 0.05) are indicated by different lower case letters down a column.

a) sealed containers b) invigoration tubes c) cryopreservation tanks

Figure 1. Conventional seed storage options for butternut nuts.
a) sealed containers; b) invigoration tubes; c) cryopreservation tanks.

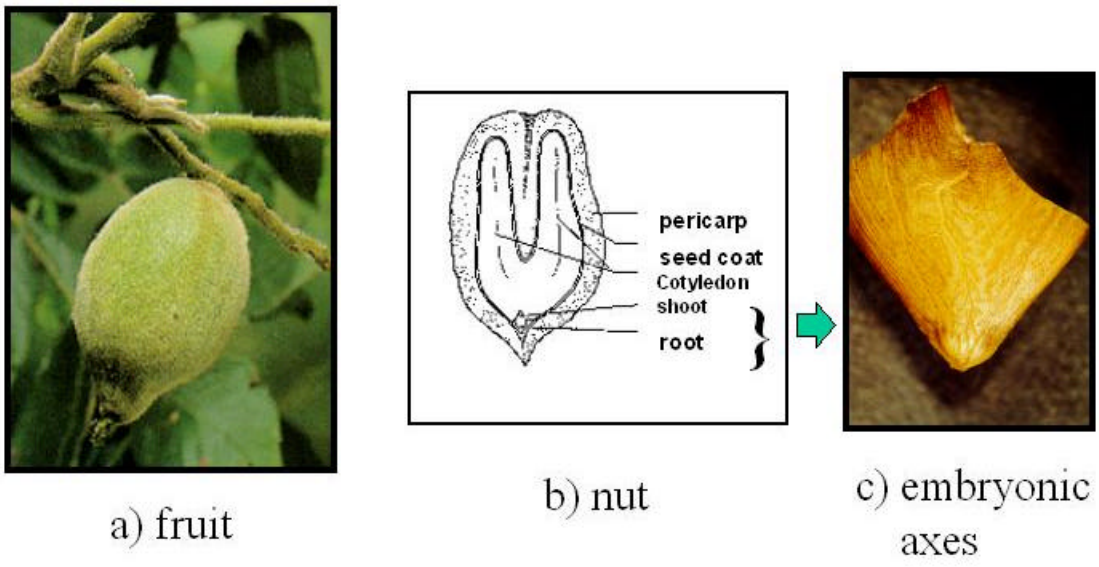

Figure 2 a) butternut fruit; b) nut and c) embryonic axes.

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Study On Genetic Transformation With *Rolb* And *Pttga20ox* Two Genes To Improve Rooting Ability And Apical Dominance Of *Populus Tomentosa*

Chen Xiaoyang, Li Wei, Liang Ji
Beijing Forestry University, P.R. China

Populus tomentosa is one kind of important afforest tree species with fast-growing and high wood quality in the northern china, which is widely used as the woody sources of fibre for the paper industry. Unlike most poplar species, *Populus tomentosa* is difficult rooting from hardwood which restrict the popularization and application seriously by using vegetative propagation technique. Genetic translation with Ri plasmid is effective way for improving the rooting ability of the plant. But the trans-genetic plants demonstrate serious phenotype variation at the same time. For example apical dominance is weakened, shortening, male sterility, early-maturing, *etc*. Studies indicated that Ri plasmid contain four *rol* genes at least. The expression of *rolB* gene of Ri plasmid can induce large amount of hair rooting alone and the trans-genetic plants with *rolB* gene have certain weakened apical dominance variation.

The gibberellins are the important regulation hormone during plant development and growth. Promoting the apical dominance of plant is the main physiology function of gibberellins. With more and more gibberellins mutants discovered, it has been confirmed in the past ten years that the GA20-oxidize was one of the main feedback regulate center on the gibberellins biosynthesis pathway. The cloning of GA20ox gene has made important progress in recent years. At present, the cDNA of GA20ox have been cloned from 7 species at least. Improving the rooting ability and apical dominance of *Populus tomentosa* with *rolB* and *GA20ox* two genes at the same time is important for the popularization of the excellent asexual cloning and for the improving growth amount of *Populus tomentosa*.

In this paper, plant binary vector containing both *rolB* and *pttGA20ox* (*Populus tomentosa GA20-oxidase*) genes was constructed for the first time; The regeneration system of the poplar leaf explants was established by orthogonal design; The gene transformation receptor system of the new poplar genotype was optimized and the experiment of antibiotic sensitiveness to it has been carried on; On the basis of constructed *rolB* and *pttGA20ox* gene plant vector by restriction endonuclease and PCR method with high fidelity DNA polymerase of pfu and pyrobest, the binary vector was constructed with both that two genes; The gene transformation of *rolB* and *pttGA20ox* with leaf dishes of *P. tomentosa* were conducted by *Agrobacterium* LBA4404; The transgenic *P. tomentosa* was identified by molecular biology method such as antibiotics, PCR and Southern blot. And also the rooting ability, apical dominance and phenotype variation of the three kind of trans-genetic *Populus tomentosa* were analysed in vitro.

The results indicated that: The optimal medium compositions for adventitious buds regeneration from leaf of *Populus tomentosa* was MS+6-BA 1.0mg/L+ZT 0.3mg/L+NAA 0.3mg/L; Plant genotype has significant influence on the regeneration efficiency among the six *P.tomentosa* clones; The regenerations ability of clone L2 and L5 is the highest, leaf regeneration frequency of adventitious buds reached 98.3% and 91.7%, respectively; The adventitious bud differentiation rate of different explants types was leaf (95.1%)>stem segment (66.3%)>root

segment (61.5%); The critical kanamycin sensitive concentration for inducing shoots and roots of *P.tomentosa* was 20mg/L and 25mg/L, respectively; Pre-culture of explants for 2days before infection with *A.tumefaciens* and co-cultivation for 2-4 days after infection would be favorable for the two genes transformation; Co-cultivation medium supplemented with 200uM acetosyringone was able to increase 3.13% for the transformation frequency; The average rooting rate of *P.tomentosa* transformed with both *rolB* genes higher remarkably than that of contrasted plants at the same hormone content; On the rooting culture medium, the rooting rate of *P.tomentosa* transformed with both *rolB* genes take up to 87.5% - 100% , whereas the rooting rate of contrasted *P.tomentosa* is only 16.2%.

The phenotype of the apical dominance and rooting ability of tobacco with both *rolB* and *pttGA20ox* two genes possess both characters of the tobacco transferred with individual *rolB* gene and *pttGA20ox* gene at the same time. The studies on genetic transformation with *rolB* and *pttGA20ox* two genes to improve rooting ability and apical dominance of *P. tomentosa, P.alba* and *P.euphratica* wich rooting are difficult are carrying out in Beijing Forestry University in P.R. China.

Leader Elongation Patterns in Control- and Open-Pollinated Loblolly Pine Families

J.H. Myszewski and F.E. Bridgwater[1]

Studying how trees achieve superior height growth by examining their leader elongation patterns could lead to a new approach to selection and breeding for height growth in loblolly pine. Previous work has shown that loblolly pine families are able to achieve above average height growth using different leader elongation strategies (Bridgwater et al. 1985; Bridgwater 1990). Some extend stem units that are longer than average and some elongate a greater number of stem units per year. If the different leader elongation strategies are under genetic control and the genes controlling them behave in an additive manner, it may be possible to combine shoot elongation strategies in control-pollinated (CP) progeny and produce greater genetic gains than achievable with the mating of individuals based solely on height. This study was designed to examine the shoot growth patterns of CP progeny resulting from a cross between parents with different leader elongation strategies.

MATERIALS AND METHODS

Two first-generation selections, 8-061 and 8-103, from the North Carolina State University-Industry Tree Improvement Cooperative were chosen for this study. Both were originally selected along the North Carolina coast and both were located in the G.W. Weyerhaeuser Seed Orchard in Washington, NC. Previous work revealed that 8-061 produced open-pollinated (OP) progeny with an above average mean stem unit length and 8-103 produced OP progeny with an above average number of stem units (Bridgwater 1990). CP seed from 8-061 X 8-103 and OP seed from each parent were cold stratified according to standard protocols, germinated on moist filter paper and sowed in Deepots™ in a greenhouse in College Station, TX. In 2001, 189 progeny from each of the three seed lots were planted in a randomized block design on the Harrison Experimental Forest in Saucier, MS. The test contained 10 blocks, each with 19 three-tree plots. In each plot, one seedling from each of the three seed lots was planted in random order. Spacing was 3.0m between blocks and 1.2m within blocks.

Total tree height and annual height increment were measured after the first and third year in the field. Following the third year, trees were harvested and the number of sterile cataphylls, fertile cataphylls (cataphylls producing needles), branches and elongation cycles in the third height increment were counted. Entire plots were excluded from the analysis if any individual tree from any of the three seed lots in the plot was dead or had been damaged by tip moths. Least squares means by family were then calculated for each variable and used to run t-tests.

RESULTS AND DISCUSSION

The results indicate that the CP progeny did indeed benefit from the combination of different leader elongation strategies. At age 3, CP progeny had significantly greater total height growth than the OP progeny of either parent (Table 1). CP progeny also had the greatest annual height increments in the first and third growing seasons. These results are not surprising since the CP

[1] USDA Forest Service, Southern Institute of Forest Genetics, Saucier, MS

progeny had 2 parents with superior height growth while the OP progeny could have resulted from crosses with slower growing parents or from inbreeding. The fact that the CP progeny produced a total number of stem units similar to that of their 8-103 half-sibs and a mean stem unit length similar to that of their 8-061 half-sibs, however, is significant. This suggests that the genes controlling shoot morphology and the different leader elongation strategies may work in an additive manner. Interestingly though, when the data is examined by elongation cycle (Table2), the number of stem units produced by the CP progeny was only similar to that of the 8-103 OP progeny in the first elongation cycle which is the cycle associated with fixed growth (i.e. the spring shoot) and the number of fertile cataphylls produced by the CP progeny was significantly lower than that produced by the 8-061 OP progeny. In the second through fifth elongation cycles which are the cycles associated with free growth, the number of fertile cataphylls produced by the CP progeny was statistically similar to that of their 8-061 half-sibs, not their 8-103 half-sibs. No statistical differences were seen in the number of sterile cataphylls after the first elongation cycle. Thus, the reason CP progeny have more stem units is not that they have an increased number of stem units per cycle (no statistical differences were observed in the mean number of branches per elongation cycle or in the majority of within elongation cycle mean number of branches) but that they have a significantly greater number of elongation cycles per year than either half-sib family. In fact, the CP family had 1.5 to 2 times as many trees (61) with 6 or more elongation cycles than either half-sib family (30 and 36 for 8-061 and 8-103, respectively). Since the number of elongation cycles per year can be influenced by environmental factors (e.g., Zahner 1962), it is not known if this pattern would hold across environments and/or across years. Therefore, this study should be repeated using additional families and locations before any strong conclusions are drawn regarding the genetics of shoot morphology.

Table 1. Least squares means for leader elongation components of control- and open-pollinated loblolly pine families (means with different letters are significantly different at α=0.05).

	Family		
	8-061 OP	8-103 OP	8-061 X 8-103
Totals for Third Growing Season:			
Total Tree Height (cm)	169.2 [a]	167.1 [a]	179.9 [b]
3rd Annual Height Increment (cm)	89.1 [a]	92.8 [a]	98.5 [b]
Number of Cycles	4.9 [a]	5.1 [a]	5.5 [b]
Stem Units	446.4 [a]	488.9 [b]	489.9 [b]
Sterile Cataphylls	107.1 [a]	116.3 [a]	134.2 [b]
Fertile Cataphylls	321.3 [a]	353.4 [b]	335.3 [a]
Branches	18.0 [a]	19.2 [b]	20.4 [c]
Mean Stem Unit Length (cm)	0.20 [b]	0.19 [a]	0.20 [b]
Mean per Cycle:			
Sterile Cataphylls	21.7 [a]	22.8 [a]	24.4 [b]
Fertile Cataphylls	67.4 [b]	70.0 [b]	62.7 [a]
Branches	3.7 [a]	3.8 [a]	3.7 [a]

Table 2. Least squares means by elongation cycle for leader elongation components of control- and open-pollinated loblolly pine families (means with different letters are significantly different at α=0.05; means in parentheses are the statistical averages of variables for which least squares means could not be estimated).

		Family		
	Cycle	8-061 OP	8-103 OP	8-061 X 8-103
Sterile Cataphylls	1	12.9 [a]	18.0 [b]	17.4 [b]
	2	19.6 [a]	17.5 [a]	19.8 [a]
	3	22.2 [a]	21.6 [a]	24.1 [a]
	4	24.7 [a]	24.8 [a]	25.6 [a]
	5	28.1 [a]	29.5 [a]	30.7 [a]
	6	(34.6)	(32.8)	(33.2)
	7	(33.2)	(46.8)	(41.2)
	8			35.0
Fertile Cataphylls	1	130.2 [b]	122.0 [ab]	115.5 [a]
	2	54.3 [a]	62.5 [b]	58.0 [ab]
	3	50.9 [a]	56.9 [b]	49.4 [a]
	4	47.1 [a]	53.8 [b]	49.1 [a]
	5	42.1 [a]	50.6 [b]	40.1 [a]
	6	(46.6)	(61.9)	(47.5)
	7	(55.7)	(53.8)	(45.1)
	8			65.0
Branches	1	3.0 [a]	3.2 [a]	3.0 [a]
	2	3.5 [a]	3.7 [a]	3.7 [a]
	3	3.6 [ab]	3.8 [b]	3.4 [a]
	4	4.1 [a]	4.1 [a]	4.2 [a]
	5	4.1 [a]	3.9 [a]	3.9 [a]
	6	(4.3)	(4.5)	(4.4)
	7	(5.1)	(4.8)	(5.3)
	8			5.0

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Growth and Genetic Variability in a Combined Provenance Test of an American Pine Species (*Pinus elliottii*) and A Chinese Pine Species (*P. massoniana*) Planted in the Southeastern USA

R.C. Schmidtling[1], J. Myszewski[2], C.D. Nelson[3] V. Hipkins[4] and L.H. Zeng[5]

[1]Scientist Emeritus, [2]Geneticist, [3]Project Leader, USDA Forest Service, SRS, Southern Institute of Forest Genetics, Saucier, MS 39574, [4]Geneticist, NFGEL Lab.,USDA Forest Service, Institute of Forest Genetics, Placerville, CA, and [5]Vice-Director, Guangdong Forest Research Institute, Guangdong, China.

The climates of southeastern China and the southeastern USA are very similar. The range in latitudes are identical, they both are bordered on the east/southeast by large oceans with northward currents offshore, and they both have large land masses to the north and west effecting similar continental climates. It is reasonable to assume that forest tree species from one country should be well adapted to the other. Indeed, pines from the southeastern USA are widely planted in China, where they generally grow better than the native species. This study seeks to evaluate growth and genetic variation of a Chinese pine compared to an American pine grown in the southeastern USA.

Growth was measured and buds were sampled for allozyme analysis in a provenance test containing 7 seed sources of slash pine *(Pinus elliottii)* and 10 seed sources of Masson's pine *(P.massoniana)* planted in two locations, south Florida and south Mississippi. The seed sources represented the full north-south geographic range of both species. The hypothesis was that because of thousands of years of clearing and high-grading, genetic variability and possibly growth would be less in the Chinese pine than in the American pine.

The overall average number of alleles per locus was identical for the two species, 2.0. The percentage of loci polymorphic was only slightly lower in Masson's pine, 56% versus 64% for slash pine. Direct count (actual) heterozygosity averaged 0.133 in the American pine. Heterozygosity averaged considerably lower, 0.069, in the Chinese pine and was highly variable among seed sources. The alleles appear to be present in Masson's pine, but are less likely to be in the heterozygous state.

Growth of the Chinese pine was much less after 10 years than the American pine in both locations. In the Mississippi planting, the slash pine averaged more than twice as tall as the Masson's pine, 24.8 feet versus 12.0 feet. The allozyme data and the growth data probably indicate a higher level of inbreeding in Masson's pine than in slash pine.

Thus, there are important differences in genetic variability and growth between Masson's pine and slash pine, probably related to the differences agricultural and cultural history. Tree improvement programs for Masson's pine in China are underway, and this may be effective in salvaging some of the existing genetic variability for the species.

Time Trends of Genetic Parameters and Provenance Variation of *Pinus halepensis* Mill. in Greece

P.G. Alizoti[1], Bailian Li[2], and K.P. Panetsos[1]

[1]Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece (E-mail of corresponding author: alizotp@for.auth.gr)
[2] Department of Forestry, North Carolina State University, Campus Box 8002, Raleigh, NC 27695, USA

Pinus halepensis Mill. is a valuable and prominent low elevation Mediterranean pine with a wide distribution ranging from southern Europe and Morocco in the West, to Syria in the Southeast and mainland of Greece in the North-East of the Mediterranean basin. The species' ability to grow in the adverse climatic conditions of the Mediterranean region; pronounced bio-seasonality with dry and hot summers, moist and cool winters, large year to year variability of total rainfall, combined with its fast growth in favorable sites, make it a very important multipurpose species for forestry. Moreover, its ability to endure forest fires through specific mechanisms, renders it irreplaceable for the special and delicate Mediterranean ecosystem. Eleven Hellenic provenances of the species, representing its natural distribution in the country, were assessed for their height growth and survival, at successive measurement ages (4, 6, 8 and 10 years of age), in two provenance-progeny trials established in Greece. Each population was represented by ten open-pollinated families. Main objectives of the study were to: (1) determine the extend and nature of genetic variation among and within provenances, (2) study the time trends of genetic parameters, (3) estimate age-age genetic correlations and determine the efficiency of early selection, (4) determine whether the eleven provenances differ for height growth and survival, as well as whether their performance differences are maintained as time progresses, (4) evaluate the level of GxE interaction *(gei).*

Remarkable differences were found among *P. halepensis* provenances. Northern provenances exhibited by far better growth for height at all measurement ages. Survival of provenances was high. Selection of the best performing provenance at successive measurement ages could yield 17%-20% increase above the average height growth at the respective measurement age, while the higher increase was recorded at age 8. Ample additive genetic variation for height growth was detected across sites at all ages, while a slight decline was recorded at age 10. Provenances' genetic variation was higher than additive genetic variation after the age of 6 years. Genetic control of height growth varied greatly among provenances. The stronger additive genetic control found in the northern provenances coupled with the marked differences among families for their height growth performance, indicated the high potential of selection within these provenances. Age-age genetic correlations were very high. Efficiency of early selection ranged among 99.2% to 100%. Rank correlation coefficients among family breeding values at different ages showed that best parents' selection could be performed with quite high precision even at the early age of 4 years. Type B provenance correlations were higher than Type B genetic correlations in all cases. There was a low ***gei*** at both the provenance (r_{Bp}: 0.93-1.00) and the family level (r_B=0.70-0.73) at all measurement ages. Results point towards the urgent necessity to take further steps for the genetic improvement of the species to secure substantially better growth combined with adaptation to future environmental changes.

Blister Rust Resistance of *Pinus strobus* and Its Interspecific Hybrids with *Pinus wallichiana*

P. Lu, R. W. Sinclair, and T. J. Boult

Ontario Ministry of Natural Resources, Ontario Forest Research Institute, Sault Ste. Marie, Ontario P6A 2E5

Extended abstract

Since its introduction from Europe at the turn of the 20th century, white pine blister rust has contributed to the decline of eastern white pine in North America. In Ontario, tree breeders have worked for decades to develop disease-resistant eastern white pine genotypes through controlled crosses among rust-free trees. Attempts were also made to introduce disease resistance genes from related white pine species in the subgenus "strobus" using traditional plant breeding approaches, such as interspecific hybridization followed by back-crossing. The objectives of current study were to: 1) evaluate blister rust resistance in advanced generations of white pine breeding populations in Ontario, 2) assess field performance of selected rust-resistant genotypes, 3) develop breeding and deployment strategies for rust-resistant white pine hybrids, and 4) investigate the mechanisms of blister rust resistance in white pine seedlings.

In this study, 6 – 18 month-old seedlings from more than 200 open-pollinated families of *Pinus strobus* and its interspecific hybrids were artificially inoculated with *Cronartium ribicola* in the greenhouse with pathogen inoculum density greater than 5,000 basidiospores per cm^2. Blister rust infection and seedling mortality were assessed biweekly or monthly. Inherited disease resistance was evaluated based on seedling mortality.

Results indicated that *P. strobus* seedlings generally lacked strong genetic resistance to blister rust and suffered high post-inoculation mortality (up to 99%) at the end of experiments. Although the selected *P. strobus* parent trees had been exposed to *C. ribicola* in previous tests, the survival of their progeny under artificial inoculation at the seedling stages was not enhanced. In contrast, some open-pollinated families of the second (F_2) and third (F_3) generations of *P. strobus* hybrids with *P. wallichiana* have demonstrated significant improvement in post-inoculation seedling survival rates (>50%), indicating the successful introduction and transmission of resistance genes over generations. An approximate 50% segregation ratio in seedling mortality for some of the hybrid families also tended to match the theoretical expectation (50%) based on the heterozygous hybrid mother trees and presumably pollen source from *P. strobus*, suggesting major gene resistance in this trait.

Field trials indicated that F_1 hybrids between *P. strobus* and *P. wallichiana* were less cold-hardy and could not grow and survive well in northern Ontario, although they performed competitively in southern Ontario. F_2 and F_3 backcross hybrids improved cold hardiness significantly and adapted much better than F_1 on more northerly sites. To improve both blister rust resistance and climatic adaptation, a multi-generation backcross strategy is being implemented in Ontario with an objective of producing F_4 backcross white pine hybrids in the next 10 -15 years, which are expected to have about 94% of their genome as *P. strobus* and almost all desirable characteristics, as well as blister rust resistance, of *P. strobus.*

Key words: Eastern white pine, *Cronartium ribicola,* disease resistance

Modelling The Impact of Adverse Correlations on Selection Response Over Generations: Some Alternative Strategies.

L. Sanchez[1] , **J.N. King**[2] and A.D. Yanchuk[2]

[1]INRA Orleans, Orleans, France;

[2] BC Ministry of Forests, Research Branch, Victoria, BC, Canada

Modelling the response of a single trait has been an important part of breeding population management in tree improvement for some time now and we have developed a comfort factor in using this tool for managing population sizes and structures, mating designs, inbreeding, and diversity. A major and realistic issue we now face is how to effectively manage for adverse correlations. We present results of a Monte-Carlo modelling project we are developing. First we outline the approach that we take with this allele-based modelling and present a range of gene models: additive models with and without pleiotropic loci of antagonistic effects, with and without between-trait linkage, cases with large-effect pleiotropic genes and some models with dominance. With these models we look at efficiencies of selection methods. Particularly the comparison of independent culling (IC) and a Smith-Hazel index selection (SH). In most scenarios it appears IC can be more efficient. Secondly we look at some particular examples we face in our programs of adverse correlations both negative and positive and add a profit function to our selection process – in order to evaluate costs as we balance gain and diversity. Adding this feature it also appears IC has some advantages. Finally we present ways we hope to develop this model using some of the new optimum breeding procedures to add to the selection processes in order to evaluate structure (single trait lines vs multi-trait lines) and population size to maintain an effective response to selection against such adverse correlations.

Bayesian Inference Of Candidate Gene Effects In Family Structured Data

Patrik Waldmann

Department of Forest Genetics and Plant Physiology, SLU, SE-901 83 Umeå, Sweden

The recent development of molecular techniques have generated a vast amount of data and it is now possible to directly infer the effects of candidate genes on phenotypic traits. Many statistical methods have been developed for estimation of candidate gene effects and QTL association. The general idea is to study if the mean phenotype differ between certain genotypes or if particular loci or alleles are correlated to the values of the phenotypes. However, individuals may have common ancestors to varying degree both within and between samples that are believed to be random. Such genetic non-randomness, for example caused by admixture, can cause biased effects and contribute to false positive associations. One way to avoid such spurious effects is to use family structured methods, the most famous is the TDT test and its extension to continuous traits. Unfortunately, the chi-square based TDT tests are not suited for estimation of candidate gene effects.

The background polygenetic variance that is introduced by the relationships among individuals within families can be accounted for by using standard quantitative genetic methods. Using a mixed model it is then possible to estimate the effect of candidate genes as fixed effects. Current advances in computing power have facilitated the implementation of MCMC methods and virtually contributed to a Bayesian revolution in statistical genetics. Here, a Bayesian Gibbs sampling approach of the single trait individual tree model is presented. One major advantage of Bayesian methods is that the full posterior distributions easily are obtained for all parameters in the model. Hence, probabilities for differences between genotypes can be deduced from the posterior distributions. The method is tested on a data set by adding simulated candidate genes to individuals from an earlier published diallel study in *Pinus sylvestris*. Posteriors for different simulated genotype effects and for the additive and dominance genetic variances of height are obtained.

Genetic Gain Predictions for Coastal Douglas-fir in the US Pacific Northwest and Their Relationships with Parent Tree Location

K.J.S. Jayawickrama and T.Z. Ye[1]

INTRODUCTION

The IFA-PNW "Progressive Tree Improvement System" emphasized forming local cooperatives to share costs, and on progeny testing lots of trees using wind-pollinated seed in small testing zones. This phase ran from 1967 till 1993, during which over 26,000 first-generation Douglas-fir parents were tested in 109 breeding units, with over 3 million progeny test trees planted. A typical breeding unit was designed for 100,000 acres of commercial timberland, extended 70km north-south and 50km east-west, but due to the varied topography in the Pacific Northwest could contain parent trees spanning as much as 600m (2,000 feet) in elevation. In some cases families originating from outside the breeding zone were included in the tests.

Since late 2003, the NWTIC began to predict genetic gains for first-generation breeding programs using Best Linear Unbiased Prediction. These first-generation programs are the basis for seed production in most orchards and for advanced-generation breeding, and estimates of gain are useful for many purposes (roguing and establishing orchards, collecting and deploying seed, valuing the contribution of tree improvement to boosting productivity of plantations and selecting parents for advanced-generation breeding). Few estimates of genetic gain have been published for coastal Douglas-fir in Oregon and Washington.

Elevation, latitude and departure information are available for most first-generation parent trees. With a dominant species covering such a wide geographic range, with possibilities of pollen flow and migration, continuous genetic variation seems likely. An early analysis of two of these programs suggested that growth rate increased with decreasing elevation, and shifts to the east or north (Silen and Mandel 1983). While the first-generation programs are individually inadequate to give an adequate estimate of clinal variation, they provide many independent estimates of variation over a small north-south and east-west range, and represent the largest sample of wild Douglas-fir parent trees that will ever be tested in the Pacific Northwest.

This paper therefore summarizes results to date in predicted gains and relationships between predicted gains and latitude, departure and elevation.

MATERIALS AND METHODS

A typical first-generation program tested 200-300 wind-pollinated families (seed collected from parent trees in the wild) on eight to 10 sites, with 12 to 16 trees per family per site. To keep replicates small, families were usually grouped into sets of 25-30 families and tested using the

[1] Director and Quantitative Geneticist respectively, Northwest Tree Improvement Cooperative, Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752.

"Reps-In-Sets" design. In this design, each set was essentially an independent experiment, with three to five replicates grouped together. Three to four trees were typically planted per family in non-contiguous plots. Sets were generally all represented on common sites. Some tests were established using the "Sets-In-Reps" design, with sets randomized in replicates; this design is better suited to compare families across sets than the "Reps-In-Sets" design. In some cases trees originating from different parts of the breeding unit were randomly allocated to sets, in others trees from different parts of the breeding unit were consciously grouped into different sets ("geographic sets").

Gains are predicted for age-10 and age-15 height, age-15 dbh and age-15 volume (as dbh^2 x height). Gain1, Gain2, and Gain3 are predicted using three linear models respectively: model1 considers set to be a fixed effect, model2 considers set to be a random effect, and model3 drops set from the model. Model1 and model2 assume that all differences between sets are due to environmental variation within test sites, while model3 does not. Since unimproved controls were generally absent, the population mean is assumed to equal zero gain. This assumption should be reasonably valid, since the Progressive Tree Improvement system stressed collecting seed from well-separated parent trees during good seed years, rather than intensive plus-tree selection.

Genotype x environment interaction is investigated using a test of crossover interaction. Tests are conducted for each pair of families evaluated across each pair of sites based on least-squares means and standard errors derived from *ANOVA* for each set at each site. Finally, family stability is expressed as the significant *COI* as a percentage of the total number of interactions for each family.

For programs analyzed to date and for which both height and volume gains could be predicted (27 programs testing 7,358 parents, which is less than 25% of all the programs), average gains for the top 1% and top 5% of parents were calculated. These proportions are relevant since 1.5-generation orchards are being established combining approximately the best 2-5% of parents from several adjacent programs. Volume Gain1 and Gain3 were regressed on latitude, departure and elevation. The average regression coefficients and correlations were calculated.

RESULTS AND DISCUSSION

Age-15 volume gain predictions for the top 1% of first-generation parents in a program typically exceeded 50%, and were typically three times as large as the age-15 height gain predictions.

Table 1. Predicted age-15 volume gains for top parents in first-generation cooperative Douglas-fir tests

Selection Intensity	Volume Gain1 %	Volume Gain3 %	Height Gain1 %	Height Gain3 %
Top 1% of parents	50.5	53.3	16.3	16.7
Top 5% of parents	37.7	39.1	12.3	13.1

Parents with the highest height gain predictions did not always have the highest volume gain predictions, and vice versa – the family-mean correlation between Ht Gain1 and Volume Gain1 was 0.86. The correlations between Gain1 and Gain3 were strong (0.94 for height, 0.97 for

volume). If top parents with the highest predicted gains in several adjacent programs are available, it seems feasible to build a 1.5-generation orchard with 40% predicted age-15 volume gain.

Within testing zones, predicted gains tended to increase with decreasing elevation (15 of 20 regression estimates with Volume Gain1 and 19 out of 21 regression estimates with Volume Gain3 were negative), but the correlation between predicted volume gain and elevation was weak. Correlations between predicted volume gain and latitude and departure were essentially zero. In some cases Gain3 was more strongly related to parent tree location than Gain1 or Gain2, which might be explained by the fact that with "geographic" sets, adjusting out set effects partially removes geographic trends. The strength of the relationships with geographic variables varied from program to program.

Table 2. Relationships with predicted age-15 volume gains in first-generation cooperative Douglas-fir tests.

Variable	Average Correlation Coefficient		Average Regression Coefficient	
	Volume Gain1 %	Volume Gain3 %	Height Gain1 %	Height Gain3 %
Latitude (km)	-0.01	-0.01	-0.037	-0.035
Departure (km)	0.01	0.03	0.023	0.090
Elevation (m)	-0.07	-0.09	-0.011	-0.017

Results obtained so far (from less than 1/4 of all the testing zones) provided little evidence to reconsider the amalgamation of first-generation zones in the second-generation testing program, at least for growth rate. Typically less than 10% of the crossover interactions in a family are significant. Any small incremental gain from progressing breeding in multiple smaller zones instead of the second-generation zones would be more than offset by increased cost. For example, we can consider the largest second-generation zone, which is 340km (210 miles) north to south and has a 600m (2,000 ft) elevation range. Based on these results, the difference in predicted volume gain north to south (for parents within the first-generation zones contributing to that zone) would be around 12%, and across the elevation range around 11%. The weak correlations between predicted gain and parent tree location also suggest it is possible to find high-gain parents throughout most first-generation breeding zones.

We plan to revisit these summaries when the remaining analyses are completed, and augment them with estimates of the variation of survival with parent tree location. The predicted gains are to be calibrated by estimates of realized gain from realized gain trials: NWTIC established one such trial in 1997 (St Clair et al. 2004) and a second one is to be planted in 2005 and 2006. NWTIC also plans to use the vast pool of progeny data for validation of gain predictions.

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Survival and Growth of *Fraxinus ornus* Provenances Tested in Reciprocal Transplants Experiments for Ecological Adaptation

K. A. Spanos[1], D. Gaitanis[1] and D. Mylonas[1]

Abstract: Six (6) provenances of the multipurpose tree species *Fraxinus ornus* have been tested in a network of reciprocal transplant experiments (RTEs) in N. Greece for growth, ecological adaptation and natural regeneration. The provenances represent six (6) indigenous populations from the following geographic areas: Stavroupolis (10FOR), Nevrokopi (11FOR), Nigrita (12FOR), Poligiros (13FOR), Kastania (14FOR) and Stomio (15FOR). Field trials (RTEs) were established in spring (March) of 2003 using one-year-old transplants (1+1). Parameters recorded at the end of the year (November/December 2003) were survival (%) and height increment (mm). Survival percentages of the tested provenances were not significantly different ((P>0.05, across all sites) whereas the differences between the planted sites were highly significant (P<0.01). The highest survival achieved in Nevrokopi (95.9%) and the lowest in Stomio (43.4%). In relation to height increment, the differences between provenances (across all sites) were significant at 10% level. Provenance 14FOR (Kastania) achieved the highest height increment (mean 72.2 mm) whereas 11FOR the lowest (57.1 mm). Differences between the planted sites (for all provenances) were also significant at 1% level. Nevrokopi planted site gave the best results (mean 81.2 mm) whereas sites Nigrita and Stomio the lowest values (51.6 mm and 53.8 mm, respectively). The interactions between provenances and the planted sites were not significantly different (P>0.05) for both parameters (survival, height increment). In general, local provenances did not outperform the distant ones in most field sites. The present case study demonstrated that it is hard to draw conclusions for the performance (growth/adaptation) of the local seed sources in this early stage of provenance trials. Long term testing (at least 10 years) is advisable for evaluation of provenances' performance in the different environments.

Key words: *Fraxinus ornus,* reciprocal transplant experiments, provenance, testing, growth, adaptation.

INTRODUCTION

Fraxinus ornus is a multipurpose tree, medium size (5-12 m) but it can become large tree (up to 20 m) in fertile humid soils. Genetic variation in species indigenous populations comprises the basis for restoring and expanding natural ecosystems. The genetic management of *Fraxinus ornus* populations is important for genetic conservation, provenance choice, seed supply and provision of certified and improved material. Species local populations are seen as desirable for both conservation and genetic improvement. Selection for production forestry is based on growth, form and other commercial criteria. In contrast regeneration under natural conditions, and particularly for ecological restoration, requires an emphasis on different traits such reproductive vigour, seedling survival, competition ability and long term adaptation (Ennos et al, 1998, Spanos et al., 2004). Adaptive variation of different populations may vary according to species, with some showing close correlation between adaptive variation and environment and others being

[1] N.AG.RE.F. – Forest Research Institute, 57006 – Vassilika, Thessaloniki, Greece

more plastic (Raymond and Lindgren 1990, Ennos et al, 1998). Reciprocal transplant experiments (RTEs) directly estimate localised adaptation to different environments by testing the fitness of 'home' (local) and 'away' (distant) genotypes within the sites from which the genotypes originate. Provenance testing is the first step to study genetic variation of a tree species and to start genetic improvement plans (Clausen 1984, Worrell 1992, Cundall et al. 1998, Ennos et al. 1998, Steinhoff 1999, Cundall et al. 2003, Spanos et al. 2004). Up to now, no complete study on genetic variation and provenance testing of *Fraxinus ornus* has been carried out in Greece. The objective of this work is to study *Fraxinus ornus* different seed sources (provenances) for growth and adaptation in a series of reciprocal provenance trials established within the populations where seed sources originate. Early selection can provide recommendations on seed source selection for natural regeneration and ecological adaptation.

MATERIALS AND METHODS

Six indigenous populations of *Fraxinus ornus* have been identified and selected in N. Greece (Fig. 1, Table 1) for reciprocal provenance trials (RTEs). Seed was collected from 30-50 trees (50-100 m) apart in each of the selected populations in November/December, 2001. Seed of each source was cold stratified (0-4° C, in moisten peat/perlite 1:1) for 10 weeks (January/February/March, 2002) in a cold storeroom at the Forest Research Institute. In March 2002, stratified seeds were removed and sown outdoors (FRI forest nursery) in the soil medium taken from each site (where seed sources originated) and left for germination. Seeds germinated in April, 2002 and remained outdoors till planting (February/March, 2003) on the selected sites. Within each of the selected populations (Fig. 1) one trial with all selected seed sources (including the local) was established for growth and ecological adaptation. In each field site 30 to 70 (depending upon availability) 1-year old seedlings per provenance were planted in a single tree plot design. Seedlings were left to grow in the field till next autumn (2003). In each planted site, manual weeding was applied in May/June once and irrigation of seedlings twice a month for the dry period July/August, 2003. In autumn (November/December, 2003) the following parameters were recorded: survival, height increment (year 2003) and diameter at root collar (unpubl. results). In this paper, survival (% of the planted seedlings) and height increment (mm) of the six (6) different seed sources in the six (6) tested sites are presented.

Table 1. Geographic details of *Fraxinus ornus* provenances planted in the tested sites.

Proven. Code	Site	Latitude (°)	Longitude (°)	Altitude (m) (a.s.l)
10FOR	Stavroupolis (10)	41.14.384 N	24.39.905 E	332
11FOR	Nevrokopi (11)	41.16.604 N	23.44.341 E	817
12FOR	Nigrita (12)	40.51.764 N	23.24.013 E	542
13FOR	Poligiros (13)	40.26.500 N	23.19.100 E	577
14FOR	Kastania (14)	40.26.370 N	22.24.412 E	300
15FOR	Stomio (15)	39.52.599 N	22.39.751 E	380

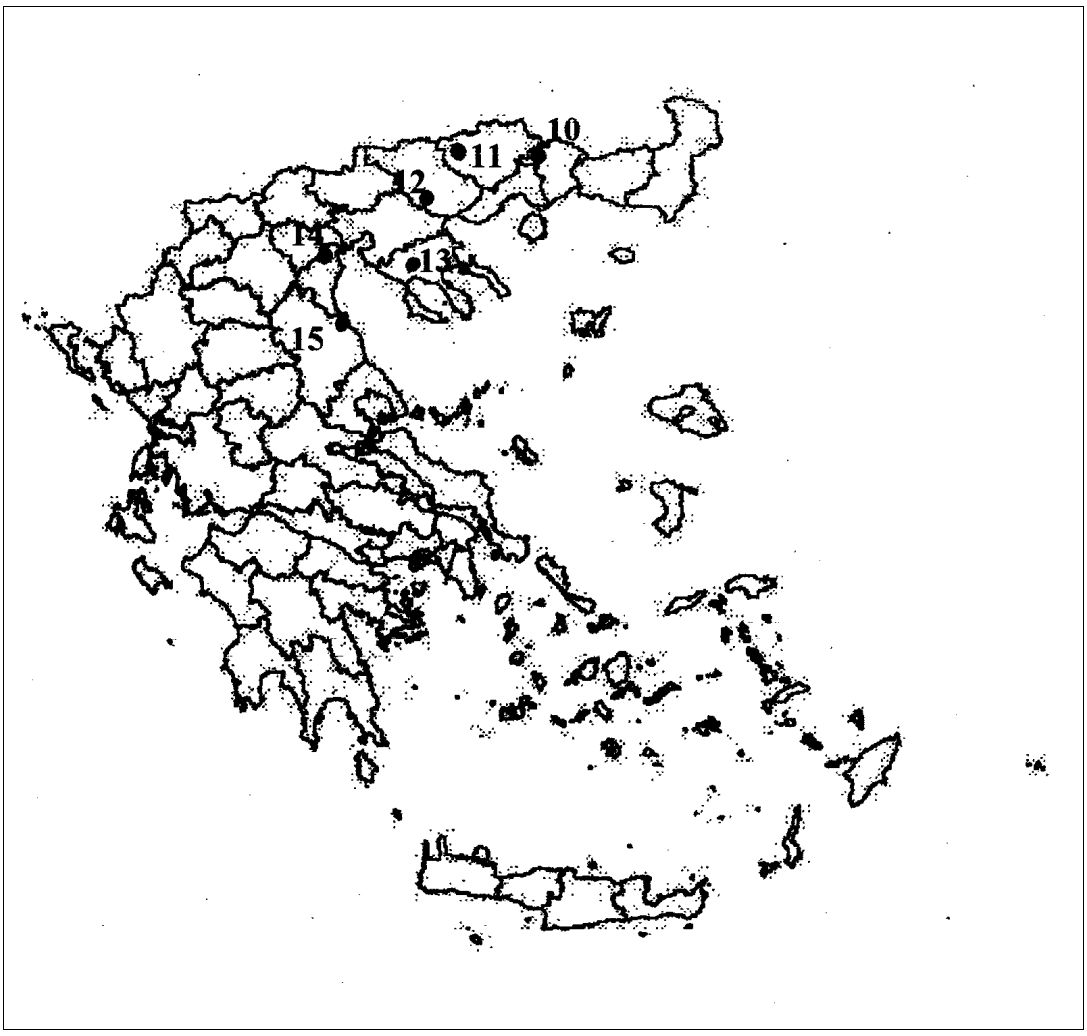

Figure 1. Provenances (10, 11,…, 15) and planted sites (•10/Stavroupolis,…..,15/Stomio) of *Fraxinus orrnus* selected in Greece.

RESULTS AND DISCUSSION

High survival rates were recorded for the six (6) tested seed sources (overall mean 71.0%) (Table 3). Analysis of variance for survival showed that there were no significant differences ($P>0.05$) between tested provenances across all sites whereas the differences between the planted sites were highly significant ($P<0.01$) (Table 2). Survival percentages of the tested provenances in the tested sites are presented in Table 3. The results demonstrate that overall survival percentages (average of all sites) were not significant different (ANOVA, $P>0.05$) between the six seed sources. The local seed sources perform well in regards of survival but in many cases did not outperform the non-locals (Table 3). Results also show that survival rates were significant different between the tested sites (ANOVA, $P<0.05$). The highest survival was recorded for Nevrokopi (94.9%) site whereas the lower for Stomio (43.4%). This can be explained by the different environmental conditions (lower altitude/drier conditions in Stomio).

Table 2. Analysis of variance for survival (provenances/sites).

Source of variation	**DF**	**SS**	**MS**	**F**	**P**
Provenance	5	550.919	110.184	0.272	0.925
Error	30	12174.828	405.828		
Total	35	12725.748			
Site	5	10319.289	2063.858	25.729	0.000
Error	30	2406.458	80.215		
Total	35	12725.747			

Table 3. Survival (% of planted seedlings) of *Fraxinus ornus* provenances planted in the tested sites [+].

SITE	Provenance Code 10FOR	11FOR	12FOR	13FOR	14FOR	15FOR	Mean **
Stavroupolis (10)	89.5	63.3	79.6	71.1	68.0	90.0	*76.9* [bc]
Nevrokopi (11)	96.9	97.0	95.3	87.1	96.4	96.8	*94.9* [a]
Nigrita (12)	62.5	41.3	60.2	62.4	58.9	52.7	*56.3* [d]
Poligiros (13)	82.6	79.3	89.0	85.9	76.5	82.6	*82.7* [b]
Kastania (14)	53.7	81.2	73.7	68.7	72.5	79.4	*71.5* [c]
Stomio (15)	35.9	26.7	50.0	35.8	52.9	59.1	*43.4* [e]
Mean [ns]	*70.2*	*64.8*	*74.6*	*68.5*	*70.9*	*76.8*	*71.00*

[+] ns - not significant difference (P>0.05), ** significant difference (P<0.01, ANOVA -test), means with the same superscript letter do not differ significantly (P>0.05, Duncan test).

From the analysis of variance (ANOVA) for the parameter height increment, significant differences (P<0.01) were found between the tested sites (Table 4). The difference between the tested provenances was significant at 10% level (P<0.1) whereas the interaction was not significant (5% or 10% level). Seed sources 14FOR and 15FOR achieved the highest height increment (across all sites - mean 72.9 mm and 69.8 mm, respectively) whereas seed sources 11FOR and 13FOR the lowest (mean 57.1 mm and 59.5 mm, respectively) (Table 5). The difference between pairs was significant (pairwise T-test, P<0.1) only in the case 11FOR vs. 14FOR. The local seed sources did not outperform significantly (P>0.05) the distant ones in most planted sites (Table 5). In relation to the differences between the planted sites, Nevrokopi (11) was the best (mean of all seed sources – 81.2 mm) whereas Stavroupolis (10) the worst (mean 31.1 mm). Differences between the tested sites are most possibly due to the different soil and climatic conditions.

Table 4. Analysis of variance for height increment.

Source of variation	DF	SS	MS	F	P
Provenance	5	29784,348	5956.870	1.963	0.081
Site	5	238306.836	47661.367	15.706	0.000
Proven. * site	25	97528.742	3901.150	1.286	0.157
Error	1203	3650641.8	3034.615		
Total	1238	4016261.7			

Table 5. Height increment (year 2003) of *Fraxinus ornus* provenances in the tested sites (mean ± standard error, mm) [+].

	Provenance Code						
SITE	10FOR	11FOR	12FOR	13FOR	14FOR	15FOR	Mean
Stavr(10)	39.5±4.8	22.2±2.0	31.6±3.0	28.7±3.4	34.9±5.9	25.2±3.3	31.1±1.7 [a]
Nevr(11)	83.3±8.8	74.8±10.6	73.2±8.1	99.8±10.8	65.7±9.5	83.1±11.1	81.2±4.1 [d]
Nigr(12)	55.1±6.6	42.6±8.9	54.5±7.2	51.5±7.8	56.0±11.0	43.3 ±6.0	51.6±3.3 [b]
Polig(13)	68.3±8.5	49.4±4.6	67.5±7.7	50.6±5.2	86.4±8.7	88.8±7.9	67.4±3.1 [c]
Kast(14)	57.6±9.8	68.5±9.1	66.1±7.6	58.5±10.0	77.1±12.3	71.8 ±8.7	67.2±3.5 [c]
Stom(15)	41.8±4.0	39.5±13.9	60.1±6.7	53.3± 9.5	72.8±11.8	54.6±10.7	53.8±3.5 [b]
Mean [1]	63.5±7.7 [fg]	57.1±7.3 [g]	62.5±7.4 [fg]	59.5±7.4 [fg]	72.2±7.9 [ef]	69.8±7.4 [fg]	63.6±7.5

[+] Means with the same superscript letter do not differ significantly (P>0.1 within row or P>0.05 within column, T- test).

Differences between different seed sources/provenances as well also between planted sites for survival and growth (Kung and Clausen 1984, Raymond and Lindgren1990, Roberds et al. 1990, Worrell 1992, Cundall et al. 1998, Savill et al. 1999, Harmer 2000, Worrell et al. 2000, Cundall et al. 2003) dates of flushing and senescence (Worrell et al. 2000) and frost hardiness (Steiner et al. 1988, Deans and Harvey 1996, Worrell et al. 2000) have been reported in broadleaves' provenance trials in the past. In other cases in trials using young progenies (*Quercus robur,* Harmer 2000) no significant variation was found between tested seed sources for growth in the early stage (6 months growth) whereas variation was significant in long term testing in the field. As in our case, it is highly possible in this early stage of provenance trials not much genetic variation in growth and adaptation has been expressed by the tested seed sources. The work described here is in progress and the long term field testing of the provenance trials will be evaluated. Geographic patterns of variation in other important parameters affecting growth and adaptation (e.g. early height growth and flushing, frost hardiness) as have been reported by others (Steiner et al. 1988, Deans and Harvey 1996, Worrell et al. 2000) is worth to study as well in a long term period.

CONCLUSIONS

Survival of the tested provenances in the planted sites where seed sources originated was high (more than 70% in many cases). No significant differences between tested provenances were

found for survival in these early trials. Survival differences between the planted sites were highly significant. Tested provenances were found to be significantly different for height increment at 10% level (across all sites). Provenance 14FOR gave the best performance in height growth. Height increment rates were significantly different between the planted sites. This is most possibly due to different site conditions (climate and soil). No significant interactions between seed sources and planted sites were found for survival and height growth in the first year of testing. Local seed sources did not outperform distant ones in most sites for the recorded parameters (survival, height growth). From these early provenance trials (one year in the field) it is hard to draw conclusions for the performance and local adaptation of the different seed sources. Long term testing (at least 10 years) is required for genotypes' expression in the different environments.

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Efficiency of Using First-generation Information during Second-generation Selection: Results of Computer Simulation

T. Z. Ye[1], K. J. S. Jayawickrama[1], and G. R. Johnson[2]

BLUP (Best linear unbiased prediction) method has been widely used in forest tree improvement programs. Since one of the properties of *BLUP* is that related individuals contribute to the predictions of each other, it seems logical that integrating data from all generations and from all populations would improve both the precision and accuracy in predicting genetic values by increasing the effective number of observations on each genotype (White and Hodge 1989; Kerr et al. 2004). However, some studies based on computer simulation (e.g. Johnson 1998) and field data (e.g. Panter and Allen 1995) showed that including historical parental information actually did little to increase the efficiency of estimating breeding values under some circumstances.

The objective of this study was to determine whether the inclusion of first-generation information sufficiently enhance the accuracy and precision of second-generation selection under different selection strategies and combinations of genetic parameters using stochastic data sets generated by computer simulation.

Simulation Scenarios

- We assumed that the 1st-generation population consisted of 300 open-pollinated families with 100 trees per family. The individual-tree heritability $h^2(1)$ was set in the range of 0.05 to 0.35.
- Two different selection strategies were adopted for the 1st-generation selection. (1) Strategy 1 (backward selection) – select top 48 parents; (2) Strategy 2 (forward selection) – select the best progeny from each of the top 48 families. All selections were based on estimated breeding values (*EBV*s).
- The 48 selections were then used as 2nd-generation parents and crossed in a disconnected 2 × 2 factorial mating design which resulted in 48 crosses in total.
- The 2nd-generation progeny trials had six test sites with 20 trees per cross per site. Different levels of heritability ($h^2(2)$ = 0.05, 0.15, 0.25, 0.35), ratio of dominance to additive genetic variance (V_D / V_A = 0, 1, 2, 3), and genotype-by-environment interaction (V_{GxE} / V_A = 0, 1, 2, 3) were assigned.

Simulation Method

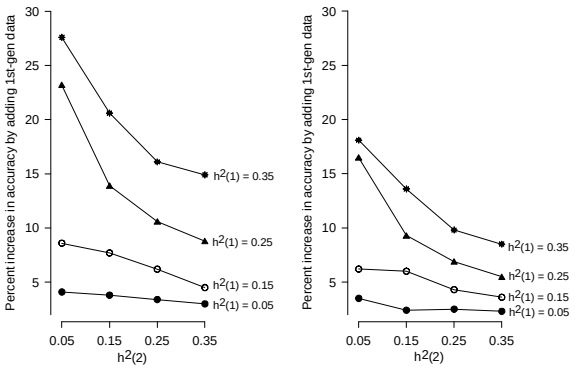
- King and Johnson's (1993) method was used to generate 500 independent stochastic data sets for each combination of genetic parameters and selection method (scenario).
- For each data set, we estimated breeding values for all the 2nd-generation parents and progeny using *BLUP* with and without integration of the 1st-generation information.

[1] Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, OR 97331, USA
[2] USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331, USA

- Kendall's rank correlation coefficients (t) between the true and estimated breeding values for both parental and tree selections were calculated. The mean and coefficient of variation of the 500 correlation coefficients from each scenario were used to quantify the accuracy and precision of selection, respectively.
- The increase in accuracy was measured as the average percent improvement in the correlation when the 1st-generation data were included for 500 simulations. Increased precision was measured as the percent reduction in the coefficient of variation of the 500 simulations.

Results and Discussion

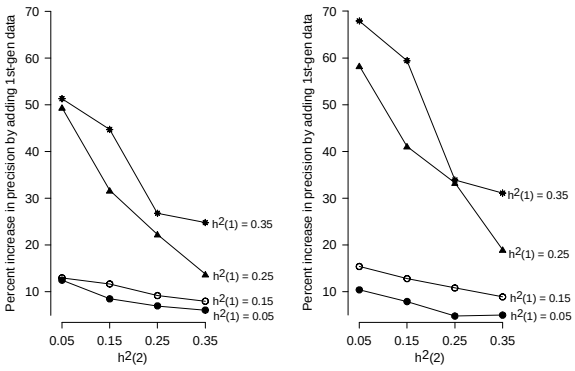
When backward selection was used in the 1st-generation, including the 1st-generation information helped increase both the accuracy and the precision of 2nd-generation selection in all scenarios. However, the amount of increase varied and generally depended more on the heritabilities in both generations (Figures 1 and 2) and less on the size of dominance and G×E effects. The value of adding 1st-generation data was high when $h^2(1)$ is high and $h^2(2)$ is low and decreased as $h^2(1)$ decreased and $h^2(2)$ increased. This is expected since the 1st-generation data contain more genetic information than random noise when $h^2(1)$ is high.



For ranking 2nd-gen parents

For ranking 2nd-gen parents

Fig. 1. Effect of heritability on the *accuracy* of ranking when *backward* selection was used in the 1st-generation selection; assuming $V_D = V_{G\times E} = V_A$



For ranking 2nd-gen progenies

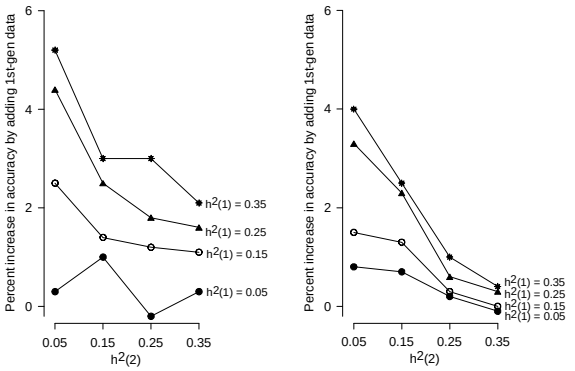
For ranking 2nd-gen progenies

Fig. 2. Effect of heritability on the *precision* of ranking when *backward* selection was used in the 1st-generation selection; assuming $V_D = V_{G\times E} = V_A$

The effects of dominance and G×E on selection efficiency followed the expected pattern. The impact of adding the 1st-generation data increased as V_D or V_{GxE} increased, but the percent increase was usually less than 5% when $V_D = V_A$ or $V_{GxE} = V_A$.

The 1st-generation information helped more in increasing the accuracy of re-selecting the 2nd-generation parents (backward) than that of selecting the 2nd-generation progenies (forward). This would be expected since the coefficient of relatedness between the 1st-generation progeny and the 2nd-generation parents is larger than the relationship between the 1st- and 2nd-generation progeny.

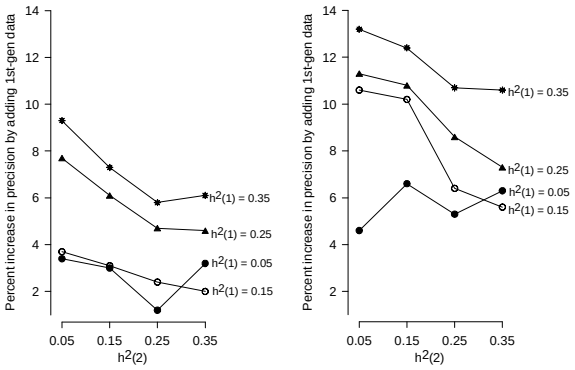
When forward selection was used in the 1st-generation, the 1st-generation information did little to increase the accuracy of ranking in the 2nd-generation (Figures 3 and 4). Because within-family selection is relatively imprecise, the rank correlation between true and estimated parental breeding values was quite low (t = 0.22 when $h^2(1) = h^2(2) = 0.25$; $V_D = V_{G\times E} = 0.5V_A$) (Table 1). When $h^2(1)$ was very low, the 1st-generation data simply added random noise for the 2nd-generation selections. The precision of ranking could increase up to 10~14% probably due to the increase the effective number of observations with high $h^2(1)$ and low $h^2(2)$.



For ranking 2nd-gen parents

For ranking 2nd-gen parents

Fig. 3. Effect of heritability on the *accuracy* of ranking when *forward* selection was used in the 1st-generation selection; assuming $V_D = V_{G\times E} = V_A$



For ranking 2nd-gen progenies

For ranking 2nd-gen progenies

Fig. 4. Effect of heritability on the *precision* of ranking when *forward* selection was used in the 1st-generation selection; assuming $V_D = V_{G\times E} = V_A$

Table 1. Kendall's rank correlation coefficient (t) between the true and estimated breeding values and the coefficient of variation (in parenthesis) for both parental and tree selections based on 500 simulations per scenario; assuming $h^2(1) = h^2(2) = 0.25$; $V_D = V_{G\times E} = 0.5V_A$.

Selection in 1st-generation	$\bar{t}$ & $CV(t)$	1st-gen parents	1st-gen progeny	2nd-gen parents	2nd-gen progeny
Backward	with 1st-gen data	0.77 (2.2%)	0.44 (3.4%)	0.52 (14.6%)	
	with 2nd-gen data			0.55 (12.7%)	0.48 (8.3%)
	with both			0.61 (10.4%)	0.52 (6.5%)
Forward	with 1st-gen data	0.77 (2.2%)	0.43 (3.8%)	0.22 (44.0%)	
	with 2nd-gen data			0.50 (14.8%)	0.45 (9.6%)
	with both			0.51 (14.2%)	0.45 (8.9%)

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Flower Stimulation Helps to Produce More Seeds of Better Genetic Quality at a Lower Cost

G Philippe

From 1985 to 2002, more than 80 flower induction trials were carried out by Cemagref in French seed orchards. They had four objectives: estimate the effectiveness of known stimulation techniques, refine the best ones and, further, determine their repercussions on seed genetic quality and seed production cost. The main species studied were Douglas-fir (*Pseudotsuga menziesii*), Norway spruce (*Picea abies*), European and Japanese larch (*Larix decidua* and *L. Kaempferi*) and maritime pine (*Pinus pinaster*).

FLOWERING AND SEED PRODUCTION

Most often, single treatments had additive effects when they were associated. Therefore, combined treatments were the most advisable. Different conditions of application were compared and, finally, recommendations can be made regarding the timing of application, the techniques and the quantity of active matter to be used (table 1). Our data suggested that biennial treatments might not be compatible with sustainable production, at least in Douglas-fir and larch. It is preferable to treat the same trees only every third year.

Species	Treatments recommended
Douglas-fir	Girdling one month before vegetative bud flushing (in conventional orchards) or severe root-pruning one month before vegetative bud flushing (in young meadow orchards) + $GA_{4/7}$ injection at the time of flushing + nitrate fertilizer (200 Kg of N/ha) combined with a weed killer at the time of flushing
Norway spruce	Girdling three weeks before vegetative bud flushing + $GA_{4/7}$ injection one week after flushing
European larch *(1)*	Girdling at the beginning of long shoot elongation + two $GA_{4/7}$ injections during shoot growth (30% and 80% total shoot length)
Japanese larch	Girdling (conventional orchards) or root-pruning (potted orchards) at the beginning of long shoot elongation
[1]Maritime pine	Application of ammonium sulfate (200 kg of N/ha) with a weed killer in March-April or $GA_{4/7}$ injection in September (increase in seed cones) or July-August (increase of pollen cones)

(1) results obtained in only one clone
NB- conventional girdling (two half-circumferential saw cuts in the stem) should be preferred to complete girdling (that cuts the phloem vessels all around the stem)
$GA_{4/7}$ quantity: 10 mg /m of height (0.5 mg/cm² stem cross sectional area for 15-year-old trees)

Table 1. Treatments recommended for Douglas-fir, Norway spruce, European larch, Japanese larch and maritime pine

[1] Forest researcher, Cemagref, Nogent-sur-Vernisson, France (gwenaël.philippe@cemagref.fr)

The treatments did not appear to disrupt flower, ovule and seed development. The treated trees tended to produce smaller cones than the controls, and that resulted in a slight decrease of the potential seed yield per cone, but it is considered as a consequence of the increased cone production and not as a direct effect of the treatments.

The treatments that promote male and female flowering are likely to enhance seed yield per cone when they are applied to large plots. In a pilot induction in a 15-year-old orchard of Douglas-fir, G+GA increased pollen production and that resulted in a 240% increase of the seed yield per hl of cones.

IMPACT OF THE TREATMENTS ON SEED GENETIC QUALITY

Induction treatments are applied to seed orchards that have been established to produce seeds of high genetic quality. Whether these quantitative and qualitative objectives are compatible is questionable. On one hand, it can be considered that the treatments favor panmixia because they increase the number of flowering trees and, thus, the number of parents in the seed lot (Wheeler *et al.* 1985). On the other hand, it has been reported that flower stimulation treatments especially favor the genotypes that have a good flowering ability. In that case, there is a risk that the treatments would increase the parental imbalance of fertility and, finally, increase the deviation to panmixia (Sweet and Krugman 1977).

The differences of production among genotypes in treated and control populations were compared, using a fertility index. This index, that corresponds to the status number for unrelated and non-inbred orchard parents, was expressed as a % of the number of parents :

$F\% = F*100 / N = (1/ S P_i^2) * 100 / N$

where P_i = proportional gametic contribution (male + female) of parent i

N = number of parents in the population

F% varies between 0% and 100%. The higher it is, the more even the parental contributions.

In Norway spruce, the index was always higher in the GA-treated populations than in the controls (figure 1). That was true in poor, medium, good and excellent flowering years. Furthermore, G+GA was often more effective than GA considering the balance of clonal contribution. In Douglas-fir, the results obtained so far are perfectly consistent with those found in Norway spruce.

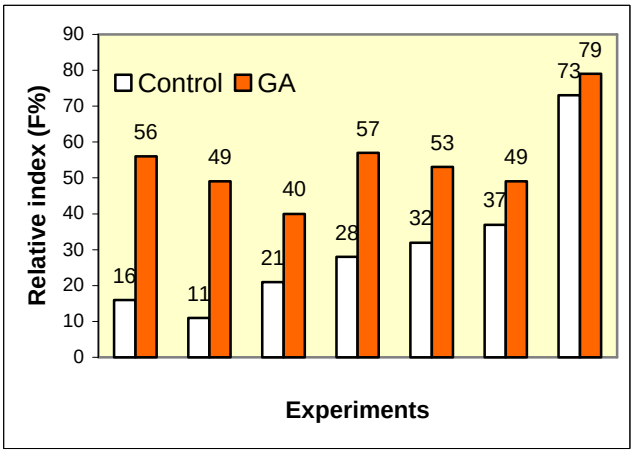

Figure 1 – Effect of GA $_{4/7}$ injection on the balance of clonal contribution in Norway spruce

As the chances that flowering clones mate depend on factors (clonal phenology, distance between clones, pollination effectiveness) that are independent of treatments, more even gamete contributions should result in an improvement of panmixia. In addition, enhancement of pollen production will lead to a reduction of both pollen contamination and selfing. Finally, flower induction treatments will provide the seed orchard manager with a better chance of obtaining the expected genetic gains because the assumptions done for the calculation of the theoretical gains are better respected.

In maritime pine, fertilization and GA had also positive effects but they were more limited. At the worst, flower induction will increase seed production without reducing seed genetic quality that is naturally at a good level.

IMPACT OF THE TREATMENTS ON SEED COST

Cost-effectiveness of 4 treatments - none, G, G+GA and G+GA+N - was assessed in Douglas-fir (Philippe *et al*. 2004). Three models of orchards, corresponding to different intensities of management, were considered. The orchards were assumed to produce during 20 years or 30 years.

The study showed that induction treatments were cost-effective in the three models of orchards and in both periods of production. Moreover, the most sophisticated and therefore the most expensive treatments were also the most cost-effective. For example, seed cost was divided by 1.6, 4.1 and 5.5 by G, G+GA, G+GA+N when the orchard was assumed to produce during 20 years.

The same kind of results is expected in Norway spruce and larch. In maritime pine also, ammonium sulfate and GA should be cost-effective but maybe less because they resulted in lower production gains. That should be studied in the future.

CONCLUSION

Finally, these results prove that there is no conflict between seed production, seed genetic quality and cost-effectiveness. It is in the seed orchard manager's interest to use flower stimulation techniques.

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Climatic adaptation in *Picea abies* progenies is influenced by the maternal temperature during zygotic embryogenesis and seed maturation. A "memory" involving DNA methylation and differential transcription of phytochrome genes?

T. Skrøppa[1], Ø. Johnsen[2], C.G. Fossdal[3], R. Baumann[4], N. Nagy[3], J. Mølmann[5], O.G. Dæhlen[6] and G. Müller-Starck[7]

In earlier experiments we have demonstrated that traits characterising climatic adaptation in Norway spruce (*Picea abies* (L.) Karst.) are influenced by the climatic conditions during the sexual reproductive process. The phenomenon occurs in particular when grafted clones are transferred from cold conditions to more a southern seed orchard site where seed production takes place. Seedlings from seeds produced in the cold environment have an advanced bud set and cold acclimation in the autumn wheras their siblings from seeds produced in the warm reproductive environment have a delayed timing of these traits. In this presentation, we will report results from more recent experiments that confirm the earlier findings and in addition identify possible molecular mechanisms behind the observed phenomenon.

MATERIALS AND METHODS

Potted grafts of Norway spruce were induced to flower and full-sib families were generated after controlled crosses. In four independent experiments seed lots of identical crosses were produced under contrasting temperatures, and in a fifth experiment both temperature and day length varied during seed production. Elevated temperatures were timed to shorter and longer periods from female meiosis, pollen tube growth, syngamy and embryogenesis, and later we compared the full-sib progeny performance for timing of terminal bud formation, cold acclimation in the autumn and initiation of growth in the spring. The phenotypic performance was compared to the accumulated heat sums during embryo development and seed production.

Real time PCR primers and probes were designed for the three phytochromes present in Norway spruce (*PhyN*, *PhyO* and *PhyP*) and the extra cellular class IV chitinase *PaChi4* in order to separately study and quantify their expression levels. Real time primers and probes for internal controls were also produced. RNA was extracted from needles of 6-8 weeks' old seedlings from pairs of full-sib families, based on seeds produced in different temperature treatments, and the transcription levels of the chosen genes were quantified for each sample. DNA was extracted for one family pair (cold and warm maternal environment) and the relative amount of methylated cytosine in genomic DNA was determined.

RESULTS AND DISCUSSION

No responses in phenotypic traits were found to short term temperature treatments during female meiosis, megaspore development, pollen tube growth and fertilization and underline that selection during female gamete formation and competition among growing pollen seems to be of little importance. Population genetic analyses based on DNA makers revealed that irregular non-random segregations are quite common and that they are more frequent when fertilization takes place under warm conditions. However, it was not possible to relate these non-Mendelian segregation events to the phenotypic effects observed. Thus, our findings weaken the hypothesis

that directional selection among haplotypes and genotypes inside the female flower could be the main explanation of the observed phenomenon in Norway spruce.

When elevated temperatures were given for a longer period of time during embryogenesis, the progenies formed terminal buds later and were less frost hardy in the autumn. The differences in progeny performance were strongly associated to the temperature sums from proembryo to mature seeds. The lowest transcription levels were found in progenies from seeds produced under warm conditions, and these progenies expressed higher level of DNA methylation than their full-sib from seeds produced under cold conditions.

These data suggest that the temperature during zygotic embryogenesis and seed maturation regulates an "epigenetic memory" involving differential expression of genes with putative functions in bud phenology, cold acclimation and embryogenesis in Norway spruce. Our data suggest that methylation of cytosine in genomic DNA is a likely candidate for such "imprinting". However, studies of methylation in the coding regions of genomic DNA, and specifically in the promoters, are necessary to confirm this hypothesis.

Results from several field tests with families produced in seed orchards located to warm sites confirm that these effects have importance under field conditions; these families have a later growth start in spring, are less damaged by late spring frost, they have a lower mortality and better height growth, and are more damaged by early autumn frosts than seedlings from assumed comparable provenances. Therefore care should be taken when seed orchards are located to sites with warmer climatic conditions than in the intended planting zone and in the use of seeds from indoor seed orchards. The effects may also influence breeding operations such as the generation of breeding populations and families for progeny testing and the use of family seed lots produced in different years.

[1]Professor, [2]Senior research scientist, [3]Research scientist, Norwegian Forest Research Institute, Høgskoleveien 8, 1432 Ås, Norway; [4]Research Scientist, [7]Professor, Technische Universität München, Am Hochanger, D-85354 Freising, Germany; [5]PhD. Student, Department of Biology, University of Tromsø, 9037 Tromsø, Norway; [6]Program manager, Biri Nursery and Seed Improvement Centre, 2836 Biri, Norway.

Characterizing The Embryo Lethal System In *Pinus taeda* L.

C. G. Williams[1] and M. M. Reynolds[2]

[1]Duke University, Nicholas School of the Environment & Earth Sciences, Durham NC, USA and
[2]Texas A&M University, Graduate Program in Genetics, College Station Texas, USA

The embryo lethal system in pines recognizes and excludes self-pollinated embryos. The question is whether the genetic basis of the embryo lethal system is composed of a random accumulation of semi-lethal mutations expressed throughout embryo development. In *Pinus taeda*, fertilization and embryo occurs through early to late summer. Using a combination of microsatellites, microscopy and fluorescent dye, we characterized the development, fertilization and embryo death in the *Pinus taeda* embryo lethal system using accessions from Summerville SC. Implications for the embryo lethal system in the *Pinaceae* are discussed.

Relation between flowering phenology of seed orchard clones and field-test performance of their open-pollinated offspring in Norway spruce

T. Nikkanen[1], S. Ruotsalainen[1] and M. Haapanen[2]

All seed orchards for northern Finland are located several hundreds of kilometers south from their area of origin, i.e., in a warmer environment. The purpose of this arrangement is to ensure frequent and abundant flowering and good ripening of the seed produced, and to achieve temporal isolation in flowering between seed orchard clones and surrounding forests. However, the phenological isolation has proved to be inadequate; a large proportion of the seed produced is sired by pollen coming outside the seed orchard (Pakkanen et al. 2000, Nikkanen et al. 2002). Some of this pollen, especially at the early stage of flowering, is likely to come from areas south of the seed orchard. The hardiness of the produced material is then further degraded compared to material that result from pollination from surrounding forests.

The objective of the study was to determine whether there was any association between the variation in female flowering phenology of the seed orchard clones and the performance (growth, survival) of their progeny in field tests.

MATERIAL AND METHODS

Reproductive phenology was studied in a Norway spruce seed orchard located in southern Finland (62º13'N, 25º24'E). The orchard consists of 67 clones from northern Finland (64º–67ºN) (Fig. 1). The seed orchard is 13.2 ha in area, and is located on a hill (Fig. 2).

The timing of flowering was observed on grafts in 1992, 1993 and 1995 (Nikkanen 2001). Observations of the phenological stage of female and male flowers were made daily on 3 grafts per clone. Female flowers were observed from the top of the graft with binoculars, and pollen shedding of male flowers from the sample branch on the southern side of the graft. Observations made in 1995 were used to study the relation between flowering phenology and field-test performance.

Temporal and spatial variation in airborne pollen was studied by means of different pollen samplers. The results obtained with a recording pollen sampler provided information about the actual timing of flowering in different years and about daily fluctuation during flowering Nikkanen 2001). A rotorod type of sampler gave an estimate of pollen density in the air (Nikkanen et al. 2002). In 1995, a total of 70 rotorod-samplers were situated on 48 masts; 37 samplers were located in the seed orchard and 33 outside it.

The rate of pollen contamination in the orchard was studied in 1989, 1992, 1993 and 1995 using paternity analysis based on isozymes (Pakkanen et al. 2000, Nikkanen et al. 2002).

[1] Finnish Forest Research Institute, Punkaharju Research Station, FIN-58450 Punkaharju, Finland
[2] Finnish Forest Research Institute, Vantaa Research Center, Box 18, FIN-01301 Vantaa, Finland

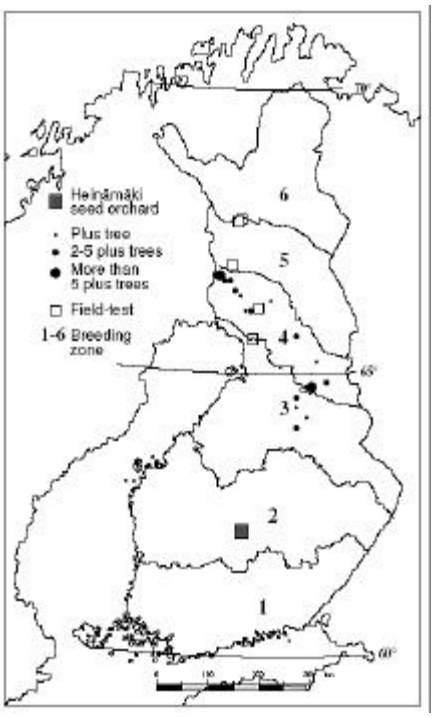

Figure 1. Location of the Heinämäki seed orchard, the field tests and the origin of the orchard clones.

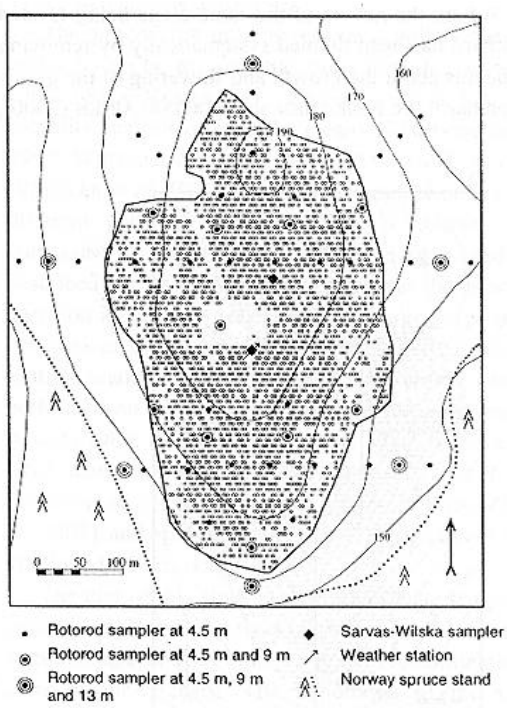

Figure 2. The seed orchard and its immediate surroundings, and the measuring device.

Survival and growth of open-pollinated seed orchard progenies of the above-mentioned clones was studied using data from five progeny tests in northern Finland. The field tests were measured at ages of 10 or 15 years. The survival of the plus tree progenies was, on average, good, and their height growth was superior to local entries (Ruotsalainen & Nikkanen 1998).

RESULTS AND DISCUSSION

The time difference in the start of the receptive period of the female flowers between the earliest and the latest graft varied from 2 (1995) to 4 (1993) days, and in the start of pollen shedding from 3 (1995) to 6 (1993) days. The clonal differences in the phenology of receptivity were statistically significant, whereas in the phenology of pollen shedding they were not. The rank correlation coefficients of the clones between the years in the start and the duration of the receptive period were positive and in most cases statistically significant. In 1995 the duration of the receptive period was 5 days, starting about one day before anthesis.

During the first two days of the receptive period of grafts in the orchard, most of the pollen caught was derived from outside the orchard. The result that the greatest increase in pollen density occurred outside the seed orchard one day later than inside it (Fig. 3), indicating phenological differences between the orchard and local forests, is one sign that the origin of airborne pollen during the first one or two days of flowering was probably not from the surrounding forests but mostly from more distant sources, i.e. from areas where the flowering of the species was in advance of that in the seed orchard area. The high rate of pollen contamination estimated from the seed orchard indicates that a strong gene flow into the seed orchards from

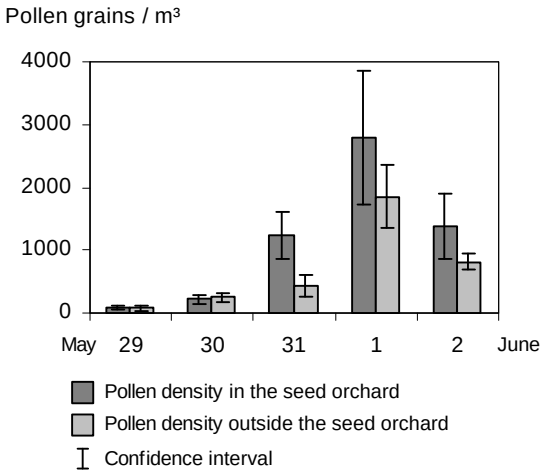

Figure 3. The average pollen densities on different days in1995 inside and outside the Heinämäki seed orchard.

outside sources is the predominant pattern at least in the orchards of northern origin established at more southern sites.

Early female flowering was found to be correlated to poor height growth of the progeny (r_s = -0.434, p = 0.004). Similar, yet weaker correlation was also observed for survival (r_s = -0.270, p = 0.083). There was a slight positive correlation between survival and height growth at the clone-mean level (r = 0.331, p = 0.023). Height growth and survival were not correlated with vegetative phenology of the clones.

Although the correlations between flowering phenology and field-test performance were not strong, the results indicate that the early flowering clones in the seed orchard were predominantly pollinated by southern background pollen, resulting in somewhat reduced adaptation of their progeny when tested in harsher northern conditions. Therefore, when the hardiness is of high priority, careful assessment of the flowering phenology of clones is advisable whenever new seed orchards are being established and old ones are being thinned.

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Topgrafting Loblolly Pine to Accelerate Breeding And Deployment Of Genetic Gain in The Southern United States

W.P. Cumbie[1], D.M. Gerwig[2], C.C. Lambeth[1], E.M. Raley[3] and S.E. McKeand[4]

[1]Weyerhaeuser Company, Hot Springs, AR, USA
[2]MeadWestvcao Corporation, Summerville, SC, USA
[3]Texas Forest Service, Texas A&M University, College Station, TX, USA
[4]Department of Forestry, N.C. State University, Raleigh, NC, USA

Topgrafting of selected loblolly pine (*Pinus taeda* L.) genotypes into the crowns of sexually mature seed orchard ramets (interstocks) has become a routine practice in southern pine tree improvement programs. The number of years for production of both male and female strobili is dramatically reduced, and this time savings is an advantage both for breeding and for operational seed orchards. Compared to traditional grafting of scions onto one- or two-year-old seedling rootstocks, topgrafting into the crowns of 5- to 15-meter trees reduces the time for strobilus production from 5 to 10 years down to 1 or 2 years. For breeding programs, this savings of time has tremendous economic advantage for relatively little cost. For operational seed orchards, entire crown replacement of large seed orchard ramets has also been economically attractive. Seedlings from high breeding-value selections can be deployed in operational plantations 5 to 10 years earlier than if traditional seed orchard management techniques are employed. Experiences from different tree improvement programs will be presented and opportunities for use of topgrafting will be discussed.

Development of Focal Point Seed Zones for White Spruce

Mark R. Lesser and William H. Parker[1]

White spruce (*Picea glauca* [Moench] Voss.) is distributed widely throughout the boreal forest of Ontario and the rest of Canada. Developing seed zones for white spruce based upon its patterns of adaptation across the landscape is a crucial step in managing this species in an ecologically sound manner in a changing climate.

Although often taken for granted or simply ignored, one of the most important decisions a forester can make is proper seed selection – no amount of intensive silviculture will produce acceptable growth if maladapted seed is used. This is why seed zones have to be developed based upon demonstrated patterns of adaptive variation on a per species level. While generic seed zones based upon climate have been established in Ontario, these can be, and most definitely should be, refined based upon biological test data representing adaptive variation as it becomes available.
The focal point seed zone methodology determines spatially explicit areas of ecological compatibility for any selected focal point. This approach will assist in properly matching natural or improved seed sources and planting sites based on current and predicted future climate conditions.

METHODS AND MATERIALS

One hundred and twenty seven provenances from Ontario and western Quebec were established at a greenhouse and six field trials throughout Ontario in the summer of 2002. Growth and phenological variables were measured over three growing seasons. Variables were assessed for significant levels of between-provenance genetic variation through analysis of variance (ANOVA) and the calculation of Intraclass Correlation Coefficient. Variables that expressed a significant amount of between-provenance variation were regressed individually against geographic and climate variables. Seventy climate variables were used in the analysis: 36 comprised of monthly maximum and minimum temperatures, and mean precipitation; and 34 comprised of derived temperature and precipitation variables. All climate data were supplied by Dr. D. McKenney, Canadian Forest Service, Landscape Analysis and Application Section, Great Lakes Forestry Centre (2004). The combination of a significant regression and a significant level of genetic variation was used as a screening process to identify only variables exhibiting adaptive variation for inclusion in further analysis.

Principal components (PC) analysis was used to summarize the main components of variation in the biological data. Seed Source PC factor scores were regressed against climate variables, and the resulting equations were used to model the PC axes. Models were converted to spatial data and reproduced as contoured grids using GIS. These grids represent the pattern of adaptation associated with the traits expressed by each PC axis. For any given point in the study area the PC axis grids can be standardized and intersected, creating zones of adaptive similarity to that point.

RESULTS AND DISCUSSION

Significant levels of between-provenance differentiation were clearly shown for the majority of the 94 variables tested by ANOVA. Of the 94 variables, 62 showed significant differences at the $p<0.05$ level. The variables that did not show significant differences were all budflush, budset and survival variables. All growth variables (height, root collar diameter, and greenhouse elongation) at all tests showed significant differences at the $p<0.05$ level.

Regressions of the 62 individual biological variables against climate variables showed significant results for all but 5 of the variables. Coefficient of determination (r^2) values for significant regressions ranged from 55 percent to 3 percent with a wide range of both temperature and precipitation variables being found pertinent.

Principal components (PC) analysis of the 57 retained variables showed the majority of the variation in the data set being explained by the first three PC axes. Results of regressions of the first three PCs' factor scores against climate variables are shown in Table 1. The first PC explained 34 percent of the overall variation and mainly represented growth potential. PC 1 has an r^2 of 25.3 percent and is explained by precipitation from the wettest period, August maximum temperature, and August precipitation. The resulting grid for the predicted PC 1 factor scores shows a strong southeast to northwest trend, with the highest growth potential being in southern Ontario and western Quebec. Overall, growth potential decreases with movement northward. PC 2 explained a further 12 percent of the variation and mainly represented phenological timing. PC 2 has an r^2 of 51.58 percent and is explained solely by June minimum temperature. The grid for PC 2 also shows a north-south trend, with southern sources flushing later in the spring and setting bud later in the fall. PC 3 explained 8 percent of the variation and mainly represented greenhouse elongation and budflush timing. PC 3 has an r^2 of 15.54 percent and is explained by mean temperature in the driest quarter and July precipitation.

Table 1. Multiple regression models of principal component analysis factor scores against climate variables

Dependant Variable	p>F	Independent Variables	Coefficient	Tolerance	p>t
Principal component 1	<0.0001	constant	-8.32296	\	<0.0001
R^2 = 25.30%		precipwp	-0.04098	0.34772	0.0114
		augmaxtemp	0.27414	0.88222	<0.0001
		augprecip	0.06647	0.3757	<0.0001
Principle component 2	<0.0001	constant	-3.51335	\	<0.0001
R^2 = 51.58%		junmintemp	0.41466	1	<0.0001
Principle component 3	<0.0001	constant	3.28565	\	<0.0001
R^2 = 15.54%		mtempdryq	-0.05738	0.70855	0.0121
		julprecip	-0.04402	0.70855	<0.0001

Focal point seed zones can be created for any point within the study area by standardizing predicted PC factor score grids to the selected point and intersecting them with each other. An example of a focal point seed zone map is shown in Figure 1. The focal point is shown with the star. Areas of greatest adaptive similarity are shown by the darkest shading. This area is within

plus or minus half a standard deviation from the focal point. Decreasing levels of shading indicate areas of decreasing adaptive similarity.

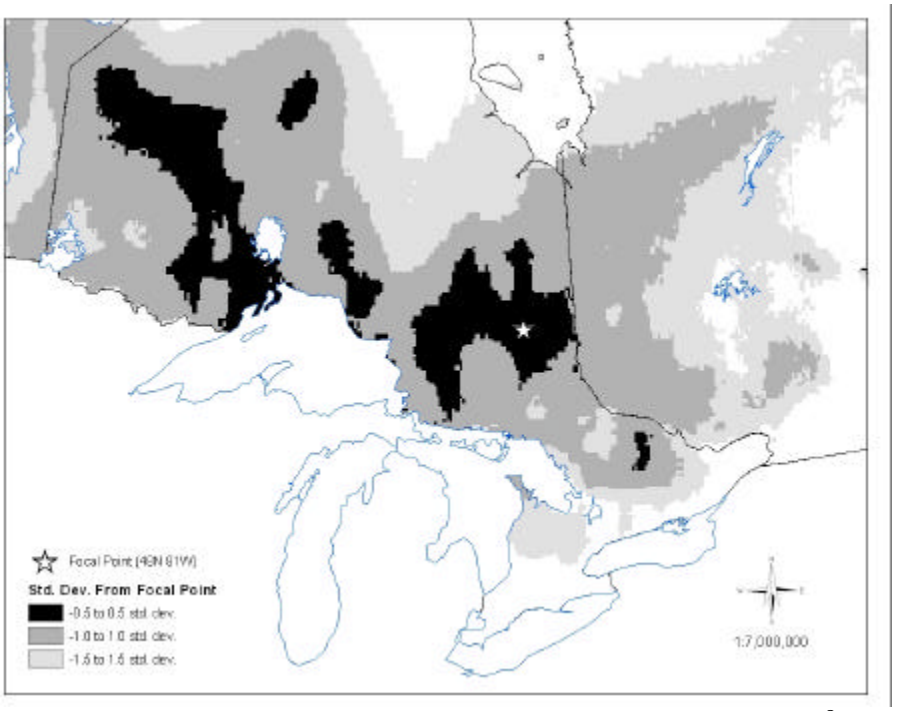

Figure 1. White spruce focal point seed zones for coordinates 48°N 81°W

Focal point seed zones created for a sampling of points selected from across the province show broad latitudinal trends and more regional longitudinal trends. Seed Zones created for points in northern areas of Ontario tend to be fairly broad, with areas within ±1.5 standard deviations extending across much of the province and only southern areas being excluded. As the focal point is moved south zones become smaller and more distinct from each other. Focal points from south-eastern Ontario generally show areas of similarity to exist in the far north-west area of the province, along with their local areas. Lake effects, most noticeably from Lake Superior, are seen along its northern and eastern shores.

Generic seed zones based upon length of growing season, when compared to the focal point seed zones, create zones that are far too specific in many cases especially through the northern areas of the province. In more southern locations generic seed zones are not nearly specific enough. Seed transfer for white spruce across traditional site region boundaries may be possible in most of north-central and north-eastern portions of Ontario.

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Genetic Thinning of Clonal Seed Orchards using Linear Deployment

F. Prescher[1], D. Lindgren[2] and M. Varghese[3]

Abstract: Linear deployment means that the number of ramets is a linear function of the breeding value of the clone. When used for thinning clonal seed orchards, there is however a higher bound set by the number of ramets in the seed orchard before thinning. Linear deployment thinning is optimal in the sense that no other thinning regime can result in higher genetic gain, higher effective clone number and lower thinning intensity without sacrificing another of these characteristics. Here three applications are described.

The first is a cutting seed orchard of *Picea abies* situated at Lagan, south Sweden. The genetic values of the clones were obtained from an inventory twelve vegetation periods after establishment of two field tests with the orchard clones, where an index combined several observations, to make the breeding values proportional to the forecasted production value. A spectacular result is that considerable improvement in *both* effective number *and* breeding value was achieved in the same time. The reduction in optimality from the practical application was vanishingly small.

The second is the seed orchard used by Bondesson and Lindgren (1993), a grafted orchard of *Picea abies* ("Maglehem"), where results from a progeny-test were available. It was thinned mainly according to the study. It was impractical to follow the algorithm exactly; the actual outcome of the thinning is reported, the reduction in optimality was minor.

The third is a three year old clonal trial of *Eucalyptus camaldulensis* situated in southern India, which will be converted to a seed orchard using the clonal averages observed on the trial site itself as genetic entries. Comparisons were done for three linear deployment thinning strategies: at the same gain; at the same effective number; or at the same thinning intensity. The preferred alternative increased the thinning intensity somewhat compared to truncation selection, increased effective number and census number much, and reduced the breeding value marginally.

Linear deployment thinning is recommended where effective number of clones is a concern and breeding values available. The algorithm is optimal and the advantage in using it may be substantial. The flexibility at thinning increases; the possibilities for additional thinning based on later observations increases (like fertility observations or repeated measurements of test trials); more clones will be archived in the orchard (e.g. for future controlled crosses); the possibility to use selective harvest will

[1] Seed manager, Svenska Skogsplantor AB, 340 14 Lagan, Sweden
[2] Professor, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden
[3] Senior Research Scientist, Institute of Forest Genetics and Tree Breeding, Coimbatore - 641002, India

improve. The added field work required for the operation is limited and practical experiences positive. Although a slightly higher genetic gain can be obtained by conventional truncation thinning at the same selection intensity, it is usually accompanied by considerable loss in effective number and number of remaining clones.

Key words: Genetic thinning, linear deployment, breeding value, seed orchard, effective number, gene diversity

INTRODUCTION

In due time a seed orchard manager comes to the point where his seed orchard is too dense spaced to be effective as a seed producing operation or when better breeding value of the orchard genotypes become available, so the genetic quality of the seed orchard crop can be improved. Until now the most common way has been to rouge the orchard either systematically, e.g. every second tree, or to use estimated breeding values and make a genetic thinning using truncation selection.

Major considerations for the genetic quality of seed orchard crops are gain and diversity (Stoehr et al. 2004). Lindgren and Matheson (1986) presented an algorithm for increasing the genetic quality of seed from seed orchards at seed orchard establishment by linear deployment. This algorithm can be said to optimally combine gain and diversity (diversity measured as effective clone number). The algorithm is optimal in the meaning that there is no way to get a higher gain without decreasing the effective clone number or to get a higher effective clone number without decreasing the genetic gain, than with linear deployment. Linear deployment means that the number of ramets is linearly related to the breeding value of the clone. The method was developed to be applicable for genetic thinning by Bondesson and Lindgren (1993).

Even if this algorithm for genetic thinning was published more than a decade ago, applications have never been described earlier, and as far as we know, it has only been applied once before 2004. In this paper, application of linear deployment thinning on three seed orchards will be presented.

MATERIAL

1) Lagan. This material is a Norway spruce (*Picea abies* (Karst.) L.) seed orchard composed of tested cutting clones, which were selected from a cutting material initially meant for operative forestry. The initial selection of ortets was done in nursery 1983 with late flushing material from Belarus. The seed orchard was established 1993 with 32 clones selected based on measurements on the current trials 1991. It was meant as a low cost seed orchard, the establishment cost was a tenth of a conventional grafted seed orchard. This was achieved as the rooted cuttings were cheap and they could be planted using a planting machine normally used for reforestation of abandoned farmland. Grafts would have been more expensive and demand more work for planting. Furthermore, the randomization of the orchard is incomplete. The clones were grouped into 6 classes; the number of clones in each group is unequal depending on the number of ramets for each clone. Group 1 was planted in row 1, 7, 13 etc., group 2 in row 2,8,14 etc…

Group 6 in row 6, 12, 18 etc. The orchard is situated in Lagan in south Sweden on an old nursery field with 5898 planted cuttings on 7 hectares, of which 5351 were alive at thinning.

The same clones are in two field trials, which were measured in 1998 after 12 vegetation periods in the field (Boije, 2001). The breeding values used for genetic thinning are an index expressed as percentages of experimental mean of predicted production value, based on observations of height, diameter and acceptability of branch angle.

2) Maglehem. This is also a Norway spruce seed orchard, localized in south Sweden. The orchard was established in 1956-60 and initially composed of grafts from 36 plus tree clones. The breeding value of these clones has been assessed based on measurements in four progeny trials (Karlsson and Danell, 1989). The breeding values for height growth were standardized and the seed orchard was genetically thinned in 1994 with the linear deployment algorithm with what seemed to be a suitable balance between genetic gain and effective clone number. The thinning did not cause any problems compared with a truncation thinning, the increased possibilities for silvicultural considerations were an asset, and the seed orchard functions well today (Karlsson, 2004).

3) Coimbatore. This material is a clonal test of *Eucalyptus camaldulensis* established at Coimbatore in south India comprising 87 clones, selected from 7 seedling seed orchards and commercially available clones. There were 15 ramets of each clone arranged in 3 tree plots with 5 replications. The test was to be converted to a clonal seed orchard based on height assessment in the trial at three years.

METHODS

The algorithm to calculate the optimal utilization of the clones combines the two desires: high effective number of clones and high genetic gain. The linear deployment is visualized as a broken line, characterized by intercept and slope (figure 1). If the breeding value of a clone is below the intercept, no ramets will be deployed from that clone. On the other hand, the total number of ramets from a clone is a limit, at thinning ramets cannot be added, only withdrawn, even if the breeding value would justify more ramets. Thus the algorithm has to be modified, see figure 1. Fortunately, the optimal line for genetic thinning has the same slope and intercept for all clones. By using the method, the genetic gain will be higher for the resulting effective clone number than any other deployment strategy.

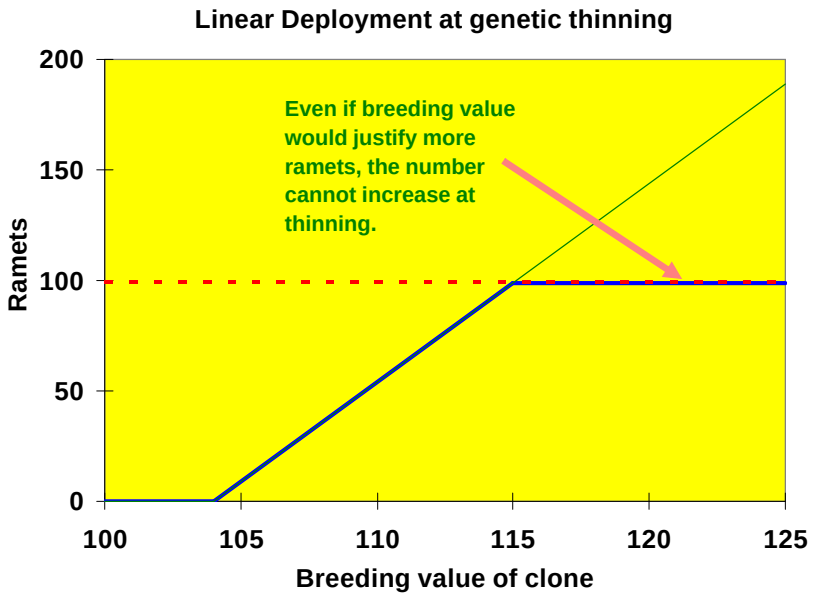

Figure 1. Application of linear deployment at genetic thinning.

Mathematics

Let g_i be the breeding value of clone i, R_i the number of ramets of clone i before thinning and r_i the number of ramets of clone i after thinning. The effective number of clones in the orchard after thinning is calculated with the following formula:

$$N_e = \frac{\left[\sum_i r_i\right]^2}{\sum_i [r_i]^2}$$

Now let G be the average breeding value of seed orchard clones weighted with their occurrence.

$$G = \frac{\sum r_i g_i}{\sum r_i}$$

The genetic thinning is optimal if ramet number is proportional to the breeding value (Bondesson and Lindgren, 1993). Linear deployment thinning is optimal in the sense that no other thinning regime can result in higher genetic gain, higher effective clone number and lower thinning intensity without sacrificing another of these characteristics. In this linear deployment thinning algorithm, g_0 denominates the intercept and b the slope of a line. The ramet number remaining after linear deployment thinning is calculated as follows:

$$\frac{R_i}{b} - g_0 < g_i \Rightarrow r_i = R_i$$

$$\frac{R_i}{b} - g_0 \geq g_i \geq g_0 \Rightarrow r_i = b(g_i - g_0)$$

$$g_i < g_0 \Rightarrow r_i = 0$$

The entries g_0 and b are chosen to get a desired combination of values for G, N_e and ramets remaining. This will be optimal in the meaning, that there is no deployment which results in a higher value of one of these three variables and can not be obtained without reducing the value of one of the others.

For calculation an EXCEL workbook, Linear_Deployment2005.xls, was used, which is available from the Internet at http://www.genfys.slu.se/staff/dagl. 'Solver', an option in the EXCEL package, was sometimes used to home in the linear deployment optimisation on preset values (e.g. for comparison with truncation selection).

RESULTS

Lagan seed orchard.

Table 1 presents the results from this study, the status of the seed orchard before thinning, the different proposed thinning schemes according to the linear deployment algorithm, thinning by truncation selection as well as the actual outcome after thinning the orchard.

Table 1. Orchard status before thinning, different linear deployment alternatives, truncation selection and status after thinning trying to use ***linear deployment resulting in N_e = 22***. N_e = effective clone no., g_0 = intercept, b = slope.

Parameter	Before thinning	Max. N_e	Max. gain at given N_e				Truncation selection*	After thinning
Clones	32	32	32	***31***	29	26	24.35	32
Ramets	5351	3644	3644	***3644***	3644	3644	3644	3644
Gain	105.96	106.89	108.18	***108.52***	108.76	108.92	108.97	108.52
N_e	19.99	26.8	24***	***22*****	20***	18***	16.82	22.00**
g_0	-∞	-667.71	94.86	***97.84***	100.37	101.15	101.82	
b	0	0.20102	16.17	***24.87***	49.05	79.89	∞	

*) Interpolated to get 3644 ramets after thinning

) Slightly higher in the third decimal compared ***linear deployment resulting in N_e = 22*

***) These values are exact, iterative methods were used to find the corresponding linear deployment

In figure 2 is shown that when increasing the number of effective clones, the breeding value decreases, at the same thinning intensity. The decrease in breeding value seem limited for increasing the effective populations size up to 22, but larger increases cost more in lost gain. That was one reason to choose ***linear deployment resulting in N_e = 22.***

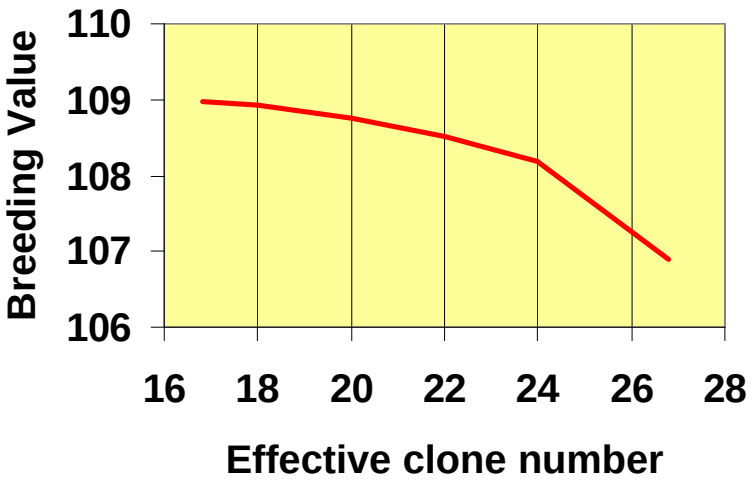

Figure 2. The dependence on the seed orchard breeding value as function of resulting effective clone number at same thinning intensity following application of linear deployment.

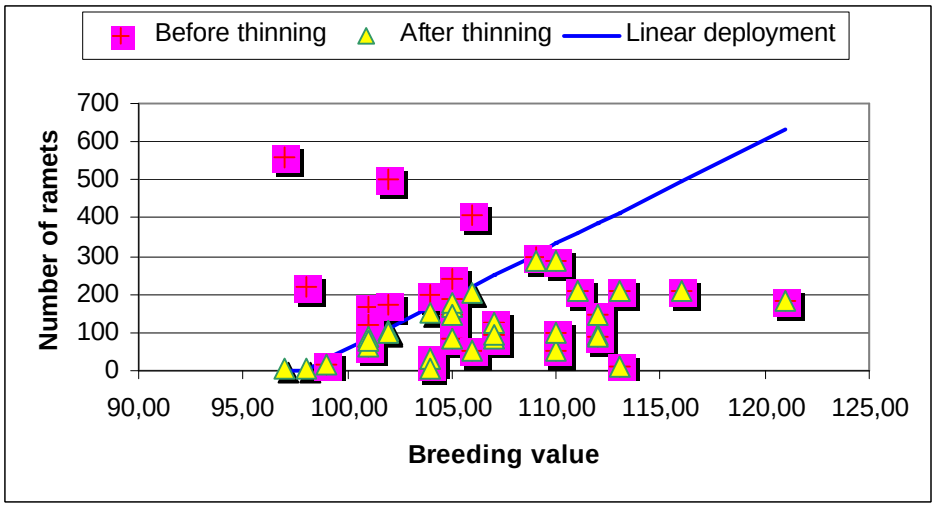

Figure 3. Number of ramets before and after thinning of seed orchard Lagan. The blue line describes the linear deployment algorithm according to ***linear deployment resulting in N_e = 22*** in table 1.

Figure 3 shows the result from the actual thinning and the proposed thinning intensity by the linear deployment algorithm. The highest increment of the gain is obtained by thinning after truncation selection. In this case both the number of clones as well as the effective number of clones is decreased. But the spectacular result from this study is, that it is possible to ***both*** increase the effective number of clones ***and*** the gain, by thinning the seed orchard using the linear deployment algorithm. We chose ***linear deployment resulting in N_e = 22*** for the practical thinning, because it combines a reasonable gain and effective number. Because of the design of the seed orchard, it has not been possible to make much silvicultural considerations during the thinning operation, only some minor changes in selected ramets, compared to the proposed linear deployment, were made. The result after thinning compared to if the algorithm had been exactly followed was that the total number of retained clones was still 32 instead of proposed 31, but gain and effective number were changed only in insignificant decimals not visible in the table.

<u>Seed orchard Maglehem.</u>

Figure 4 and table 2 presents the results from this study.

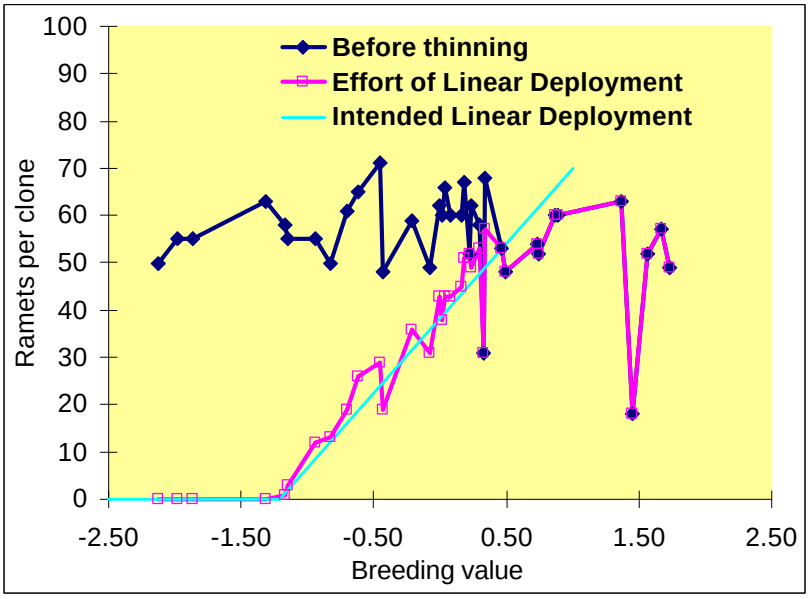

Figure 4. Genetic thinning in Maglehem seed orchard

Table 2. Thinning at Maglehem. N_e = effective clone number (status no.).

	Before thinning	Linear deployment	Truncation 1	Truncation 2	After thinning
Clones	36	32	28	23	32
Ramets	2006	1260	1565	1261	1260
Gain	-0.03	0.49	0.36	0.56	0.48
N_e	34.9	26.8	27.0	22.1	26.8

Linear deployment produces a much higher gain than truncation, if compared at the same effective number and a somewhat higher number of retained clones. Truncation gives maximal gain at the same thinning intensity, but at a high cost in retained clones and effective number. The real thinning was somewhat inoptimal, but the loss in gain because of that was marginal. No problems were noted, which would not have been likely to occur with conventional truncation selection. The seed orchard now functions well. The improved possibilities for seed orchard considerations (spacing, condition of graft, cone set) with linear deployment thinning were regarded an asset (Karlsson 2004).

Eucalyptus camaldulensis clonal trial conversion to seed orchard in India.

The results are presented in table 3.

Table 3. Different thinning strategies of clonal trial in Coimbatore.

Parameter	Before thinning	Linear deployment, same no. of ramets	Linear deployment, same gain	Linear deployment, same N_e	Truncation selection
N	87	72	70	62	43
Ramets	1157	**573**	429	396	**573**
Height, m	7.05	7.49	**7.56**	7.65	**7.56**
N_e	85.7	57.3	50.5	**42.4**	**42.4**

The results show that at the same thinning intensity there is much higher retained number of clones and effective number, but a marginal loss in gain. When thinning to the same gain, there are much higher retained number of clones and effective number, but a more intensive thinning is required. Finally, when the same effective number is desired, there are a higher retained number of clones and more gain, but a more intensive thinning is required.

DISCUSSION

Genetic thinning using truncation selection has the advantage that the genetic gain can be increased more than with other methods at the same thinning intensity. But in the same time the number of clones as well as the effective number of clones, and thus the gene diversity in the produced seed, may be much reduced. Since the original number of clones in a seed orchard often is small, the selection when thinning the orchard is weak. Comparing truncation selection and thinning by linear deployment to the same relative effective number, linear deployment preserves much more of the variance which gives the operator flexibility in future.

The results in this study show, that using the linear deployment algorithm at thinning is theoretically optimal. The loss from optimality because of practical difficulties, such as silvicultural considerations, especially spacing of ramets, is marginal. In the Lagan application there were no possibilities to make big changes in the thinning operation due to the odd design of the orchard. In a orchard with random distribution of clones, such as the Maglehem application, one has to take such considerations. Anyhow, the added flexibility in the method may offer advantages that excel the disadvantages.

Linear Deployment results in a larger variance for the considered character than truncation selection at the same effective clonal number (Lindgren 1993). The effect of this can be debated in terms similar to the value of genetic diversity, there are pros and cons. A change in variance of the index selected for affects only that and correlated characters and can thus be expected to have rather limited consequences, while coancestry affects the whole genome.

The increase in gain and retained clones at the same effective clone number are substantial. It is sometime possible to make significant increases at ***both*** the genetic value ***and*** effective clone number with a moderate genetic thinning. These entities have earlier been seen as incompatible.

There are other factors than analysed in this study which may matter for the optimal number of ramets. Among other factors that can influence are variations in male and female fertility of ramets of different clones, pollen contamination, selfing and relatedness among clones.

We recommend linear deployment thinning for cases where the effective number of clones is a concern. A major reason is that a high effective number is accompanied by high gene diversity of the seed crop. Especially the gene diversity is a political matter for regulatory authorities to cope with, e.g. when deciding about a seed orchards certification for trade. Furthermore, the flexibility in thinning increases since a greater number of ramets are available for rouging than in a truncation selection thinning procedure. Also the possibilities for additional thinning based on later observations are improved (like fertility observations or repeated measurements of test trials at higher age) due to the higher effective number of clones (Varghese et al., 2005), (Kang et al. 2001). Since more clones will be archived in the orchard the seed orchard operator has more options to expand on the clones which will be seen as desirable later, e.g. mass production of controlled crosses or selective harvests for specific goals. There will be improved possibilities for crosses to carry on the long term breeding or to harvest scions for new grafts. If the last copies of clones are removed, options are lost, if it is present in only a few copies, options to expand remain. That more clones are retained is likely to be viewed positively from a legal point of view. The added field work required for the operation is limited and practical experiences positive.

ACKNOWLEDGEMENT

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Selection of Japanese Cedar With Few Allergens and Less Pollen In Akita Prefecture

Y. Sasaki[1], and Y Hotta[2]

[1]Assistant Research Supervisor, Akita Forest Technical Center, Akita, Japan,
[2]Professor, Department of Health and Nutrition, Niigata Health and Welfare University, Niigata, Japan

Japanese cedar (*Cryptomeria japonica*) plantations cover a very large area in Akita Prefecture, Japan, and the spread of cedar pollen has resulted in a recent increase in the number of pollenosis patients in Akita, especially among schoolchildren. We are therefore looking for cedars that produce little pollen or fewer allergens. As Cry j 1 is a major cause of pollenosis, we have focused on reducing quantities of pollen and this allergen. Using sandwich ELISA, we found a seven-fold difference in the Cry j 1 content of eleven strains of elite Japanese cedar in Akita (from 9 to 63 mg Cry j 1/100 g pollen) and grouped them into four classes based on their mean and standard deviation. It may be possible to select trees with fewer allergens from among these trees and their offspring.

The quantity of pollen has been considered a cause of pollenosis. Usually, the Japanese cedar in our seed orchard flower in a random fashion, regulated by the hormone gibberellin. In 1995, however, a large number of elite trees in Akita Prefecture naturally flowered simultaneously, and these elite cedars showed a normal distribution between pollen productivity and the number of trees. However, the correlation between pollen productivity and the content of pollenosis-inducing allergen (Cry j 1) has not been investigated. We believe that the pollen allergens and pollen productivity of Japanese cedar are under independent genetic control.

Plotting Cry j 1 content against pollen productivity in 1995 produced an interesting result. Of 11 strains of elite Japanese cedar, three had high allergen/abundant pollen, two had high allergen/little pollen, three had little allergen/abundant pollen, and three had little allergen/little pollen. The last group is the focus of our research to reduce cedar pollenosis.

Genetic Assessment of Orchard Crops in Japanese Black Pine Clonal Seed Orchard consisting of Pinewood Nematode-Resistant Clones

S. Goto[1], F. Miyahara, Y. Mori, A. Watanabe

During the last five decades, pine forests in Japan have been severely damaged by pine wilt disease caused by pinewood nematodes (*Bursaphelenchus xylophilus*). Based on the severity of the pest and the importance of pine forests, a research project on selection and production of pine-wood nematode resistant plant material was initiated in 1978. Under this project, sixteen resistant trees were selected from 14,620 candidate trees of Japanese black pine. Clonal seed orchards using grafts of these trees have played an important role for the reforestation in the coastal area damaged by pine wilt disease.

In the previous study, we used RAPD markers for clonal checking of ramets in the Japanese black pine clonal seed orchard located on Fukuoka prefecture of Japan and detected one non-orchard clone N1 within the seed orchard (Goto et al., 2001). In this present study, we evaluate the male reproductive success of orchard clones and pollen contamination from inside (N1) and outside source and discussed the influence of pollen contamination on the performance of orchard crops.

MATERIALS AND METHODS

We collected 384 open-pollinated seeds from 24 trees included 6 clones (16 seeds x 4 ramets x 6 clones) in the seed orchard, October 2002. Needles of 16 orchard clones and one non-orchard clone N1 were also collected for the paternity analysis. Genotypes of 384 seeds and 17 candidate paternal clones were determined in five microsatellite loci. Paternity assignment basically relies on the simple exclusion method. Possible paternal alleles at every locus, inferring by subtracting the maternal alleles from offspring alleles, were compared with 17 candidate clones. Clones that did not share the possible paternal alleles at locus were excluded from candidate clones. When all 17 clones were excluded (no match), we judged their paternity was pollen contamination from the outside source. When all but one male tree from them was excluded (exact match), we determined the non-excluded clone as paternal clone of a given offspring.

The observed genetic contribution P_O of the non-orchard clone (N1) was defined as the proportion of the seeds sired by N1 through the paternity analysis. The density of pollen dispersed from individual trees drops off rapidly from the source and can be approximately described by a negative exponential distribution (Lian et al., 2001). Then, we assumed that the expected genetic contribution P_E of N1 will be defined according to the normal distribution with variance t^2 as follows,

$$P_E = aEXP(-(d^2/2t^2))$$

where a and d indicate coefficient of the function and the distance from N1 to each mother tree, respectively. We calculate the log-likelihood of the difference between P_O and P_E, subsequently we optimized the parameters a and t to maximize the log-likelihood using the Solver function of Microsoft™ Excel™.

RESULTS AND DISCUSSION

The average of number of allele and the expected heterozygosity is 9 and 0.795, respectively. High polymorphisms of the microsatellite loci are sufficient for the precise assignment of their paternity. The pollen contamination from outside and inside source (N1) was calculated 9.9% and 3.6%, respectively. The male reproductive success varied widely from 0.0% in Ei(t)-495 and Bizen(t)-143 to 10.5% in Tsuyazaki(t)-50 (Fig.1). Deviated mating will be common in most seed orchards (Goto et al., 2002a; Moriguchi et al., 2004).

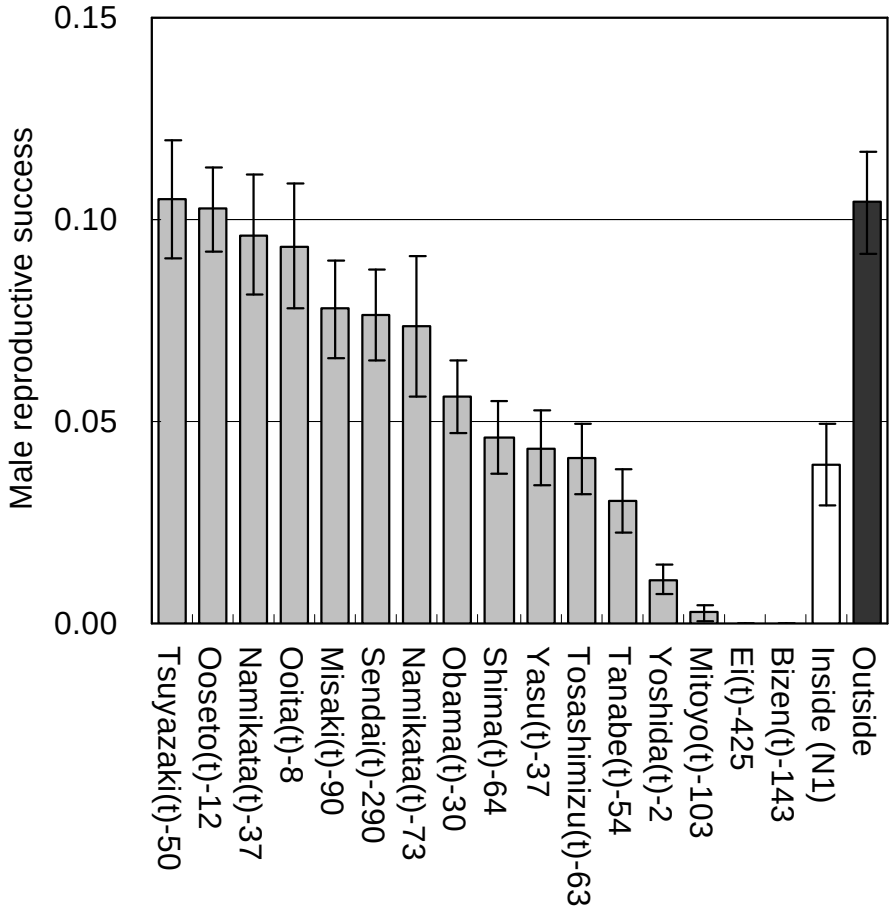

Fig.1 Male reproductive success of orchard clones and pollen contamination from inside (N1) and outside source

To maximize the log-likelihood, the parameters a and t were estimated 21.8% and 24.1m, respectively. This pollen dispersal curve is reasonable compared with the previous reports in Japanese red pine (Lian et al., 2001). When we applied this model to the all 159 matured trees within the seed orchard, P_E for the total orchard crops was estimated 9.0%. On the other hand, outside pollen contamination of each mother tree was not related to the distance from the edge of the seed orchard, therefore outside pollen contamination for the total orchard crops is substituted the value of the seeds surveyed in this study (9.9%).

Nematode-resistant materials of Japanese black pine are produced by inoculating the pinewood nematode to the seedlings produced from the orchard. The survival rate of seedlings through the inoculation is about 60% when mating was occurred within the seed orchard without pollen contamination, but those of seedlings sired by pollen contamination decrease as 25% (Goto et al., 2002b). In this present study, the proportion of seedlings with mating among orchard clones, inside pollen contamination, and outside pollen contamination was estimated 81.1%, 9.9%, and 9.0%, respectively. Therefore, the realized survival rate was calculated as 53.4 % (Fig.2). Recently, the inoculation of pinewood nematode has been conducted to about 50,000 seedlings in Fukuoka prefecture, Japan. Therefore, 30,000 seedlings would expect to survive through the inoculation without pollen contamination, while only 26,689 seedlings will survive in the realistic situation. Economic loss was calculated as 1,655,325 Japanese yen where the cost per plant is supposed as 500 Japanese yen. This study shows that impacts of pollen contamination from inside and outside source will be serious and genetic assessment of pollen contamination is essential for breeding against pinewood nematode.

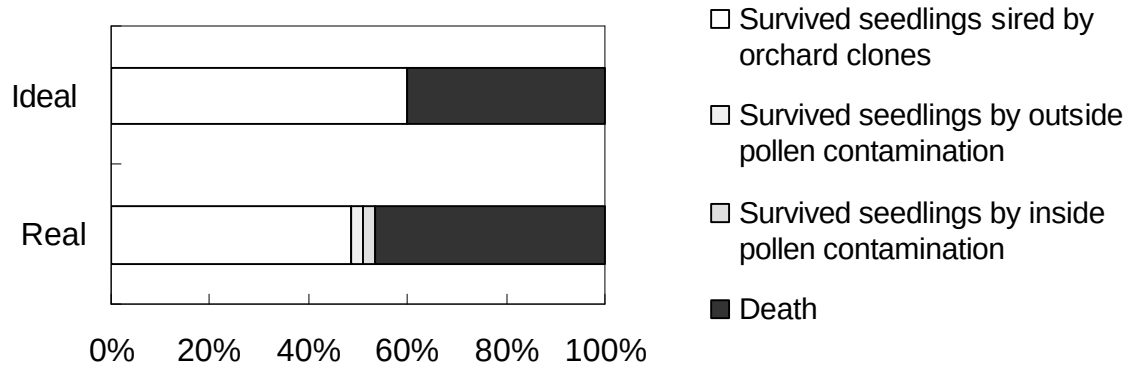

Fig.2 Comparison of survival rates between real and ideal situation

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Peach: A Model Genome For Fruiting Deciduous Trees

Albert G. Abbott

Professor, Dept. of Genetics and Biochemistry
Clemson University, Clemson SC, 29634

Although *Prunus* is an economically and biologically important genus, little was known about the genome structure and organization of its members prior to DNA marker technologies. However, peach (*Prunus persica* L. Batsch) is considered the best genetically characterized species in the genus, and one of the best genetically characterized fruit trees. With the application of DNA marker technologies to the problem of developing genetic resources in trees, peach has distinct advantages that make it suitable as a model species for comparative and functional genomics in the family. Peach has a relatively short juvenility period, 2-3 years compared to most other fruit tree species, such as, apple, pear, and citrus that have a juvenile phase ranging from 6-10 years. While some *Prunus* species such as cultivated plums and sour cherries are polyploid, peach is a diploid with n = 8 and has a comparatively small genome: 5.9 x 10^8 bp or 0.61pg/diploid nucleus (about twice the value for *Arabidopsis thaliana*).

A large number of genes controlling fundamentally important traits have been genetically described in peach. These include genes controlling flower development, fruit development, tree growth habit, dormancy, cold hardiness, and disease and pest resistance. Extensive and detailed molecular genetic mapping efforts are being carried out worldwide, and many of these traits (both single gene and QTL) have been mapped.
The goal of our research is to develop peach as a model genetic resource for the identification, characterization and cloning of important genes of Rosaceae species and for other deciduous tree species.

Comparative genomics research has substantiated that the genomes of *Prunus* species are highly conserved in structure and organization and that markers for traits generated in one species can be used to screen for similar characters in other species crosses. Thus, markers flanking traits of interest in one species are directly useful for identifying the corresponding physically mapped genomic regions of peach which directly provides the tools necessary to genetically dissect these trait containing regions for the purpose of obtaining: 1) more tightly linked markers; 2) genomic BAC contigs spanning the region for gene identification and cloning studies, and 3) already previously identified candidate genes located in these marked regions.

The utility of having as complete a genomics database (integrated genetic/physical map and mapped EST database) for a Rosaceae pioneer species genome is clearly without question. A physical map serves as the tool to cross compare maps of different species and to identify cloned genomic regions containing important gene loci thus facilitating the process of gene marking and gene discovery in related species. For this reason, we have completed an initial framework peach physical map anchored on the general *Prunus* genetic map. Work is underway to complete assignment of the *Prunus* EST unigene set onto this physical/genetic map resource. This physical map will also serve as substrate for the whole genome sequencing of peach as a pioneer species for the family and a model fruit tree genome.

Linking Functional Genomics to Tree Improvement: Growth and Fitness Tradeoffs in *Populus* and *Salix*

S.A. Harding, F.L. Casado, H. Jiang, M. Lee Jeong, H.-W. Lin and C.-J. Tsai[1]

The genera *Populus* and *Salix* (family Salicaceae) are populated by fast-growing woody species that are widely planted as short-rotation tree crops for biomass. Their uses range from pulp and bioenergy production that have commercial value, to phytoremediation and carbon sequestration that are of environmental significance. Since biomass productivity depends on simultaneous selection for growth and 'fitness' traits, such as those affecting nutrient retention and protection against biotic and abiotic stresses, tree improvement programs can benefit from a thorough comprehension of the growth-fitness tradeoffs. Foliar phenolic glycosides (PGs) and condensed tannins (CTs) are important fitness determinants in *Populus* and *Salix* (Lindroth and Hwang 1996; Driebe and Whitham 2000; Orians, 2000). They are the predominant secondary metabolites in these species and can accumulate to high levels (e.g., up to 35% leaf dry weight) that correctlate negatively with growth and, therefore, may incur growth-impacting metabolic costs (Lindroth and Hwang 1996; Kleiner et al. 1999; Ruuhola and Julkunen-Titto 2003). To understand the molecular mechanisms orchestrating resource allocation between growth and fitness, natural cottonwood and willow hybrids were investigated using traditional analysis of leaf phenolics coupled with metabolic profiling and cDNA microarray hybridization.

RESULTS AND DISCUSSION

Seven backcross lines of *P. fremontii* and *P. angustifolia* exhibiting growth/CT-PG phenotypes ranging from fast/low to slow/high were characterized by GC-MS metabolic profiling (Lee Jeong et al. 2004). The lines were separable on the basis of principal component analysis (PCA) of their primary metabolite profiles, with higher and lower CT lines tending to segregate according to their underlying metabolism. The results revealed the potential for distinct metabolic links between primary and secondary metabolism among the various CT-PG phenotypes. Hydroponic N deprivation was used as a means to perturb resource reallocation, and three cottonwood lines exhibiting differential CT-PG responses to nitrogen starvation (i.e., non-responsive, CT-responsive, and PG-responsive) were investigated further by metabolic profiling and microarray analyses.

The CT- and PG-responsive lines substantially increased their phenolic levels during stress, but at greatly differing overall metabolic costs based on key metabolite levels and gene expression changes. For instance, levels of amino acids remained essentially unchanged in the CT-responsive line, but were greatly reduced in the PG-responsive line. The other line exhibiting a weak phenolics response also mounted a strong amino acid response, suggesting a reprogramming of primary metabolism. Global gene expression analysis revealed a reduced expression of genes directly associated with photosynthetic light harvesting and carbon fixation in this line during N stress.

[1] Biotechnology Research Center, School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931.
Author of correspondence: chtsai@mtu.edu

Differential gene expression changes in response to N starvation were also observed in the PG- and CT-responsive lines. Specifically, phenylalanine ammonia-lyase (PAL1) expression increased in the PG-responsive line, but not the CT-elevated line, even though both PG and CT biosynthesis involves PAL (Kao et al., 2002). Anthocyanidin reductase, required for CT biosynthesis, increased in the CT-elevated line, but did not change in the PG-responsive line where CT remained constant. Genes belonging to the protein synthesis and protein fate categories were also severely affected during stress of the PG-responsive line.

Variable metabolite and global gene expression changes were also observed in natural willow hybrids exhibiting a wide range of basal and stress-induced CT-PG levels. Our results suggested that stresses that can effect fitness sinks (e.g., CT and PG pools) do so in a broad physiological and metabolic context, and interface variably with photosynthesis, protein synthesis and primary (amino acid) metabolism among various *Populus* and *Salix* hybrids. Ongoing analysis of these results is expected to reveal important insights into the interface of primary and secondary metabolism with respect to growth and stress management in these species.

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Functional Genomics of Wood Formation and Beyond

MacKay J.[1], Bousquet, J.[1], Cooke J.[1], Pavy N.[1], Séguin A.[2], Isabel, N.[2], Regan, R.[3], Retzel, E.[4]

Abstract

Wood formation in forest trees is a dynamic process that is modulated by a variety of genetic determinants and environmental cues. We are using a functional genomics approach to characterize to delineate groups of coordinately regulated genes and putatively associated regulators of gene expression in two forest tree taxa, poplar and white spruce. Our goal is to provide a deeper and broader knowledge of candidate genes to integrate into association mapping studies aiming to uncover the molecular basis of naturally-occurring phenotypic variation, and ultimately lead to applications in marker assisted breeding.
In this report, we summarize our work in EST sequencing, conducted as a pre-requisite for large-scale gene expression analysis. We also present our functional analysis approach of putative transcription factors and other proteins that we hypothesize to be regulators of wood formation. As a means to further define the role plaid by these genes, we have developed a suite of transgenic poplar and spruce trees that mis-express each of the candidate genes. Large-scale transcript profiling with custom microarrays is being used to assess how putative regulatory genes and environmental conditions modulate gene expression. We are also using activation-tagging for large-scale functional discovery of genes in poplar. This unbiased gain-of-function approach is being used to produce up to 10,000 mutant poplar lines to be intensely screened and provide a unprecedented resource for forest genomics, to be exploited over the next several years.

Large-Scale EST Sequence Analysis in White Spruce (*Picea glauca L.)*

We have synthesized and sequenced ESTs (Expressed Sequence Tags) from 17 spruce and 8 poplar non normalized cDNA libraries (Tables 1 & 2). Libraries were made from not commonly sampled tissues or conditions, and each represented several developmental stages, manipulative treatments, and/or time points. Standard library synthesis methods were optimized for long insert selection. Here, we report the results of spruce EST sequence analysis.
Approximately 50,000 spruce cDNA clones were randomly selected and sequenced from the 3' end. Clones from the highest quality libraries were also sequenced from the 5' end to accelerate gene discovery. Sequencing of 3' ends is ideal for discovery of DNA polymorphism, and was chosen as it offers robust clustering. Although read quality and length varied considerably between libraries, the processed sequences were generally long, averaging over 600 bp (Table 2). Sequence assemblies encompassing all of the sequences from random clones gave 16,578 spruce consensus sequences (singlets and contigs). Sequencing from 3' and 5' ends has lead to superior average consensus sequence length compared to previous publicly available datasets, and is improving our ability to assign putative gene functions. In total, 45% of the clones have been

[1] Centre de Recherche en Biologie Forestière, Université Laval, Québec City, Canada.
[2] Natural Resources Canada, Laurentian Forestry Centre, Québec City, Canada
[3] Department of Biology, Queen's University, Kingston, Ontario, Canada
[4] Center of Computational Genomics and Bioinformatics, Univ. of Minnesota, Minneapolis, MN

completely sequenced, at least in one direction. In conjunction with microarray manufacture, we have now re-arrayed and resequenced a poplar unigene of 3400 clones, and a spruce unigene of 9000 clones, both of which have been printed onto microarrays. We have also released 28,882 spruce sequences to Genbank. The Arborea web page (www.Arborea.ulaval.ca) provides library description, an overview of sequencing results, release dates and links to sequence assemblies.

Table 1. EST sequence analysis and release summary statistics (as of September 2004).

Processed Sequences	**Spruce: 73,658**				**Poplar: 14,208**	
	3' reads 17 libraries		**5' reads** 11 libraries		**5' reads** 8 libraries	
	All	Quality	All	Quality	All	Quality
NUMBER of READS	49536	32418	24096	19205	14208	11657
Average READ LENGTH (bp)	386	580	305	381	458	554
PROPORTION QUALITY reads		65%		80%		82%
DATA RELEASED quality reads	----	18560	----	10322	----	8907

Table 2. WHITE SPRUCE (*Picea glauca L.*) - cDNA libraries and EST sequencing

Library ID#	**LIBRARY NAME (Treatments & tissues)**	**Number of Quality Reads[1]**	**Average Length Processed Quality reads**
GQ001	Male STROBILI development sequence	2589	527
GQ002	Female CONES development sequence	2324	500
GQ003	Vegetative BUDS development sequence	1062	560
GQ004	Secondary XYLEM - mature trees	7735	600
GQ006	CAMBIUM, PHLOEM - mature trees	6705	635
GQ007	Secondary XYLEM - girdled seedlings	1053	556
GQ008	CAMBIUM to BARK - girdled seedlings	937	577
GQ013	Elongating ROOT tips - saplings	2050	470
GQ016	Primary, sec. SHOOT - N fert. treatments	3031	736
GQ017	Immature somatic EMBRYOS	2220	692
GQ018	Clean ROOTS systems - N treatments	1645	659
GQ019	Clean ROOTS systems - P treatments	3776	705
GQ020	Clean ROOTS systems - Diurnal cycle	8601	757
GQ022	ROOT secondary XYLEM - mature trees	1532	598
GQ025	Annual flush SHOOTS diurnal cycle - trees	5164	658
GQ026	NEEDLES - N fertilization treatments	461	686
GQ027	Mature somatic EMBRYOS	281	490
TOTAL		**51623**	

[1]Quality Sequences are those that contain >100 bp of Phred20 sequencing after vector trimming.

Genome-Wide Analysis and Discovery of Gene Function

Microarray RNA transcript profiling affords the opportunity to study the expression of thousands of genes at once, and thus, identify groups of coordinately regulated genes, make inferences relating to mechanisms controlling gene expression, or genetic and molecular processes underlying phenotypes of interest. We have developed an experimental framework which relies on the mis-expression of putative regulatory genes, primarily transcription factors, to help dissect the complex information obtained through large-scale transcript profiling. For this purpose, we have generated transgenic spruce and poplar trees which over-express members of the HD-zipIII, R2R3-MYB, LIM, SCARECROW and KNOX transcription factor families. The experimental pipeline that we have established is illustrated in Figure 1. We based the choice of these genes upon their implication in vascular growth and development in model angiosperm plants, like *Arabidopsis thaliana*. Thus the overall approach aims to test specific hypotheses related to the control of gene expression and vascular development.

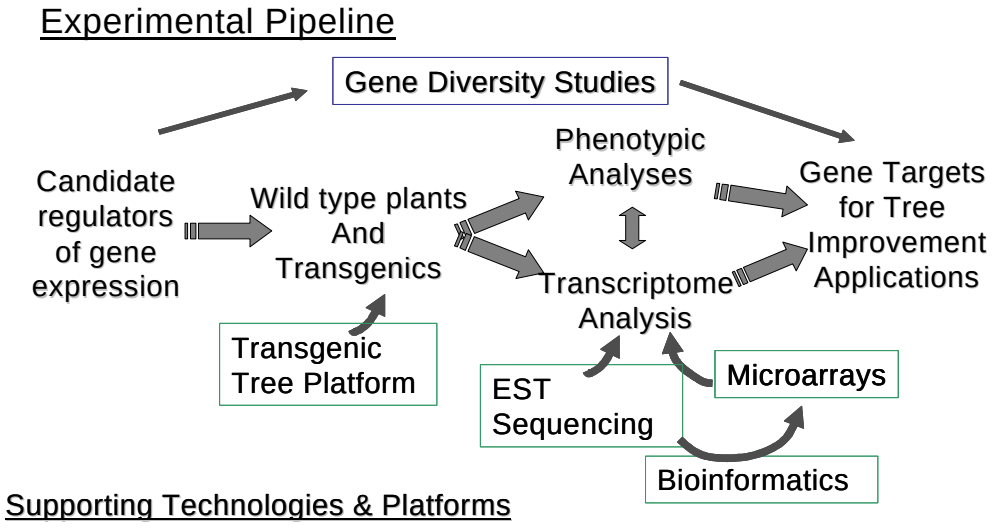


Figure 1. Experimental pipeline for genome-wide analysis of regulatory gene function.

We are also using an alternative approach, that of activation-tagging which is not premised on hypotheses and thus is unbiased as to which genes may play key roles in wood formation and properties. Activation tagging, is a gain-of-function approach, where transgenic lines are obtained in which a strong promoter sequence has been inserted randomly throughout the genome. When the insertion occurs next to a gene, its expression may be activated ectopically or at a higher level than normal, thus causing a dominant mutant phenotype. The mutant lines are screened to uncover altered xylem phenotypes, leading to the isolation and characterization of the tagged genes. We are currently actively producing tagged lines with the goal of obtaining 10,000 lines for submission to a thorough screening process.

Conclusion and Future Perspectives.

While model plants provide tremendous insight into xylem differentiation, we are conducting functional genomic analyses directly in softwood and hardwood forest trees, to best inform

association mapping and QTL-EST co-localization studies in trees. Functional investigations of tree genes are indeed essential, as many key regulators are part of large gene families that are not orthologous between angiosperm plants and softwood trees (gymnosperms). Although much remains to be learned about the genetic control of wood formation in trees, we are seeking to integrate functional genomic results, gene diversity analyses (Figure 1) and genetic mapping to enable association mapping experiments. In turn, high throughput or large-scale genotyping assays remain to be developed for efficient association mapping based upon the screening of SNPs in hundreds or thousands of genes.

Genetic Regulation of Wood Formation

Kyung-Hwan Han[1], Jae-Heung Ko[2], Jaemo Yang[3], Sunchung Park[3], Costas Prassinos[3], and Andrew H. Park[3]

[1]Associate Professor, [2]Post-doctoral Research Associate, [3]Graduate Student, Department of Forestry, 126 Natural Resources, Michigan State University, East Lansing, MI 48824-1222, USA

Trees undergo secondary growth and produce a woody body as a result of growth and differentiation of the cells produced by the cambial meristem. This secondary growth is one of the key target traits in tree domestication efforts. The ability to achieve enhanced diameter growth and/or to maintain healthy stems is especially critical in high density, intensive plantation cultures. The overarching goal of our study is understand the molecular mechanisms involved in the control of cambial meristem activity and the genetic regulation of annual growth cycle.

We are using a two-model-system strategy to achieve the goal. First, we take advantage of the advances in *Arabidopsis* functional genomics and publicly available genetic resources to identify candidate genes that regulate wood formation. Recently, we established novel experimental conditions that induce secondary growth in *Arabidopsis* plants, causing them to express all of the major components of wood formation during ontogeny (Plant Physiology 135: 1069-1083). Using this system, we demonstrate that the weight carried by the stem is a primary signal for the induction of cambium differentiation and the plant hormone, auxin, is a downstream carrier of the signal for this process. The *Arabidopsis* whole-transcriptome GeneChip analyses have provided an unprecedented view of the flux that occurs in the transciptome of the wood forming-stems of *Arabidopsis* plants, which further support the hypothesis that increasing body weight in a growing tree triggers the transition from primary growth to secondary growth. However, *Arabidopsis* is not a tree, so it is important to conduct parallel investigations using a tree species. The second phase of our study is to apply the molecular information gained from the *Arabidopsis* work to poplar, a tree species that is especially well suited for this study.

In addition, we are also focusing on the genetic control of annual growth cycle in poplar, which is closely timed with seasonal changes in daylength and temperature. As the first step toward understanding the molecular basis of this process in trees, a series of transcriptional profiling experiments were carried out to identify batteries of genes whose expression patterns are associated with the annual growth cycle. In order to gain further insights into the genetic mechanisms that govern the onset and release of dormancy during the annual growth cycle, monthly profiles of the genes expressed in the xylem and bark tissues of field-grown poplars were obtained using cDNA microarrays and compared with those of controlled environment-grown poplars. Cluster analyses of the differentially expressed genes confirmed the previously held notion that trees regulate seasonal growth cycle by integrating environmental factors with developmental state. The genes identified in this study can become the focus of tree improvement strategies for exploitation of maximum productivity and manipulation of physical property of the wood for production of value-added forest products.

Genomics, Biotechnology and Domestication in Poplar

Amy Brunner, Caiping Ma, Richard Meilan[1] Victor Busov[2], Steven Strauss

Department of Forest Science, Oregon State University, Corvallis, OR, USA
[1] Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA
[2] School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, USA

Because of its transformability, clonability, rapid growth, and extensive genomic resources, including a publicly-available genome sequence, poplar is an extraordinary system in which to investigate the potential for transgenic biotechnology, and to advance tree domestication. Towards these goals, we use comparative genomics, microarray expression analysis and gene function analysis in transgenic trees. The attributes of the poplar system has allowed both reverse genetics, where specific genes are chosen based on sequence and then mutants generated by altering expression of those genes, and forward genetics, where mutants are first generated and the affected genes then identified.

Our laboratory has generated more than 6,500 independent gene-transfer events in 17 different genotypes of *Populus*, and field-tested more than 1,600 of these lines. Specific traits that we study include maturation, flowering and stature. We have demonstrated the potential of poplar forward genetics by producing a population of 627 independent transgenic lines using activation tagging that were screened in tissue culture, greenhouse, and field. After identifying 10 mutants in the lab/greenhouse, we identified 32 additional mutants in the field with altered morphological, phenological, and physiological traits. Key lessons from our experience include: 1) Stable gene expression is the rule in vegetatively propagated transgenic poplars; 2) Somaclonal variation is modest and manageable; 3) Transformation and field tests are extraordinary functional genomics methods; 4) The value of transgenic traits look high, but await careful, broad evaluation; 5) Sterility systems can be developed via diverse means; and 6) Domestication transgenes can provide new avenues to promote biosafety. In sum, transformation in poplar is extremely reliable, making it a logical choice for developing new technologies and providing commercial demonstrations that pave the way for broader adoption in forestry.

Marker-directed Population Improvement

C.D. Nelson[1] and C.S. Echt[1]

[1]USDA Forest Service, Southern Institute of Forest Genetics,
23332 Miss. Highway 67, Saucier, MS 39564 USA

If increasing the frequency of favorable alleles in populations is the goal of tree breeding, then combining marker-trait correlated selection with accelerated breeding should offer a most efficient means for achieving this goal. We have initiated a project to study the various factors involved in developing a framework for the implementation of marker-directed population improvement (MDPI) in tree breeding programs such as those practiced for loblolly pine in the southern U.S. These factors include the number, distribution, and information content of genetic markers, the number of parents and mating designs for producing progeny populations, the number of progeny evaluated and selected in each generation, the number of traits evaluated and their inheritance mode, and the technical opportunities for efficiently detecting and utilizing quantitative trait variation with genetic markers. Our basic model works at the breeding population level within the context of multiple population breeding programs. Each population consists of multiple families in which linkage dis-equilibrium is maximized over large stretches of the genomes of the progeny trees. The association of marker and trait alleles are determined in the population and thus all relevant parameters for MDPI exist— linkage phase known and allelic (both marker and trait) variation present. A set of informative markers well spaced across the genome permits robust quantitative trait variation detection, linkage phase determination, and accurate prediction of genetic merit (i.e., multi-trait breeding value). Selecting individuals with the highest breeding values provides advanced generations with ever increasing frequencies of favorable alleles, providing better parents for deployment populations in less time than under traditional selection schemes. We will explore these factors and provide a new framework (MDPI) for developing marker-based selection in tree improvement, both from the perspective of utilizing existing populations as well as developing new populations specifically for MDPI.

Marker Aided Selection of Sitka Spruce in Great Britain.

S.J. Lee[1], S.W. A'Hara[2] and J. Cottrell[2]

[1]Conifer Breeding Programme, [2]Molecular Biologist,
Forest Research,
An Agency of the British Forestry Commission,
Roslin, EH25 9SY, Scotland.

Forest Research has embarked on a programme of Marker Aided Selection for the principal conifer species grown in Britain - Sitka spruce (*Picea sitchensis*). The overall objective is to increase the efficiency of Sitka spruce breeding and selection by screening in the laboratory for economically important traits relating to wood quality and volume. The initial objective is to find markers for wood density although it is expected that the programme will be extended to include a large number of markers relating to a range of traits. Screening with markers will improve the gains available to the forestry industry by increasing the selection intensity and identifying at a very early age those rare genotypes that combine generally conflicting traits such as fast growth rate and high wood density. Cryopreservation and somatic embryogenesis will assist in delivering the gains to industry.

The new programme involves establishing an accurate relationship between phenotypic field performance and markers identified in the laboratory. To this end, a large clonal trial is planned which will be replicated across climatically contrasting sites. The trees for this trial will consist of 1,500 genotypes from within each of 3 full-sib families; 4,500 genotypes in all. There will be 4 ramets of each genotype at each site. The same genotypes will be planted at all three sites.

Work has started on the development of a molecular map in one of the full-sib families. A framework map will be established, based on Simple Sequence Repeats (SSRs). These are being sourced from the literature and by data-mining Expressed Sequenced Tags (ESTs) available in Genbank. In addition, novel genomic SSRs have been developed specifically for this project. The primary objective is to have a set of 200 framework SSR markers and then to use Single Nucleotide Polymorphisms (SNPs) to increase marker density.

Microarray Analysis of Gene Expression in OP Pine Families in Field Plantings

R. W. Whetten, Z. Li, and S. E. McKeand[1]

Abstract: Adaptation of genomic science methods to exploit available experimental materials is an important strategy for tree biologists, and is necessary to leverage the extensive investment already made in establishment and measurement of test plantings over the past several decades. This study was conducted to test the utility of microarray analysis of gene expression for tissues sampled from six-year-old field trials of open-pollinated loblolly pine families. Genes were chosen for use in this study based on reported differential expression in model plants responding to increased nitrate availability, and putative pine homologs were identified in collections of partially-sequenced cDNA clones. The microarray experiments were conducted with replication at both technical and biological levels. Steady-state levels of mRNA transcripts for 63 genes were compared between three different open-pollinated loblolly pine families grown under two different nutrient regimes at the SETRES-2 study in Scotland County, NC. The resulting data were analyzed using linear models incorporating both fixed and random effects, to account for the various experimental factors and sources of noise. Significant differences in gene expression were detectable for many of the contrasts tested, between families as well as between control and fertilized plots. We conclude that microarray analysis of gene expression differences can be applied to samples collected from field plantings of open-pollinated pine families, provided that sufficient replication is included in the experimental design to accommodate the inevitable variation due to genetic and environmental heterogeneity.

Keywords: gene expression profiling, nutrient response, *Pinus taeda* L., fertilization.

INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the most widely-planted tree species in the United States, due to its importance in plantation forestry in the southern US (McKeand et al, 2003). The response of loblolly pine to nutrient treatment is an important part of ongoing genetic improvement research with loblolly pine. A study was established in 1993 to compare the performance of open-pollinated families of Atlantic Coastal Plain (ACP) and Lost Pine – Texas (LPT) provenances with and without nutrient supplementation. Initial results showed increased growth in response to the nutrient treatment, with differences in the degree of response at both the provenance level and family level (McKeand et al., 2000).

The genetic basis of increased growth is an important question in forest genetics. The traditional polygenic model assumes that differences in growth or other quantitative traits is due to differences in many genes, each of small individual effect. Molecular genetic studies in annual plant species, in contrast, show that a few genes of major effect can play important roles in controlling some biological processes (Remington and Purugganan, 2003). Experiments to test these alternative perspectives in forest trees require the ability to conduct molecular genetic

[1] Department of Forestry, N. C. State University, Raleigh, NC 27695-8002, USA

experiments in contexts relevant to tree breeding, such as open-pollinated families in field trials. We report here results of a preliminary study to test the ability of microarray analysis to detect effects of nutrient application to loblolly pine in a field site. The basis for comparison of gene expression patterns is the open-pollinated family level, with comparisons between families and between treatments.

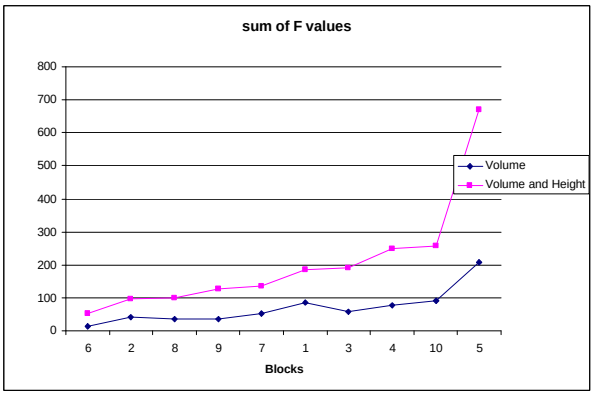
The study used methods of sample collection, RNA extraction, and data analysis chosen specifically to deal with the high levels of environmental and genetic heterogeneity expected within and between open-pollinated pine families growing in the field. The objective of the study was to identify differences in gene expression attributable to genetic differences between families or to different treatments. Statistically significant differences in gene expression levels were detected, both between families and between treatments. The number of genes tested was very small, so few detailed conclusions can be drawn about the genetics or physiology of pine growth response to fertilization. The results are sufficiently robust, however, to justify a larger future survey of gene expression differences in response to fertilization.

MATERIALS AND METHODS

Collection and processing of tissue samples

Foliage samples were collected from the SETRES-2 study site in Scotland County, NC (McKeand et al., 2000). Height and stem volume were considered when selecting the families and individual trees to be analyzed. These two characters are widely used for evaluation of pine growth (Li et al, 2000). The ACP provenance outperformed the LPT provenance in both the fertilized and control treatments (McKeand et al., 2000), and so three ACP families were chosen for analysis, representing the extremes of growth response in both fertilized and non-fertilized treatments within that provenance. Family A ranked first in mean height and second in mean volume, family B ranked first in mean volume and second in mean height, and family C ranked lowest for the mean values of both height and volume.

Blocks were selected for sampling according to two criteria. A lower significance of the plot with block effects was considered to indicate lower environmental variability, which was considered desirable for genetic analysis. The height and volume observations were fitted in a linear model with family plot as a fixed effect, using either volume alone or volume plus height as the response variable. The blocks were then ranked by increasing F values of the family effect (Fig 1). Blocks 2, 6, 7, 8, and 9 appeared to be the least variable. The second criterion was experimental convenience: blocks 6, 7, 8 and 9 are close to each other and tissue samples could be collected conveniently.



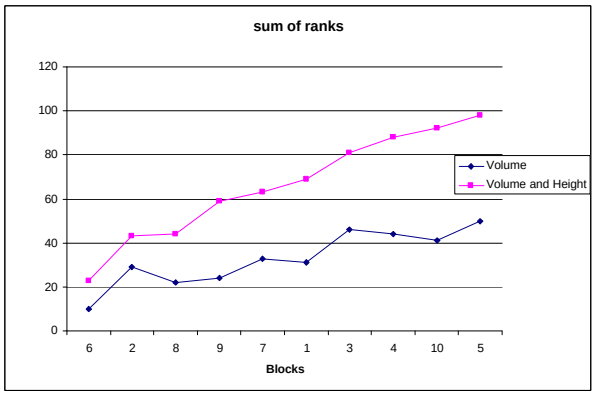


Figure 1 Sum of family effect F-values (left) and sum of family effect F-value ranks (right) for the ten blocks

Individual trees were selected from within these four blocks based on similarity of individual-tree measures to the mean of that block. Mean and standard deviation were calculated for tree volume for each family plot from which samples were to be harvested. Ten to fifteen individual trees with trait measures closest to the plot mean were selected, and then further screened for the five trees with the lowest deviation from the plot mean height.

Sample collection times were kept to the minimal possible, to reduce environmental variation between samples. Sample harvest work started at 9 am and finished at about 2 pm. Samples were collected by block, in order of the spatial arrangement of blocks at the planting site. The families are randomly arranged in the provenance sub plot, so the sequence in which the tissue samples were collected is therefore random with respect to family. Each sample harvested was the current spring (2001) shoot tip of the first flush of the previous year (2000), taken from branches that point north. Shoot tips were collected from the trees with a pole pruner, and the needles were removed and cut into small pieces, then frozen immediately in liquid nitrogen. An equal amount of tissue was collected from each of the five trees sampled in each subplot. After sample collection in a subplot was complete, the tissue was transferred from liquid nitrogen to a paper box stored in a cooler with dry ice. Each paper box contained samples from a single family × fertilization × block combination.

RNA extraction

Samples from blocks 6 and 9 with the same family × fertilization treatment were pooled for extraction of RNA, as were samples from blocks 7 and 8 with the same family × fertilization treatment. This pooling step was carried out to average the environmental differences between set of two blocks, and reduce the number of microarray hybridizations required to analyze the samples.

Each pooled tissue sample was ground with dry ice in a coffee grinder. The powdered tissue sample was collected with a spatula into a disposable centrifuge tube and kept at -80º C in an ultra-low freezer. Before RNA extraction the powdered tissue was finely ground with liquid nitrogen in a mortar, and weighed with a scale to assure that uniform amounts of tissue were used. The CTAB extraction procedure (Chang et al., 1993) was used to isolate total RNA from the powdered tissue samples. The final pellets were dissolved with 50 µl DEPC treated water, the

concentrations were determined by absorbance at 260 and 280 nm, and the RNA solutions were stored at –80º C.

Selection of probe sequences

Genes selected for use as probes on the microarrays were selected to test the hypothesis that fertilization in pine plantations would affect mRNA levels of pine genes homologous to *Arabidopsis* genes shown to be differentially expressed in response to nitrate application (Wang et al, 2000). The following steps were used to identify candidate pine genes for use as probes on the microarrays.

1. The locus identifiers of *Arabidopsis* genes from Wang et al (2000) were used to search for physiological pathways in the KEGG database, and at the same time the corresponding protein sequences were used as TBLASTN queries to search the pine EST collection for possible homologs.
2. DNA sequences of candidate pine homologs of *Arabidopsis* genes were translated to predicted amino acid sequences and used as BLASTP queries in searches of *Arabidopsis* proteins. Pine candidates that identified the putative *Arabidopsis* homolog as the most similar protein were used on the microarrays, while pine candidate cDNAs that identified a different protein as the most similar in the BLASTP search were discarded as potential probes.
3. Genes in physiological pathways related with the metabolism of nitrate were also included in the searches, in addition to the nitrate-responsive genes identified by Wang et al. (2000). The seven pathways from which genes were selected are:

- energy metabolism
  - photosynthetic carbon fixation pathway
  - nitrogen reduction and fixation pathway
- carbohydrate metabolism
  - pentose phosphate cycle
- amino acid metabolism
  - glutathione metabolism
  - glycine, serine and theronine metabolism
  - glutamate metabolism
  - selenoamino acid metabolism

Yeast protein sequences of enzymes in these pathways were retrieved from the database, and candidate pine homologs were identified in the pine EST collection using the same methods described above.

Array experimental design:

We used a balanced incomplete block design for the microarray experiment, in which arrays are blocks of size two, and not every family and treatment combination can appear in the same array (Kerr and Churchill, 2001). The hybridization design is shown in directed graph format below, in which each arrow connecting two samples represents a microarray to which labeled targets derived from those two RNA samples were hybridized. The direction of the arrows shows which fluorescent dye was used in labeling each target; the arrowhead represents Cy5 labeling of one sample and the arrow base represents Cy3 labeling of the other sample.

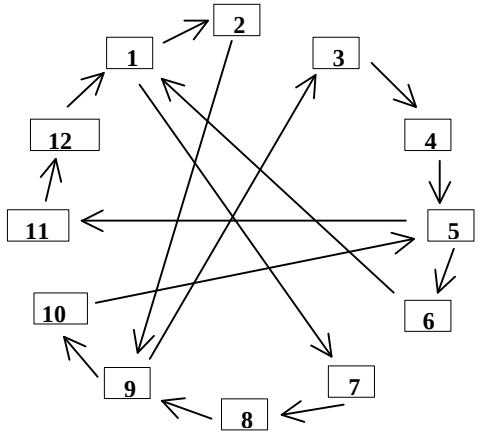


Fig 2. The balanced incomplete block hybridization design gives equal emphasis to comparisons of treatments and families.

Table 1. Identities of samples in array design

Number	Family	Treatment	Blocks
1	B	control	7, 8
2	B	fertilized	7, 8
3	B	control	6, 9
4	B	fertilized	6, 9
5	C	control	7, 8
6	C	fertilized	7, 8
7	C	control	6, 9
8	C	fertilized	6, 9
9	A	control	7, 8
10	A	fertilized	7, 8
11	A	control	6, 9
12	A	fertilized	6, 9

Probe and microarray preparation

Stock solutions of plasmid cDNA clones were diluted one-hundred fold and clone inserts were PCR amplified in 50 µl reactions in 96-well reaction plates (MJ Research) using M13 forward and reverse universal primers. Samples of each PCR product were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized using UV light. This step was necessary to confirm both quantity and quality of the PCR reactions. The primary criterion for quality in this case is the presence of a single band. PCR reactions that contain two equally abundant products were considered likely to have arisen from contaminated templates, and were not used in the microarray experiment.

Unincorporated nucleotides and primers were removed from the PCR products using 96-well multiscreen filter plates (Millipore Corp. Bedford, MA). Purified DNA was recovered in 40 *m*l H2O (Sigma, St. Louis, MO) and dimethyl sulfoxide added to a final concentration of 50%. Denatured purified DNA was transferred to v-bottom shaped 384-well plates (Genetix, Dorset, UK), and printed on CMT-GAPS aminosilane-coated glass microscope slides (Corning, Corning, NY, USA) using an Affymetrix 417 Arrayer (Affymetrix, Woburn, MA). Twelve replicate spots of each gene were printed on each array, two spots each by six different print tips. After printing, slides were allowed to dry and spotted DNA was bound to each slide by UV-crosslinking (250 mJ/cm^2) using a Stratalinker® (Stratagene, LA Jolla, CA, USA). Slides were then baked at 75º C for 2 hours and stored in provided slide containers at room temperature.

Target preparation and hybridization

Twenty micrograms of purified total RNA were used for each cDNA synthesis reaction. First and second strand cDNA synthesis were performed according to the manufacturer's recommendations (Gibco-BRL, USA). Reactions were then precipitated using equal volumes of 2-propanol, dried and re-dissolved in 63 *m*l H2O. Re-dissolved cDNA was denatured for 5min at 95 °C and kept on ice until use. Labeling of the cDNA was carried out in a 100 *m*l reaction volume and incubated using a miniCycler (MJ Research, USA) at 37 °C for 1 hour in the dark, according to previously published methods (van Zyl et al., 2002). Labeled target DNAs were purified using Qiaquick spin columns (Qiagen, USA), and then dried and re-dissolved in 20 µl

hybridization buffer containing 50 % formamide, 5 x SSC, 0.5 % SDS, 5 x Denharts solution (Sigma, USA), 0.5 mg/ml poly A-RNA (Sigma, USA) and 0.5 mg/ml calf thymus DNA (Sigma, USA). Targets were denatured for 2 min at 95 °C and kept on ice until use.

Microarray slides were pre-hybridized for 45 min at 42° C in a solution containing 5 x SSC, 0.1 % SDS and 1% BSA, Fraction V (Sigma, USA), then washed in water, rinsed in 2-propanol, and dried by centrifugation at 500 rpm for 5 min. The denatured labeled targets (20 ml) were then added to each pre-hybridized slide, covered with a plastic cover-slip (HybriSlip, Surgipath Medical Industries Inc, USA), placed in a hybridization chamber (ArrayIt, TeleChem International, USA) and incubated in the dark for 16 to 20 hours at 42° C. After hybridization the slides were washed for 4 min each in 1x SSC, 0.2% SDS, followed by 0.1x SS, 0.2% SDS and then rinsed with 0.1x SSC. Slides were dried by centrifugation at 500 rpm for 5 min and scanned using a ScanArray 4000 Microarray Analysis System (GSI Lumonics, Watertown, MA, USA).

Data acquisition and analysis

The images were processed using Quantarray software (GSI Lumonics), and intensity data and position data acquired for every spot on each slide. Perl scripts, an MS Access database and MS Excel workbooks were used to preprocess and manage the microarray data. A concatenated data file with array, position, and probe information and intensity measurements was produced from the database for analysis with SAS system software.

Analysis of the microarray data used mixed linear models incorporating both fixed and random effects (Wolfinger et al., 2001). This approach centers around two interconnected analysis-of-variance (ANOVA) models: the normalization model and the gene model. The normalization model analyzes the experiment-wide variance, while the gene model analyzes the gene level variance.

The normalization model accounts for systematic effects that occur over the entire experiment, such as variation between labeling reactions or between different hybridizations. Such differences are not specific to individual genes, but could bias inferences made on the data from the individual genes. The model is:

$$Y_{jk}=m+A_j+D_k+A\times D+\varepsilon$$

Y_{jk} is the base 2 logarithm of the background corrected signal for array j and dye k, m is the overall mean value, A is the random effect for arrays, D is the fixed effect for dyes, A×D is the interaction effect of array and dye, and ε is stochastic error. The residuals from the normalization model are the "normalized data" used for analysis of gene-specific effects, because most of the experiment-wide variation has been removed.

The residuals from the normalization model are analyzed separately for each gene, using data from all replicate spots and all arrays. The gene model is a combination of the models typically used in forest genetics and the gene model previously used for analysis of microarray data at the gene level (Wolfinger et al., 2001). The gene model is:

$$R_{gjklmn}=\mu+ S_l + D_k + F_m + E_n + F\times D + F\times E + D\times E + F\times D\times E + A\times S + D\times S + \varepsilon$$

R_{gjklmn} is the residual value from the normalization model for spot l of gene g on array j with dye k hybridized with target from family m sampled from treatment n, m is the overall mean value, S_l is the spot effect, which accounts for random variability among the twelve replicate spots of each

gene on each microarray, D_k is the fixed dye effect as in the normalization model, F_m is the fixed family effect, and E_n the fixed nutrient treatment effect. The family and treatment factors for each gene, and their interactions, are the key experimental effects of interest. $A_j \times S_l$ and $D_k \times S_l$ interaction terms account for the variance of repeated measurements for each spot across different arrays and dyes, including the contributions of local background, and ε is stochastic error.

Statistical inferences based on the gene model were used in multiple comparisons for each gene based on least-squares-means estimates of differences in gene expression between pairs of treatments. The probability of differential expression was adjusted for multiple comparisons using the Bonferroni correction.

RESULTS

Model fitting results

The dye effect is the only fixed effect in the normalization model, and it is not significant. There are three fixed effects and four interactions of fixed effects in the gene model. Among them, fertilization, family and fertilization × family interaction are the effects of greatest interest. Many of the 63 genes tested show significant differences due to the fixed effects (Table 2). All but three genes show significant differences in expression between families, all but seven show differences due to fertilization, and all but one show a family × fertilization interaction effect.

Table 2. Significance levels for type 3 estimates of fixed effect in the gene model[2]

Probe	fam	fam*fert	fert	Probe	fam	fam*fert	fert	Probe	fam	fam*fert	fert
AA556420	****	****	**	AW290405	****	****	****	BE496409	****	****	****
AA556491	****	****	|	AW290590	****	****	****	BE496464	****	****	****
AA556526	|	****	****	AW290829	****	***	***	BE496557	****	****	|
AA556674	**	****	****	AW495754	****	*	****	BE496574	****	****	****
AI812344	****	***	****	AW626636	****	****	****	BE582124	****	****	****
AI812383	****	****	****	AW783974	|	|	****	BE582359	****	****	****
AI812384	****	****	****	AW870043	****	****	****	BE758619	**	*	|
AI812720	****	****	****	AW870079	****	****	****	BE758652	|	****	****
AI812956	****	****	****	AW888011	****	****	*	BE762095	****	****	****
AI813214	****	****	*	AW981957	****	****	****	BE996826	****	****	****
AI919914	****	****	****	AW985161	****	****	****	BF010516	****	****	****
AW010502	****	****	***	AW985206	***	***	*	BF010833	*	*	****
AW043196	****	****	****	BE049788	****	****	****	BF010960	****	****	**
AW289686	****	****	****	BE187245	****	****	**	BF060555	****	****	****
AW289826	***	****	|	BE187467	****	****	****	BF186004	****	****	****
AW289857	**	****	|	BE209169	****	****	****	BF221418	****	****	*
AW289907	***	***	**	BE209239	****	***	|	BF517276	****	****	***
AW290112	****	*	****	BE458101	****	**	****	BF517705	****	****	****
AW290149	****	**	****	BE458113	****	****	|	BF517951	****	****	****
AW290158	****	****	****	BE496282	****	****	****	BF609673	****	****	****
AW290257	****	***	****	BE496329	****	****	***	BF778290	****	****	*

Comparison of fertilization effects with *Arabidopsis*

One hypothesis guiding this experiment was that results from *Arabidopsis* studies of differential gene expression after treatment with low levels or high levels of nitrate might be predictive for which genes would show differential expression between pine families with different levels of fertilization in the field. Comparison of the ratios of differential expression observed in *Arabidopsis* by Wang et al. (2000) and the results of this experiment show that the predictive value is low.

The first three columns of Table 3 summarize the results of Wang et al (2000). They applied two treatments – "low", consisting of a 20 min treatment with 250 *m*M nitrate, and "high", consisting of a two-hour treatment with 5 mM to 10 mM nitrate. Each treatment was compared to a control treated with an equivalent concentration of chloride ions for an equivalent time. The first column in Table 3 specifies which treatment was found to result in consistent differences in signal intensity for a particular cDNA probe across the replicates of each treatment. The second column gives the ratio of the nitrate-induced level of expression to the control level of expression, on a linear scale. The third column lists the putative functions of the proteins predicted from the DNA

[2] **** denotes adjusted $p < .0001$, *** denotes adjusted $p < .001$, ** denotes adjusted $p < .01$, * denotes adjusted $p < .05$ and | indicates a non-significant difference (adjusted $p > .05$)

sequences of the probes at the time of the experiment. The fourth column of Table 3 shows the significance level and direction of the change in expression of the putative pine homolog of the same gene. The magnitude of differential expression was much lower in the pine experiment, with no genes differentially expressed at greater than a two-fold difference on a linear scale. There is little if any relationship between the response of a particular gene in the *Arabidopsis* study and the response of the putative homolog in the pine experiment. The correlation between the ratio estimated by Wang et al. and that estimated in this study is 0.21.

Family and family × fertilization effects

A second hypothesis tested in this experiment was that open-pollinated pine families would show consistent differences in gene expression, and a third was that the change in gene expression in response to fertilization would be different for different families. The family and family × fertilization effects were significant for almost all genes tested (Table 2), supporting both these hypotheses. Family C showed the greatest number of genes responsive to fertilization (23 genes had higher mRNA levels in fertilized plants, 24 genes had lower levels), family A an intermediate number (14 genes higher, 14 genes lower), and family B had the least genes (2 genes higher, 1 lower). Only three genes showed different directions of responses in different families, and all three had higher mRNA levels in response to fertilization in family A, lower levels in family C, and were unchanged in family B. One gene was differentially expressed (lower mRNA levels in fertilized plants) in family B but unchanged in the other two families, and eleven genes were differentially expressed (two with lower mRNA levels and nine with higher mRNA levels) in family A but unchanged in the other two families. Only two genes responded to fertilization across all families: Genbank accession AW290112, predicted to encode a ferredoxin-nitrite reductase [E.C. 1.7.7.1], detected lower mRNA levels in fertilized plants, and Genbank accession AW290257, predicted to encode a glutathione synthetase [E.C. 6.3.2.3], detected higher mRNA levels in fertilized plants.

Table 3 Comparison of Wang et al (2000) results to pine results [3]

Treatment	Ratio (nitrate/control)	Gene	pine
High	-2.7	Phosphoglycerate dehydrogenase	+
High	2.4	GF14 protein ?-chain	+++
High	2.7	Putative carbonic anhydrase	++++
High	2.3	Putative NPK1-related protein kinase	++++
High	3.2	Nicotianamine synthase	++++
High	4.8	Asparagine synthetase (ASN2)	++++
High	-3.2	Putative copper amine oxidase	++++
High & low	3.7 & 2.4	Putative auxin-induced protein	++++
High & low	3.2 & 1.9	NADH-dependent glutamate synthase (GOGAT)	++++
High	-4.1	Homolog of No Apical Meristem gene	|
High	2.6	Putative anthranilate N-benzoyltransferase	|
High	-2.9	Vacuolar processing enzyme (g-VPE)	|
Low	2.7	Nitrate transporter (NRT1/CHL1)	|
High & low	2.2 & 2.3	High-capacity calcium antiporter (CAX1)	|
High & low	4.9 & 2.3	Transaldolase (TAL1)	-
Low	2.2	NAD-dependent malate dehydrogenase, chloroplast	---
High	3.2	Putative CoA-ligase	----
High	2.9	Response regulator ARR6	----
High	-2.8	Putative auxin/aluminum-regulated gene	----
High	2.5	Glutamine synthetase	----
High	-2.5	Osmotin	----
High & low	13.3 & 11.4	Uroporphyrin III methyltransferase (UPM1)	----
High & low	9 & 6.2	Ferredoxin NADP oxidoreductase (FNR)	----
High & low	7.1 & 6.8	6-Phosphogluconate dehydrogenase (6PGDH)	----
High & low	8.3 & 5.5	Ferredoxin NADP oxidoreductase (FNR)	----
High & low	4.9 & 3.5	Nitrate reductase (NIA2)	----
High & low	6.3 & 5.5	Nitrate reductase (NIA1)	----
High & low	16 & 10.5	Nitrite reductase (NiR)	----
High	2.8	Ketol-acid reductoisomerase subunit	----
High & low	2.8 & 1.6	Transketolase	----

[3]

| no significant difference between control and fertilized

+ , increase in fertilized with adjusted P <.05 - , decrease in fertilized with adjusted P <.05
++ , increase with adjusted p <.01 -- , decrease with adjusted p <.01
+++ , increase with adjusted p <.001 --- , decrease with adjusted p <.001
++++ , increase with adjusted p <.0001 ---- , decrease with adjusted p <.0001

Table 4. Significance levels for fertilizer response and family differences for 63 probes. The first column lists Genbank accession numbers for the pine cDNA sequences used as probes, the next four columns show significance levels for the fertilized-control comparison and three pairwise family contrasts, with symbols as in Table 3, and the fifth column lists the locus ID of the most similar *Arabidopsis* gene.

Probe	Fert-Con	A-B	A-C	B-C	Ath locus ID	Probe	Fert-Con	A-B	A-C	B-C	Ath locus ID
AA556420	++	----	----	|	At1g63290	AW985206	-	--	|	++	At1g56190
AA556491	|	----	++	++++	At1g72140	BE049788	----	---	++++	++++	At4g11600
AA556526	++++	|	|	|	At5g53460	BE187245	--	++++	|	----	At1g23190
AA556674	++++	|	+	++	At1g68560	BE187467	----	----	++++	++++	At2g16570
AI812344	----	++++	+++	----	At3g17390	BE209169	++++	|	-	----	At3g04790
AI812383	----	---	++++	++++	At3g48990	BE209239	|	++++	|	----	At4g32940
AI812384	----	+++	--	----	At5g65780	BE458101	----	++++	|	----	At3g23810
AI812720	----	++++	+	----	At5g37600	BE458113	|	----	++	++++	At1g01720
AI812956	++++	|	----	--	At3g04790	BE496282	++++	|	+	++++	At2g42490
AI813214	+	++	|	----	At4g34200	BE496329	---	|	----	----	At3g47520
AI919914	----	--	++++	++++	At3g01120	BE496409	----	++++	++	----	At5g37600
AW010502	+++	++++	++	---	At1g65930	BE496464	----	|	++++	++++	At3g02360
AW043196	++++	|	----	--	At5g26710	BE496557	|	----	++++	++++	At4g35630
AW289686	++++	--	----	----	At1g63290	BE496574	++++	-	----	----	At4g35630
AW289826	|	|	++	+++	At1g12230	BE582124	----	|	++++	++++	At4g24520
AW289857	|	|	++	++	At3g13320	BE582359	----	----	++++	++++	At3g20330
AW289907	++	|	-	---	AT1G11860	BE758619	|	+	|	--	At5g48930
AW290112	----	++++	|	--	At2g15620	BE758652	----	|	|	|	At5g19140
AW290149	----	+	--	----	At3g60750	BE762095	----	----	++++	++++	At5g40850
AW290158	++++	|	----	----	At2g43750	BE996826	++++	-	----	---	At2g46690
AW290257	++++	----	----	|	At5g27380	BF010516	----	|	++++	++++	At3g58610
AW290405	++++	----	----	|	At5g07440	BF010833	----	|	|	-	At5g17770
AW290590	----	---	++++	++++	At5g08570	BF010960	--	+	----	----	At5g50850
AW290829	+++	|	----	----	At5g38480	BF060555	++++	----	----	|	At5g14740
AW495754	++++	++	----	----	At3g24170	BF186004	----	---	++++	++++	At5g65690
AW626636	++++	----	----	|	At5g55090	BF221418	+	|	+++	+++	At5g42740
AW783974	++++	|	|	|	At5g65010	BF517276	---	|	--	----	At3g48000
AW870043	++++	--	----	----	At4g35630	BF517705	----	++++	|	----	At4g11650
AW870079	++++	|	----	----	At1g18640	BF517951	++++	----	----	|	At4g24520
AW888011	-	----	++++	++++	At5g11520	BF609673	----	----	++++	++++	At1g74890
AW981957	----	++	----	----	At3g55440	BF778290	-	|	+++	++++	At5g13420
AW985161	++++	|	----	---	At3g24090						

DISCUSSION

The insignificance of dye effects in both the normalization model and gene model indicate that the procedures used in image scanning and data acquisition were effective in minimizing the differences between Cy3 and Cy5. The significant family × dye interaction for most of the genes indicate that labeling efficiency differs from family to family. This is probably due to variation in the quality of the RNA preparations.

Most of the genes studied showed significant differences in expression between the fertilization and control treatments. This is consistent with the simple hypothesis that differences in gene expression underlie the differences in growth rate between families in response to the nutrient treatment. The pattern of differences in gene expression levels between fertilized and control field plantings of loblolly pine is quite different from the patterns of differential gene expression induced by nitrate treatment of *Arabidopsis* as reported by Wang et al (2000). While this result is not surprising, given the differences between the treatment regimes and between the species themselves, it does suggest that simple extrapolation of findings from model plants to more applied settings will not provide much guidance for future experiments.

The largest difference in gene expression observed in any single contrast in this experiment was less than two-fold, which stands in marked contrast to other microarray experiments with pine that have reported differences of 10-fold or greater (Brinker et al., 2004). The differences in gene expression estimated in this experiment, however, are average differences between open-pollinated families, rather than differences between single individuals. The level of technical and biological replication employed is sufficient to give these estimates some credibility for the one season in which samples were taken. The next step in developing microarray analysis for open-pollinated, field grown material is to test the same families for differences in the same genes in a different growing season, to determine how stable the differences in expression are across years.

Gene expression differences in this study have not yet been confirmed by real-time PCR or Northern blots, due to the preliminary nature of the study. Previous studies with pine microarrays have shown that carefully replicated microarray experiments give estimates of differential gene expression that are highly correlated with estimates from real-time PCR, although the magnitude of differential expression estimated by real-time PCR is usually greater than that estimated by microarrays (Brinker et al., 2004; Yang et al., 2004). Precise estimation of the magnitude of differences in gene expression was not an objective of this experiment, although it could be incorporated into future experiments to test associations between gene expression levels and growth responses across larger numbers of families.

Some differentially-expressed genes detected by this approach will be variable between families or between environments due to chance alone; others may be associated with genetic differences between families related to differences in performance. Joint application of microarray analysis and genetic marker approaches to explore the genetic basis of variation in existing progeny tests has the potential to contribute significantly to our understanding of the genetic basis of tree growth and development.

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Genotype:Phenotype Association for Loblolly Pine Disease Traits

A.M. Morse[1], G.C. Kayihan[1], D.A. Huber[1], E. Ersoz[3], D.B. Neale[3,4], T.L. White[1], and J.M. Davis[1,2].

Fusiform rust (biotrophic pathogen: *Cronartium quercuum* f. sp. *fusiforme)* and pitch canker (necrotrophic pathogen: *Fusarium circinatum*) are major diseases of pines in the southeastern U.S. The major symptom of fusiform rust disease is the formation of stem galls that result in decreased survival, wood quality and growth. Losses due to fusiform rust are estimated to range from $25 to $35 million per year (Cubbage et al. 2000). Pitch canker is also a significant, although episodic, disease problem. Pitch canker symptoms include resinous lesions on stems and branches, seedling mortality, decreased growth rates and crown die-back. The contrasting disease symptoms of displayed by pitch canker and fusiform rust reflect the distinct life history strategies of the two pathogens that incite these diseases. Genetic variation in host resistance has been demonstrated for both fusiform rust (Kuhlman and Powers 1988; McKeand et al. 1999) and pitch canker (Kuhlman and Cade 1985).

We measured the phenotypic responses of loblolly pine (*Pinus taeda*) to these two distinct endemic pathogens in a screen involving over 1,000 clonally replicated genotypes. Genetic parameters were calculated for a variety of disease phenotypes to evaluate their respective heritabilities and to assess correlations among phenotypes. Heritable phenotypes were associated with allelic variation in candidate genes, using mixed models to estimate SNP genotype significance and magnitude of effect. Allelic effects of small magnitude were detected. Thus, association genetics is a promising approach to identify and quantify the effects of loblolly pine alleles on disease phenotypes.

MATERIALS AND METHODS

Sixty-three loblolly pine families were generated using a circular mating design and clonally propagated. Disease screens were performed for both pitch canker and fusiform rust on ramets pruned to provide multiple shoots for inoculations. Pitch canker screens were carried out using a single clonal isolate of *F. circinatum* (University of Florida Forest Pathology Laboratory). Fusiform rust screens used a ten-gall or one-gall mix of *C. quercuum* spores for inocula.

Pine gene expression arrays were constructed and used as described (Morse et al. 2004). Genes whose transcript abundance varied significantly between treatments (tissue types) were identified, and both treatments and genes were grouped using hierarchical clustering to visualize co-regulated genes. Heritable phenotypes were associated with allelic variation in candidate genes, using mixed models to estimate SNP genotype significance and magnitude of effect.

[1] School of Forest Resources and Conservation and [2] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, USA
[3] Department of Environmental Horticulture, University of California, Davis, CA, USA
[4] Institute of Forest Genetics, Pacific Southwest Research Station, US Department of Agriculture Forest Service, Davis, CA, USA

RESULTS AND DISCUSSION

Positive genetic correlation between family rankings in the two pitch canker screens indicate that genotypes performed consistently across screens. The genetic correlation between the two fusiform rust screens was also high, suggesting a general consistency in performance between the ten-gall and one-gall mixes. In contrast, there was a lack of genetic correlation between pitch canker resistance and fusiform rust resistance. This implies distinct genetic architectures underlying resistance to the two diseases, which may reflect the distinct life history properties of *F. circinatum* and *C. quercuum*.

Gene expression arrays were screened to evaluate how the pine transcriptome responds to challenge by *F. circinatum* and *C. quercuum* at the level of gene expression. Most genes regulated by the pitch canker pathogen were related to plant defense, such as genes encoding pathogenesis-regulated proteins previously identified in model systems. In contrast, these same host defense genes appeared suppressed by the fusiform rust pathogen. Thus, it appears fusiform rust is down-regulating host defenses that are induced by pitch canker. This is consistent with the emerging view that biotrophic pathogens actively suppress host defenses in order to complete their life cycles and is consistent with hypothesis that fusiform rust and pitch canker disease traits are regulated by distinct genes and pathways.

Host genes regulated by pathogens are candidates for involvement in disease resistance. We measured allelic variation (as single-nucleotide polymorphisms, or SNPs) in candidate genes for disease resistance. We associated a SNP in COMT2-1, a gene encoding an enzyme in the phenylpropanoid pathway, with pitch canker (but not fusiform rust) disease resistance. An epistatic interaction was also detected with another locus involved in the same pathway, suggesting that phenylpropanoid products may play an important role in conditioning pitch canker disease resistance. Association genetics offers exciting opportunities to identify the specific alleles responsible for disease resistance in loblolly pine.

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Investigating Genetic Interactions In The Pine-Fusiform Rust Pathosystem

H.V. Amerson[1], T.L. Kubisiak[2], S.A. Garcia[3], G.C. Kuhlman[4], and C.D. Nelson[2]

[1 & 3]Associate Professor and Research Analyst, Dept. of Forestry, N.C. State University, Raleigh, NC, USA, [2]Research Geneticist, USDA-Forest Service, Saucier, MS, USA and [4]Retired Research Pathologist, USDA-Forest Service, Athens, GA, USA.

Fusiform rust disease of pines, caused by *Cronartium quercuum* f.sp. *fusiforme* (*Cqf*), has been and remains the most destructive disease in pine plantations of the southern US. The NCSU fusiform rust program, in conjunction with the USDA-Forest Service in Saucier, MS and Athens, GA, has research underway that is elucidating some of the genetic interactions in this pathosystem.

Major genes (R-genes) for fusiform rust resistance, primarily in loblolly pine and to a lesser degree in slash pine, are being recognized and tagged with genetic markers. These genes, termed *Fr* genes, are being defined in resistant selections (clones) and are heterozygous in the studied selections. Thus the resistance (R) and the non-resistance (r) alleles of these major *Fr* genes segregate in the progeny of the selections and can be followed with the genetic markers that have been developed for each specific *Fr* gene. Typically 1 or 2 different heterozygous *Fr* genes have been found in a given selection and to date 8 different *Fr* genes (*Fr1----8*) are known among several different resistant loblolly selections.

Some of the major *Fr* genes discovered in loblolly pine are being used to investigate virulence composition in pathogen populations. In that work, progeny from selections (families), showing segregation for 1 or 2 different *Fr* genes, are being challenged with *Cqf* either artificially in greenhouse studies or naturally in field studies. Infection of progeny with a genetic marker defined resistance allele (R1, R2, R3 etc.) denotes virulence in the pathogen population against the specific R-allele of a given *Fr* gene. The percentage of R-individuals infected, for a given *Fr* gene, provides a measure for the level of virulence in a particular inoculum.

In concert with genetic marker mapping of *Fr* genes in the host, an effort is underway to genetic marker map the avirulence gene (pathogenicity locus) *Avr1* that corresponds to the *Fr1* gene in loblolly pine. If this avirulence mapping approach is successful, other *Avr* genes, apart from *Avr1*, will be similarly mapped. Markers linked with *Avr* genes in our mapping populations can serve as the subjects of linkage dis-equilibrium assessments in inoculation trials, and as starting points for chromosome-walking procedures, that potentially may yield sequence information for *Avr* genes.

A synopsis of the works noted herein will be the focus of the conference presentation.

Strategies for Breeding Blight-Resistant, Timber-Type Chestnuts (*Castanea* Miller)

R. C. Leffel[1]

Abstract: In 1995 the Pennsylvania Chapter of The American Chestnut Foundation (PA-TACF) initiated a regional backcross breeding program cooperative with TACF. TACF's goal is to restore the American chestnut [*Castanea dentata* (Marsh.) Borkh.] to its original range in the U.S. PA-TACF produced more than 8400 third-backcross (BC3) seed, 1996 – 2003, utilizing TACF pollen from two sources of resistance (BC2's) and PA American chestnut trees. BC3 trees are being selected on basis of: (1) reaction to inoculations with two strains of the chestnut blight [*Cryphonectria parasitica* (Murr.) Barr]; and (2) desirable timber tree form and other American chestnut characteristics. Selected BC3 trees are being intercrossed for BC3F2 seed. Uncertainties emerging in the current program warrant consideration of other breeding strategies for breeding blight-resistant, timber-type chestnuts. Other strategies presented for consideration are: (1) hybridizing screened BC2F2 trees with local American chestnut trees to produce BC3's, and utilization of natural selection to screen their BC3F2's; (2) cytoplasmic male sterility (CMS) as potential backcross methodology; and (3) multispecies hybrids among American, Chinese (*C. mollissima* Bl.), and Japanese (*C. crenata* Sieb. & Zucc.) chestnuts.

Keywords: Chestnuts, *Castanea* sp., chestnut blight [*Cryphonectria parasitica* (Murr.) Barr.], 'Clapper', 'Graves', backcrossing, cytoplasmic male sterility (CMS), blight-resistance, timber-type, genetic diversity.

INTRODUCTION

Chestnut blight [*Cryphonectria parasitica* (Murr.) Burr.] was first discovered in New York City in 1904 as a pathogen of American chestnut [*Castanea dentata* (Marsh.) Borkh.] By the 1950's the disease had eliminated American chestnut as a major timber species throughout its natural range in Piedmont and Appalachian eastern U.S. Burnham (1981) suggested transfer of blight resistance of Chinese chestnut (*C. mollissima* Bl.) to American chestnut by backcross breeding. Burnham et.al. (1986) reviewed previous chestnut breeding programs and proposed steps and methodology for a backcross breeding program for blight-resistant American chestnut populations. The American Chestnut Foundation (TACF) was established in 1983 by Burnham and colleagues with the goal of restoration of American chestnuts. Hebard (1994) presented the beginning and intermediate steps in TACF's breeding plan. The Pennsylvania Chapter of TACF (PA-TACF) was reorganized in 1995 and immediately participated in a regional backcross breeding program with TACF.

[1]Research Agronomist, Agricultural Research Service, U.S. Department of Agriculture (Retired) 13275 Laurel Road, Brogue, PA 17309-9488

PA-TACF REGIONAL BACKCROSS BREEDING PROGRAM FOR BLIGHT-RESISTANT AMERICAN CHESTNUTS

Initially in the TACF backcross breeding program at Meadowview VA, two surviving first backcross (BC1) trees from the previous breeding programs, 'Clapper' (USDA) and 'Graves' (CT A.E.S.), were utilized as most advanced sources of resistance from Chinese donor parents. By 1995, TACF had produced, inoculated, and then selected Clapper and Graves BC2 lines for blight resistance and American chestnut characteristics. TACF supplied pollen from screened BC2 trees to enable PA-TACF to establish 20 or more BC3 lines from each source of resistance, 1995-2004, approximately 100 BC3 seed or more per BC3 line. A BC3 line is defined as the progeny of an American chestnut tree pollinated by a screened BC2 tree. Progress to date in the most advanced part of the PA-TACF backcross program is summarized in Table 1.

Table 1. Clapper and Graves BC3 germplasm developed by PA-TACF, 1995-2004

Number of:	*Sources of Resistance:*	
	Clapper	*Graves*
Orchards	29	14
BC3 lines	25	21
BC3's seeded	4957	3512
BC3's surviving	2689	2524
BC3's inoculated	509	20
BC3's selected	59	0
BC3F2s seeded	1108	0
BC3F2 seed - single crosses 2004	1979	0
BC3F2 seed - open pollination 2004	995	0

A great challenge remains: the establishment, management, inoculation, and screening of BC3F2 orchards for each of the two sources of resistance. Hebard (2002) presented the BC3F2 seed orchard planting technique and mating designs of BC3 selected trees to produce a recommended number of BC3F2 seed for each source of resistance. He recommended 1350 seed per BC3 line via open pollination (OP), or 1350 seed per single cross (SC) between pairs of BC3 lines. For 20 BC3 lines this requires 27,000 OP or 13,500 SC BC3F2 seed for each source of resistance. This number is based on the 95% level of probability of capturing most of the alleles in each of the 20 BC3 lines in the restoration of species effort. Leffel (2003) questioned the advisability of attempting to produce such large populations and recommended use of selected BC3 lines in the development of synthetic varieties. He recommended 500 BC3F2 seed per SC or 1000 BC3F2 seed per maternal line via OP, a total of 5000 SC or 10,000 OP BC3F2 seed per source of resistance. Eriksson et.al. (1995) conclude that the Multiple Population Breeding System "is the most efficient method for forest tree gene conservation but suffers from the costs associated with planting."

It has been suggested that: (1) capturing most of the alleles or building a large genetic base is not necessary initially; and (2) the proposed TACF breeding strategy devotes too much effort to

avoid inbreeding, is large and cumbersome, is largely based on unproven hypotheses, and poses serious logistical and cost challenges. There are also concerns that TACF's efforts: (1) use only three theoretically different sources of resistance, initially; and (2) select for resistance to only two strains of the pathogen. McKechnie (2003) suggested testing that TACF Clapper resistance genes apply equally to all strains of the fungal pathogen, prior to establishing such large populations as proposed by TACF. Research objectives of TACF, 2004 – 2014 include: (1) two or more sources of resistance for 13 chapters of TACF, as many as a total of 810,000 O.P. BC3F2 seed: and (2) planting and monitoring of blight-resistant, American chestnuts in the Appalachian Mountains with a goal of planting 200,000 acres over the next 30 years. TACF breeding strategy, for each source of resistance, can be tested efficiently. Initially, only one or more screened and selected BC3 trees from each of four BC3 lines and 500 BC3F2 seed from each of two SC's are required (Leffel 2001a).

The need to conserve most alleles sampled by the breeding program is an open question subject to debate. Hebard (personal communication, 2003) stated: "Regarding rare alleles, it is important to remember that they may not be of any adaptive significance whatsoever. While they are a reservoir of adaptations that may be useful in the future, these adaptations may also never have any use. And they may be duplicated by de novo mutation more rapidly than they can spread within this species. It also is important to remember that the functions of most rare genes are unknown, and probably will remain unknown."

As is frequently the case in taxonomy, there is disagreement on the identification of American chestnut trees selected for backcrossing. Introgressive hybridization, difficult to detect, may have occurred in PA for the past 100 years or more. Can this explain the increased resistance of F2's from intercrosses between large surviving American chestnuts? Plant breeders consider introgressive hybridization as enriching a species to a small degree with genes derived from other species, but advocates of purity of the species may be concerned. Inheritance of resistance to chestnut blight is not yet ascertained, but is assumed to be attributable to two or three genes with Clapper or Graves sources of resistance. Selection for resistance to chestnut-blight is based on phenotype of cankers, the reactions to inoculations one year after inoculation, and not on progeny testing. Leffel and Peifer (2003) reported that some BC3 lines may lack sufficient resistance and their resistance varies across locations. Estimates of heritabilities of resistance and tree form are generally lacking in chestnuts. Results to date in PA-TACF orchards include death of some of the most resistant BC3 trees prior to flowering. Clapper BC3 lines included trees with "Clapper defect": abnormal bark and premature death of trees. Some screened, selected BC3 trees have not yet flowered after nine years, suggesting a generation time of ten or more years for more northern locations (6 years were assumed originally). Some breeders suggest that selection of earliest flowering BC3's will result in less timber-type chestnuts. In light of problems and uncertainties in present BC programs, other strategies for breeding blight-resistant, timber-type chestnuts should be considered.

BC2F2 ORCHARDS AND NATURAL SELECTION IN BREEDING BLIGHT-RESISTANT AMERICAN CHESTNUTS

TACF reported (TACF newsletter, The Bark, Fall 2003, pg.7) that Alabama provisional chapter utilized BC2F2 trees for breeding: two native trees were used to pollinate BC2F2 trees. The 7-

year-old BC2F2 trees were previously screened and rated for their blight resistance. By August 1999, TACF listed a total of 13,070 Clapper BC2F2 seed planted 1996-98 at 37 locations from Alabama to Maine and as far west as Wisconsin. A chestnut breeding plan, utilizing a single "donor" tree and a population of local American chestnut trees has been proposed (G. Miller. Feb. 2002. The genetic journey of a king. Mimeo, 8 pg). The donor tree would be a source of high resistance with all or mostly American chestnut characteristics, such as a Clapper BC2F2 or BC2F3 tree. Preferably, the tree would be progeny tested, presumably homozygous for blight-resistance genes, and as resistant as the Chinese grandparent of Clapper. (The Chinese grandparent of Clapper is no longer available). The thousands of Clapper BC2F2 trees in out-plantings should include desirable donor trees: trees homozygous for blight resistance should occur in 1/16 or 1/64 of BC2F2 populations, 2 or 3 gene hypothesis for blight resistance, respectively. The oldest Clapper BC2F2 trees in out-plantings are now 8 years of age. Considerable natural selection may have occurred already within these plantings, eliminating the more blight-susceptible genotypes. If blight-free, American-like Clapper BC2F2 trees are first selected and then inoculated, selection of donor tree(s) for resistance can be made 1 year subsequent to inoculation.

A possible alternative to inoculations subsequent to BC2F2, utilizing only natural selection in BC3 and subsequent generations is:

1. Collect pollen from as many local American trees as possible, at least 20, and pollinate BC2F2 donor tree to produce BC3 seed: 10 seeds per cross, full sibs within crosses and half sibs between crosses, should be adequate. If more than one BC2F2 donor tree can be utilized, be sure it traces to a different BC2 line, and cross to a different set of American trees.
2. Theoretically, all BC3's should be equal in blight resistance, as all should be heterozygous for blight resistance. Do not inoculate. Establish all BC3 trees in an orchard, IN ISOLATION. Allow BC3 trees to inter-pollinate, producing BC3F2 seed in great abundance. Visual selection can be practiced within and among BC3 half-sibs for timber-type, etc., but as many BC3 half-sib lines should be maintained as possible.
3. Harvest equal amounts of BC3F2 seed per maternal BC3 tree and seed BC3F2 seed at rates that allow surviving BC3F2 trees to serve as a seed orchard, to produce BC3F3 seed.
4. Allow natural selection to prevail in BC3F2 orchards. Eventually, blight-resistant BC3F2 trees will produce BC3F3 seed for plantings of blight-resistant chestnuts with timber form.

The foregoing program will include sibbing and half-sibbing, and if one includes more than one donor BC2F2, matings between half-first cousins. Despite inbreeding, natural selection can improve the resulting population – and combining two or more "breeding units" as defined by Miller, upon intercrossing, can restore vigor lost by inbreeding depression. The program will produce BC3F3 seed for blight-resistant American chestnuts with minimum effort and expense, and will provide germplasm pools for future generations of chestnut tree breeders, IF Clapper is adequate for such purposes.

CYTOPLASMIC MALE STERILILTY (CMS) IN CHESTNUTS: POTENTIAL METHODOLOGY FOR BACKCROSS BREEDING?

Male sterility is the absence or nonfunctioning of pollen in plants. CMS is sometimes found in the progeny of interspecific crosses, where the nuclear gene(s) of one species is put into the cytoplasm of another species. Cytoplasm, the part of the cell other than the nucleus, including

mitochondria and chloroplasts DNA, is usually inherited from the female parent only, via the egg cells. Thus inheritance of CMS is maternal whereas the restoration of fertility is controlled by nuclear factors from both parents inherited in a regular Mendelian fashion. Burnham et.al. (1986) cited unpublished data by Jaynes indicating frequent occurrence of male sterility and occasional female sterile trees in interspecific hybrid chestnut trees: "Male sterility in a few trees was said to be cytoplasmic". Shi and Hebard (1997) reported that in TACF breeding program at Meadowview VA, all F1 progenies of Chinese (C) x American (A) were male fertile (MF), but all F1 progenies of A x C were male sterile (MS): "We propose that the male sterility of *C. dentata, C. mollissima,* and their hybrids is controlled by both nuclear and cytoplasmic factors". Reinterpretation of past data and more recent experimental data at Meadowview (P.H. Sisco, personal communication) also indicated that CxA F1 hybrids are MF, but AxC F1 hybrids are MS. Leffel (2001b) proposed CMS as potential methodology for breeding blight-resistant, timber-type chestnuts. CMS in chestnuts may provide a method of emasculating trees genetically to aid in the control of the pollen parent, eliminating the need for hand pollinations beyond the F1 generation. Chestnuts are diploid, self-incompatible, and wind pollinated: a species well adapted to utilization of OP in isolation from other undesired parents, for seed production. The terminology utilized herein is:

A is American; C is Chinese; and J is Japanese chestnut;

MF is male fertility and MS is male sterility;

(*cms-Amer*) is the cytoplasmic genotype of American chestnut that confers MS in the presence of Chinese alleles of certain nuclear gene(s), but confers MF in the presence of the corresponding American alleles of these genes;

(*cms-Chin*^{F}) is the cytoplasmic genotype of Chinese chestnut trees. So far as known, trees with Chinese cytoplasm are MF regardless of the nuclear genotype;

S is the Chinese allele of a nuclear gene that confers MS when in combination with (*cms-Amer*) cytoplasm;

s is the American allele of the same nuclear gene. This allele confers MF in any cytoplasm;

S is dominant to *s*. The only combination of cytoplasm and nuclear gene in the backcross methodology presented herein that will be MS is (*cms-Amer*) Ss; (*cms-Amer*) ss will be MF.

ASSUMPTIONS AND PROCEDURES

1. CMS occurs in AxC F1 hybrids, but not in the reciprocal CxA F1 hybrids;
2. Chinese chestnut trees vary in resistance to chestnut blight. Thus not all loci for resistance to chestnut blight are in any one chestnut tree and there may be a number of alleles for resistance at a specific locus within a population of Chinese chestnut trees;
3. Blight resistance will be treated as a quantitatively inherited character;

4. Identity of AxC F1 hybrid trees and all subsequent generation trees are identified by maternal tree, allowing selection within and among maternal lines;
5. AxC F1 hybrid trees and all American trees utilized are verified as to identity to eliminate possible contaminants;
6. CMS orchards must be adequately isolated from all other chestnuts except American chestnuts when CMS orchards include American chestnuts;
7. Plant CMS F1's/A's orchards in RCB design with as many replications as possible to reduce sibbing;
8. GROW CHESTNUT TREES OPTIMALLY, as suggested by Rutter (1992).

A CMS plan for three generations of backcrossing is presented in Fig. 1

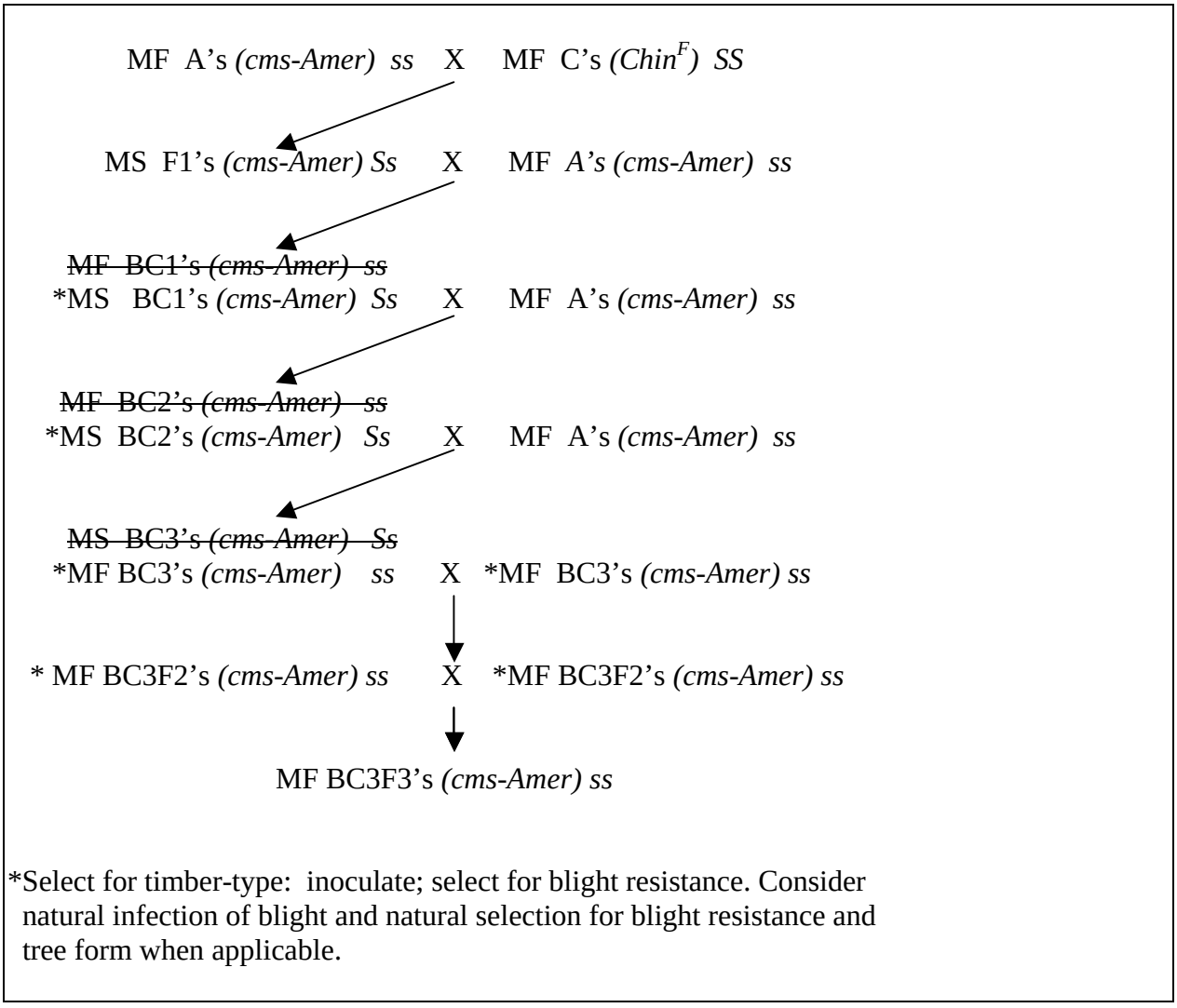

Fig. 1. Backcrossing three generations utilizing CMS and open-pollination in isolation.

As many AxC F1 hybrids as possible are made via controlled crosses, utilizing local A and old, blight-resistant C or J chestnut trees (to date an AxJ F1 is also MS). The MS F1's produced are established with as many local A's as possible, to allow MS F1's to be pollinated by MF A's. BC1, BC2 and BC3 generations segregate for MF and MS. MF BC1's and MF BC2's are eliminated prior to pollination of MS BC1's and MS BC2's by A's. MS BC3's are eliminated prior to intercrossing of MF BC3's to produce MF BC3F2's. For each generation beyond F1, selection is practiced for timber-type and then inoculated and selected for blight resistance. BC3F3's produced by intercrossing of selected MF BC3F2's, should be MF and resistant to chestnut blight. Recurrent selection can be practiced for further progress if needed. A CMS plan for one or two generations of backcrossing is presented in Figure 2.

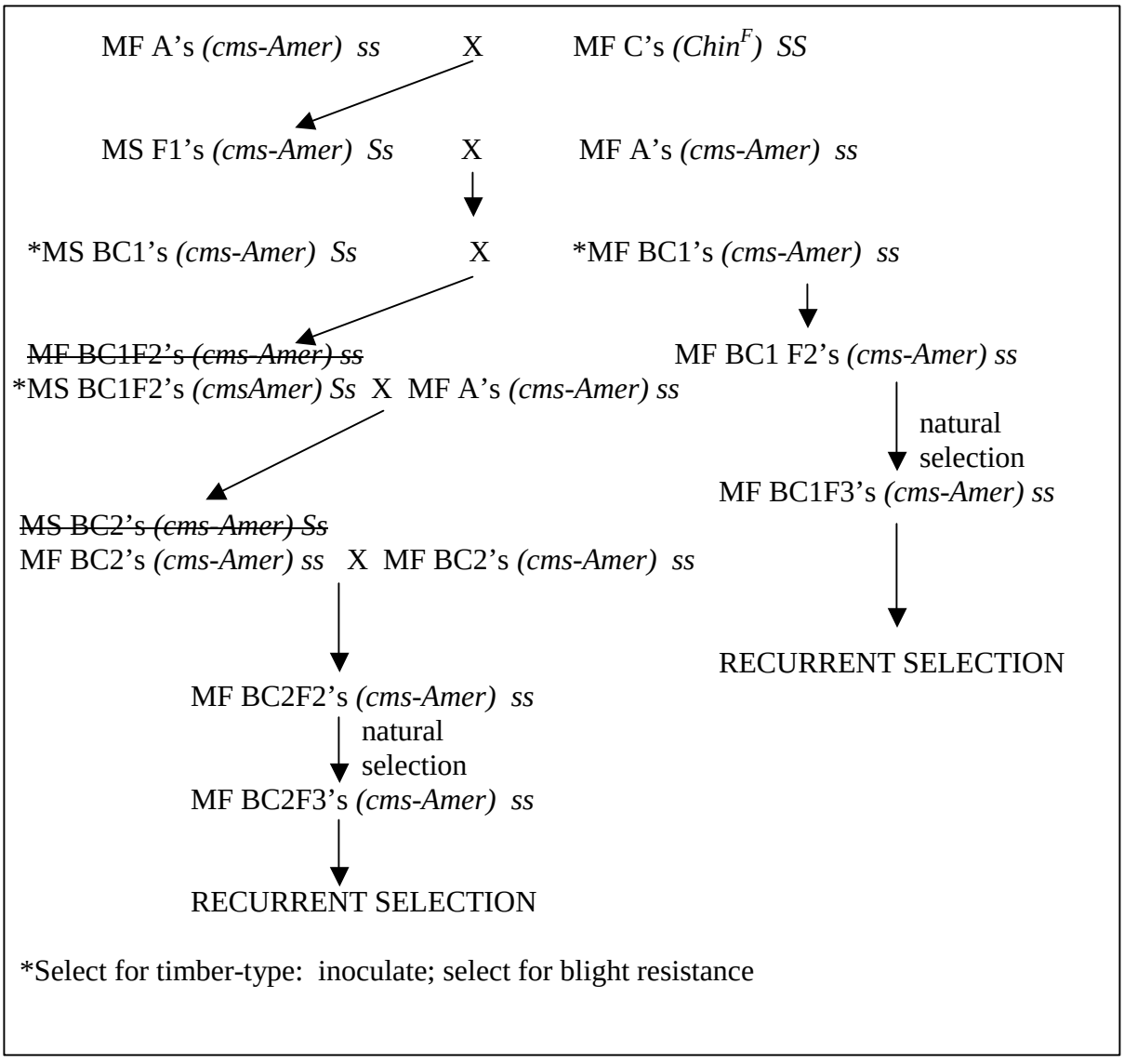

Fig. 2. Backcrossing one or two generations utilizing CMS and open-pollination in isolation.

An F1's/A's orchard is established as proposed previously for Figure 1, producing MS and MF BC1's. An orchard of exclusively BC1's is established (Figure 2.) to allow, after selection: (1) pollination of MS BC1's by MF BC1's, and (2) pollination among MF BC1's. Thus two sources of BC1F2 seed are available: (1) seed from screened MS BC1 trees for two generations (or more) of backcrossing; and (2) seed from screened MF BC1 trees, one generation of backcrossing. For two generations of backcrossing, the screened, selected MS BC1F2's are pollinated by concurrently established A's, to produce MS and MF BC2's. The selected MF BC2's may not require inoculation as they should be highly heterozygous for blight resistance assuming a high degree of homozygosity in the previously selected BC1F2's. Intercrossing among selected MF BC2's produces MF BC2F2's. These BC2F2's can be subjected to natural selection for blight resistance and timber type, assuming that the tallest, most timber-type trees will prevail in producing BC2F3 seed. Recurrent selection can be utilized subsequently if needed. For one generation of backcrossing, (Figure 2), seeds harvested from screened MF BC1's are MF BC1F2's. A BC1F2 orchard can be established at appropriate seeding rates and allowed to stand for natural selection, subsequent production of MF BC1F3 seed, and recurrent selection if desired.

In CMS backcross programs, selection may be practiced for MS or MF each BC generation. As many generations of backcrossing can be conducted as desired utilizing a large and diverse population of locally adapted American chestnuts each cycle of backcrossing as recurrent parents. In the BC generation in which MF is selected, MS trees are eliminated from the program along with any possible undesired donor parent genes closely linked with its S gene of Chinese origin. As few as ten AxC or Ax J F1 seed per cross may be sufficient; thus the number of Chinese or Japanese donor parents with local adaptation can be maximized. A germplasm pool is thus formulated with maximum sources of resistance. Seed harvested from American trees established with MS F1's or only MS BC's after elimination of MF BC's perpetuate germplasm pools of American chestnuts. The only seed produced by OP among American trees are American: the MS trees produce no pollen. CMS in chestnut was reported as controlled by one or more genes by Shi and Hebard (1997). CMS will be most efficient when governed by interaction of American cytoplasm with only one dominant Chinese locus from the donor parent. It may be possible to select for a single-gene system only, if more than one gene are involved in some crosses. When BC2 tree Graves pollinated local American trees in the Meadowview program, the BC2 populations segregated 1:1 for MS:MF, indicating only one gene involved. Graves pollen is available from CT A.E.S. and one more generation of backcrossing may be adequate, with large BC2F2 populations, as suggested by Borlaug (2000). CMS in other species has been subject to environmental variation, such as temperature, moisture, and day length. An individual plant can vary from MS to partial MS to MF, depending on environmental variation. Obviously, CMS will be successful when not subject to such environmental variation. Will the breeder be able to select for CMS that is sufficiently stable over environments to meet the requirements of the program? Only experimentation can determine the future of CMS as a breeding methodology in chestnuts. Crosses of AxC and Ax J chestnut trees will create germplasm pools with multiple sources of resistance and regional adaptation regardless of the fate of CMS as a breeding methodology (Figure 2, one generation of backcrossing).

CMS, IF it succeeds, offers advantages in breeding blight-resistant chestnut trees:

1. Genetic emasculation can be obtained when desired, eliminating the requirements of thousands of controlled crosses for BC and BCF2 populations;
2. The gene(s) from the Chinese parent that causes male sterility is dominant. Thus MS is easily eliminated by selecting for fertile trees in American cytoplasm;
3. Male sterility is an easy trait to score;
4. There is at least a 10-fold increase in seed production using OP vs. controlled crosses at far less expense and risk of injuries. More seed can be produced than can be utilized in a single program, but surpluses can be distributed for new programs;
5. Seeds produced on pure American trees within F1 and BC orchards can perpetuate American germplasm pools, because the MS hybrid trees produce no pollen;
6. MS AxC F1 hybrids or MS BC's may be established in clear-cuts and shelter cuts known to contain a good population of sprouts of American chestnuts. In this case, no seeding of Americans will be required; and
7. It is easier to include multiple sources of resistance in the breeding program, as recommended by TACF 1999 Science Review by Mehlenbacher et.al. (2000).

In year 2000, 30 F1 crosses (10 AxC & reciprocal = 20; 1 AXJ & reciprocal = 2; and 8 additional AxC) were made to establish five CMS F1's/A's orchards in 2001. The entries for five orchards decreased in number as F1 seed per seed lot was depleted. Data on fertility and confirmation of F1 trees is available to date on only the largest of the orchards seeded, originally 150 F1's (30 F1 crosses x 5 seeds each) and 150 PA –A's (30 PA-A's x 5 OP seeds each), a total of 60 entries x 5 reps, RCB design. This orchard was reseeded, as losses occurred, in subsequent years to date. Current stands are 148 F1's, 123 A's, and 29 BC1's (MS AxC F1 x A.) The older, larger trees were classified (1 July 2004) as F1's or A's, or as contaminants in attempted hybridizations. Confirmed F1's producing catkins were classified as MF or MS. Among 122 putative F1 hybrid trees, 36 were classified as Chinese or American, 29 percent contamination! Status of flowering of the 86 confirmed F1 hybrid trees is summarized in Table 2.

Table 2. Flowering and fertility of 86 confirmed F1 hybrid trees in Gratland orchard, 1 July 2004

	F1 Hybrids:				
Classification of F1's	*AxC*	*AxJ*	*CxA*	*JxA*	*Total*
Non-flowering	39	8	8	0	55
*Male Sterile	19	2	0	0	21
**Male Fertile	0	0	7	3	10
	58	10	15	3	86

* 6 PA-A trees as female and 8 C or J trees as male parents

** 7 C or J trees as female and 7 PA-A trees as male parents

Thus, based on limited production of catkins per tree, complete MS appears in the AxC and AxJ F1 hybrid trees. Also, eight 7-to-9 year-old AxC F1 hybrid trees in Brogue orchard have

exhibited complete MS to date. BC1 seed (MS AxC F1 x A) has been produced and seeded to determine the segregation of MS:MF for some of these trees. The female fertility of the MS F1's appears normal in most instances of backcrossing to A's: exceptions may be gametophytic incompatibility suggested by Burnham et.al. (1986). CMS was first observed at Brogue in Tree BR97-161 (seeded in 1997) in year 2001, as well as in its full-sibs. BC1 seed produced by tree BR97-161 for the past three years is presented in Table 3.

Table 3. BC1 and OP seed production of MS AxC F1 hybrid tree BR97-161

Year	*Male A's*	*BC1 Crosses*			*BC1 Controls*			*OP*	
		Bags	*Burs*	*Seed*	*Bags*	*Burs*	*Seed*	*Burs*	*Seed*
2002	1 tree FaYo	125	523	189	17	82	0		479
2003	*7 trees	108	276	144	36	72	0		45
2004	1 tree FaYo	95	311	130	12	53	4	340	693

* OP progeny of PA-A's from 6 PA counties, BC1 seed obtained in all 7 crosses.

Thus, the proposed CMS strategy of breeding blight-resistant, timber-type chestnuts is promising to date, but as Aristotle (384-322 B.C.) commented: "Nothing is certain; not even that."

BREEDING BLIGHT-RESISTANT, TIMBER-TYPE CHESTNUTS VIA F1-F2-F3 GENERATIONS

From the standpoint of plant breeding, is insistence on recovery of American characteristics via successive backcrosses the best strategy? Why insure the susceptibility of American chestnut to Phytophthera rot, gall wasp, and possibly other pests rather than utilizing the resistances to these pests found in Chinese and Japanese chestnut? If the multitude of leaf, twig, etc. characteristics of American chestnuts cannot be justified economically and ecologically, why consider them? What is the inheritance of tree form, timber-type American x fruit tree Chinese or Japanese, in chestnuts? Burnham et. al. (1986) comments on Jaynes earlier quote of: "There appears to be linkage of traits for poor form with blight resistance, and conversely, good form and vigor with blight susceptibility," as follows: "To corroborate his statement regarding an unfavorable linkage, data from large numbers of F2's from CxA F1's, and for backcrosses to A and C would be needed. The difference in growth form between the low stature Chinese tree and the American chestnut is probably governed by many independently inherited genes. Only if each gene for one trait were closely linked to one gene for the other and most of them closely linked with each other would there be a genetic linkage between the original parental traits, in this case poor form with blight resistance. Hence linkage is highly improbable. A pleiotropic effect of the gene(s) for blight resistance that results in low statured growth form is unlikely". IF desirable tree form (timber-type) can be obtained in large F2 populations of Oriental x American chestnuts, multispecies blight-resistant, timber-type hybrid chestnuts can be bred as follows:

1. Locate and cross old, highly blight-resistant, local C or J x local A's to produce a maximum of MF F1's, thus avoiding CMS;
2. Seed F1's in isolation blocks for intercrossing via OP. Harvest F2 seed by maternal F1 tree and seed equal numbers of F2 seed per F1 cross;
3. Seed F2 populations, as many as possible, in isolation block at appropriate seeding rate to provide F2 trees as a seed orchard subsequent to selection for resistance and tree form. Natural selection can be utilized: highly resistant F2 trees with good tree form should eventually prevail.
4. Harvest F3 seed from F2 orchards for reforestation or for subsequent recurrent selection.

Such an F1-F2-F3, three generation program, with multiple sources of resistance will provide a germplasm pool with more genetic diversity for blight resistance, other desirable characteristics, and greater local adaptation than can be achieved by current backcrossing programs. Seeds of 17 F1 crosses, CxA or JxA, were obtained in 2004.

CONCLUSIONS

The feasibility of restoration of the American chestnut as a species throughout its original range of adaptation is beyond the scope of this paper! In the PA-TACF backcross breeding program (part of the restoration effort), uncertainties have emerged that require clarification. The adequateness of the blight resistance of BC1 donor tree parents, Clapper and Graves, and of the purity of recurrent American parents in PA is questionable. Other breeding strategies can be considered by forest-tree breeders that may contribute to: (1) restoration of the species; or (2) development of blight-resistant timber-type chestnuts. The latter is more easily achievable. The current program of backcrossing three or more generations (Clapper and Graves sources of resistance) can be evaluated initially via development of synthetic varieties requiring greatly reduced numbers of BC3F2 trees. CMS with multiple sources of resistance may be more efficient in backcross breeding in contrast to present backcross methodology. Multispecies hybrids with multiple sources of resistance, via F1-F2-F3 generations with subsequent recurrent selection, should be considered for development of blight-resistant, timber-type chestnuts, and may be superior to pure species. Breeding strategies should consider use of natural epiphytotics (the greatest in history!) or more efficiently induced epiphytotics, and natural selection for blight-resistance and timber-type when applicable.

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White Pine Blister Rust Resistance in North America White Pines: Current status and Research needs

Richard A. Sniezko[1] and Paul Zambino[2]

White pine blister rust (caused by *Cronartium ribicola*) was introduced to North American approximately 100 years ago. All nine species of native white pines are susceptible, and several species have been heavily impacted. Resistance programs have been underway for over 40 years for western white pine (WWP), sugar pine (SP) and eastern white pine (EWP). Recent concerns about the susceptibility of the other six North America species of white pines to blister rust, and the high levels of mortality in whitebark pine and limber pine have raised awareness of the need to formulate a strategy for all North American species (Samman *et al*. 2003).

The WWP and SP resistance programs have been the most active in recent years, evaluating thousands of phenotypic selections via artificial inoculation of progeny. Programs with WWP have advanced the furthest, due to the relatively high levels of resistance that can be obtained from rare individuals, and the relatively short time to reproductive maturity, which speeds breeding and orchard production. The USDA Forest Service western white pine programs in Region 1 (Idaho) and Region 6 have produced operational orchard lots for several decades. An array of resistance responses has been discerned from the inoculation tests (see Sniezko and Kegley 2003 and Zambino and McDonald 2004 for overviews of results and literature).

The most well- documented resistance response is the hypersensitive response (HR) in the needles of western white pine, sugar pine, and southwestern white pine (Kinloch *et al*. 2003; Kinloch *et al*. 2002). This response has been shown to be controlled by different R-genes for WWP (Cr2) and SP (Cr1). Pathotypes virulent to Cr2 (vcr2) and to Cr1 (vcr1) have been documented (Kinloch *et al*. 2004). Less information is available on the underlying nature, inheritance and durability of other resistance responses (Sniezko and Kegley 2003; Zambino and McDonald 2004). The frequency of resistance is very low in species (WWP, SP, and EWP) that have been tested to date. Unlike hypersensitive cell death, the expression of other resistance responses can vary depending upon amount of inoculum and environment. Field tests may be important, not only for their ability to identify the potential variation in the response of pines from different resistance backgrounds to different natural levels of rust inoculum, but for other, unrecognized factors that vary among sites that may affect the pathogen, host response and disease development. Most replicated field trials examining field resistance of individual families have been established since 1996 (e.g. Sniezko et al. 2004). These field tests are just beginning to yield information on relationship between resistant responses observed in short-term inoculation tests to field resistance, and on durability of resistance.

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Prototype Screening of *Acacia koa* Seedling Families For Resistance to Koa Wilt (Fusarium oxysporum f. sp. koae)

N. S. Dudley[1], R. Anderson[2], R.A. Sniezko[3], A. Oguchi[4]

[1] Hawaii Agriculture Research Center, 99-193 Aiea Heights Drive, Aiea, Hawaii 96701, USA
[2] USGS-Biological Resources Division, Pacific Island Ecosystems Research Center, 3190 Maile Way , Honolulu, HI 96822, USA
[3] USDA Forest Service, Dorena Tree Improvement Center, 34963 Shoreview Road, Cottage Grove, Oregon 97424, USA
[4] California Polytechnic State University, Forestry and Natural Resources, 1 Grand Avenue, San Luis Obispo, California 93407, USA

Acacia koa is the most important native tree in the State of Hawaii from both an economic and environmental perspective, but there is high mortality of young trees at low elevation sites due to a vascular wilt caused by *Fusarium oxysporum* f. sp. koae. A recent report also indicates that mortality is occurring in natural stands at higher elevation (which is caused by an unknown pathogen) and the origin of the pathogen is currently unknown. At Maunawili, Ohau, of the thirty *Acacia koa* families tested, survival percent ranged from a low 4.0% to a high 91.6% at 48 months. The average family survival percent was 35.4%. However, the two best families had survived percentages 91.6% and 75.0% respectively. Family variation in *A. koa* in field trials strongly suggests the presence of resistance of *F. oxysporum f. sp. koae*. To confirm whether genetic resistance exists, a short-term greenhouse test involving artificial inoculation was initiated. A subset of seed lots from the Maunawili trial were selected based on seed availability and representing a large range in survival. The results from this screening trial provide the first formal evaluation of existing genetic variation within koa for disease resistance. It is anticipated, that this trial will also provide the prototype for a larger operational resistance screening and development of resistant populations of Koa.

Resistance and Virulence Interactions Between Two White Pine Species and Blister Rust in A 30-Year Field Trial

B.B. Kinloch[1], Jr, D.A. Davis[2], and D.C. Burton[2]

[1]Institute of Forest Genetics, USDA Forest Service, Pacific Southwest Research Station, Berkeley CA 94701; USDA Forest Service, Klamath National Forest, Happy Camp, CA 96039

We monitored infection and mortality of full-sib families of sugar pine (*Pinus lambertiana)* and western white pine (*P. monticola)* selected for different mechanisms of resistance to white pine blister rust over nearly three decades in a field test in northern California. Natural infection was deliberately enhanced by interplanting alternate host *Ribes* spp. among test seedlings. Parents of the families were from three different geographic provinces, representing the northern Rocky Mountains of Idaho (western white pine), the Cascade Range of Oregon and Washington (western white pine) and the Siskiyou Mountains of California and Oregon (sugar pine). Several families of each pine species had major gene resistance (MGR), or suspected genes for partial resistance (PR), or both types of resistance. PR consisted of low infection frequency, or reactions in bark tissues that inactivated the pathogen. Open-pollinated seedlings from susceptible seed parents were included as controls. Under the chronic epidemic conditions that prevailed, controls of both species became infected within a few years after planting and died soon afterwards. Families with MGR segregated in expected Mendelian ratios (resistance:susceptibility), but each R gene (Cr1 in sugar pine and Cr2 in western white pine) was eventually defeated by biotypes of the rust with specific virulence to them. The two biotypes, with corresponding virulence factors vcr1 and vcr2, appeared spontaneously on their hosts about 6 years apart, then rapidly increased in frequency through natural selection. Neutralization of MGR unmasked underlying PR factors. Western white pine families from Idaho, which lacked Cr2, performed the best overall, ranging from 44 to 64% rust-free or with aborted infections after final inspection. Some western white pine families from the Cascades, both with and without Cr2, performed comparably (17 to 68% rust-free). These families were no more vulnerable to vcr2 than they were to wild type inoculum. Relatively simple inheritance of PR can not be precluded, but it is not influenced by Cr genes in either species. Specific combining ability may play a more important role than general combining ability. PR was less frequent in sugar pine than western white pine, and sugar pine families had steeper infection rate curves than western white pine. Tolerance was infrequent in both species, with few trees with any normal symptoms surviving. The data indicate that genotypes with durable resistance exist at low frequencies in both species, but could be increased for commercial culture or species restoration through forward selection and breeding for PR.

Evolution of Genetic Parameters and New Breeding Strategies for Maritime Pine (*Pinus Pinaster,* Ait.) in South Western France

A. Raffin , P. Pastuszka, P. Alazard , L. Bouffier, P. Garnier-Géré, C. Plomion, and A. Kremer[1]

In South Western France (Aquitaine), a cultivated forest of 1 million ha of maritime pine (*Pinus pinaster*) has been planted or sowed since 1850, just neighbouring some 40.000 ha of natural populations of the same species on coastal dunes. Today 8,5 millions m3 are harvested per year (85% of the biological production) and are largely processed locally. The breeding programme started in the early 60s and has led to a number of seed orchards.

The aim of this presentation is to report on the genetic evolution of the breeding population, the recent advances in the long term breeding strategies to manage this evolution, and the genomic studies for MAS application.

Evolution of genetic gain, diversity and genetic variance over generations of the breeding population were evaluated, for the two traits under selection : total height (volume predictor) and stem straightness.
Genetic gain of the breeding population compared to non improved material could only be estimated in a progeny test of plus trees (generation G0) with unselected controls (Danjon, 1995). Gain on total height and stem straightness at 22 years old (2/5 of rotation age) was respectively 6% (25% on volume) and 20%. Though estimated on small tree plots, these genetic gains were close to the evaluation of realized genetic gain of the first set of seed orchards, observed in 11 experimental trials between 9 and 19 years old on large tree plots (between 250 and 700 trees per treatment on each site) : +15% on volume and on stem straightness. These 3 family seed orchards are a representation of the breeding population since they were also progeny tests of the plus trees and contained almost all the diversity of their generation before roguing. Pollen pollution may account for the decrease in estimated genetic gain between the breeding population and the seed orchards (open pollinated SO).
Diversity in the breeding population can be evaluated through effective population size. We used the status number (Ns) parameter (Lindgren et al., 1996). For a plus tree population of 250 founders, the first cycle of selection reached Ns = 93 and the second cycle Ns = 64. This drastic decrease (-63% and –31% respectively) was mainly due to the coancestry between selections and the number of full sibs selected in the best families.[1]
Heritabilities, genetic additive and phenotypic coefficients of variance of height and stem straightness were studied over 3 populations (Landes unimproved provenance, plus tree generation, and first cycle selections) through 20 progeny tests between 8 and 10 years old. In average, 88 families of 63 trees were assessed. Confidence intervals for the evaluated parameters were computed by jack-knife or analytical methods. A meta analysis was performed to compare

[1] Research Engineer Tree Breeder, INRA, Research Engineer Tree Breeder, AFOCEL, PhD student, INRA, and Scientists, INRA, respectively. INRA, UMR Biogeco, 33612 Cestas Cedex, France. AFOCEL, Domaine de Sivaillan Les Lamberts, 33480 Moulis en Médoc, France

the 3 populations for each trait and each parameter, based on a Chi2 test : sum of squares were weighted by the inverse of their variance, to account for the accuracy of each estimated parameter. The results showed a significant ($p<0.05$) decrease in heritabilities for both traits over populations. For height, CVa also decreased significantly ($p<0.05$) while no evolution in CVp was observed. A loss of additive variability in the first breeding generations is in agreement with the expected effect of selection. However limitations of this preliminary study could also account for this result including the large variance of estimations within populations, different mating systems between populations, and relationships between families within progeny trials.
Overall, the study of genetic parameters in the breeding populations showed good genetic gains and an expected decrease in variability. However managing the accumulation of coancestry relationships within the population and rationalization of between vs. within family selection could greatly improve the stabilization of genetic variability.

A sublining strategy is proposed for managing pedigrees within the breeding population of maritime pine.
For different reasons, the usual seed orchards for this species today is an OP seed orchards with 30 to 40 clones : concerns on the diversity present in production populations and on possible interactions between the large cultivated forest and neighbouring natural stands of the same species, cost of controlled pollinations in seed orchards, unavailability of an efficient vegetative propagation method for maritime pine. This situation impose to select a large number of unrelated clones to build a seed orchard, and management of pedigrees is a priority. Sublining, by creating unrelated groups within the main population is then a well adapted strategy.
With an objective of a total size of the breeding population between 300 and 400, and a status number to reach 100, 10 to 20 sublines could be created. Clones dispatching between sublines will be optimised based on real pedigrees and breeding values.

Eventually, recent advances on genomics of maritime pine are presented.
A large programme of candidate genes detection has been led, with gene expression studies in contrasting conditions for water stress and for wood formation, mainly within controlled crosses. To find genes that really account for variation of adaptive traits, a Candidate Gene approach is now in progress : CG screening (sampling natural populations to identify polymorphism, SNP discovery panel), and CG validation (association tests between CG variants and trait variation : linkage desequilibrium mapping). Validated CG would then be useful for Marker Assisted Selection application.
CG screening was performed for wood formation in 9 maritime pine natural populations. 173 SNPs were identified in 8 lignin pathway genes with 1 polymorphic site per 40 base pairs on average. GC validation was then studied in a clonal test of the maritime pine breeding population, by association between SNP of CG and phenotypic variation of wood quality traits. Significant associations were found between lignin content and SNPs of CAD and C4H genes, explaining between 15 and 20 % of the phenotypic variation.
The same approach is worked on for drought resistance traits.

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Experimental Inbred Population in Radiata Pine: A Potential New Breeding Strategy

Harry X. Wu, A. Colin Matheson, David. J. Spencer, John. V. Owen, and Aljoy Abarquez[1]

Abstract

For an inbreeding and hybrid breeding strategy to be successfully implemented in tree improvement it is necessary 1) to demonstrate theoretically that such a strategy is more efficient than more conventional breeding strategies and 2) to show experimentally that inbreeding (selfing) can produce superior inbred lines (effective purging of deleterious alleles), that inbreeding will not substantially reduce reproductive ability, that early selection for inbred lines is effective, and that there is heterosis in the F1 hybrid. In this paper, the theoretical advantage of using inbreeding as a breeding strategy will be demonstrated and experimental results from a long-term radiata pine inbreeding experiment are summarised.

The theoretical advantage of using an inbreeding and crossbreeding approach was simulated using a simplified locus model. Selfing and subsequent selection was more efficient than conventional outcrossing and selection due to the higher segregation rate of recessive alleles through selfing. Examination of radiata pine inbreeding trials has revealed:
(1) although inbreeding decreased growth it increased dispersion for growth rate; the largest trees were from the most inbred population;
(2) superior trees can be identified early from their growth curves and from age-age genetic correlations. The populations at higher inbred levels showed higher age-age correlations and thus early selection was more efficient among inbreds than among outcrosses;
(3) inbreeding did not affect wood density significantly, but variation in wood density among trees was increased by inbreeding;
(4) the effects of inbreeding depression on fecundity were higher in young trees than when they were older, but overall inbreeding depression on fecundity was low in radiata pine. It was observed that fecundity varied more widely between the eight founder clones than between inbreeding levels (self and outcross);
(5) Heterosis was observed.

Based on the simulated theoretical advantage and our observations in radiata pine inbreeding experiment, it appears there is great potential to develop high-quality inbred lines and produce highly productive crosses among such lines in radiata pine. An experimental inbred population has been established in radiata pine by selfing 51 elite clones from a commercial breeding population and, through continued selfing of S_1 and S_2 and crosses among the best S_2 and S_1 lines the potential for heterosis in radiata pine has been created.

Introduction

Since Shull (1909) and East (1909) first developed the idea of inbreeding-outcrossing in maize

[1] CSIRO Division of Forestry and Forest Products, PO Box E4008, Kingston, Canberra ACT 2600, Australia INB6b.DOC; 26 September 2004

(*Zea mays* L.) to produce uniform and highly productive hybrids, selfing and subsequent cross-breeding has became a principal breeding method for improvement of many outcrossing agronomic species. There are three major advantages of using a single-cross hybrid: high yield, uniformity and stability as follows:
1. Inbreeding and subsequent cross-breeding with selected inbred lines have produced superior growth in maize, for example, Martin and Hallauer (1976) reported that yield of hybrid maize obtained from four sets of inbred lines exceeded 150% of the parental average.
2. A cross between two relative homozygous genotypes would produce an uniform genotype, very attractive for its uniform appearance, maturity and harvesting characteristics.
3. Progeny produced from single crosses are heterozygous and have more stable yield in variable environments.

On the other hand, the advantage using inbred-hybrid method relative to population improvement (outcross and mass selection) has been questioned by a number of influential scientists, including R.C. Lewontin (1990). Theoretically, if overdominance is the principal mode of heterotic gene action, then the only way to capture the higher yield permanently is the inbred-hybrid method, because that is the only way to produce seed that are heterozygous at all loci. Experimental studies in maize revealed that overdominance was not evident for most traits while partial dominance of gene action was more common (Moll *et al*. 1964). Lewontin argued that if partial dominance was the principal mode of gene action, then the highest possible yield would not be the heterozygote, but the homozygote for the superior alleles. Such homozygotes can be isolated through mass selection. Under these conditions the inbred-hybrid method would not have an advantage relative to mass selection and would not lead to greater improvement in yield. In comparing the advantage of mass selection relative to inbred-hybrid approaches under the partial dominance model, Lewontin neglected the differential selection intensity between outcrossing/mass selection and inbreeding/mass selection approaches. Such differential selection intensity could lead to the inbred-hybrid being more efficient breeding method in trees. The theoretical efficiency of using an inbred-hybrid approach relative to conventional outcrossing/mass selection will be demonstrated in this paper.

Selfing as a breeding tool for forest trees was first advocated four decades ago, using experience with maize as a justification (Matthews and Mclean 1957, Righter 1960, Barker 1966, Bingham 1973). Righter (1960) suggested the development of selfed lines for conifers and the production of hybrid seeds for plantation forestry. Subsequently, Wilcox (1972) and Lindgren (1975) suggested selection of the best selfed lines for inclusion in an outcrossing program to enhance genetic gain. However, the inbreeding and cross-breeding approach has not been used as a practical breeding tool in conifer tree improvement. There are three major reasons for this:
1. Most conifer breeding programs in the last three decades are still in their infancy and most resources have been devoted to assembling and evaluating plus trees selected in the wild or in plantations.
2. Long generation turnover makes one or several generations of selfing to produce inbred lines time-consuming and expensive (Snyder 1968, Franklin 1969).
3. Early results from inbreeding experiments have revealed severe inbreeding depression in conifers, including seed production, growth and adult fecundity (see Williams and Savolainen 1996 for review).

For these reasons early conifer breeding programs worldwide have been managed for inbreeding avoidance and have relied upon open-pollinated production seed orchards. Sib or random-mating was recommended in early generations of conifer domestication to reduce inbreeding depression in the breeding population. Simple recurrent selection in a single, large breeding population was preferred at that time, although population subdivision strategies are more commonly used today (Burdon and Namkoong 1983, McKeand and Bridgewater 1993, White 1993). Selfing as a breeding tool has been reviewed recently because of the growing interest in small elite breeding populations (Williams and Savolainen 1996) and the possibility of purging deleterious alleles (Namkoong and Bishir 1987, Hedrick 1994) although selfing was not recommended for early generations of breeding program. However, all these debates lack any theoretical comparison of efficiency among the various breeding strategies using different models of gene action. Similarly, there is no empirical evidence that inbreeding-outcrossing is not a better breeding strategy for tree improvement.

Whether inbreeding-outcrossing is a more efficient breeding strategy and can be applied to tree breeding successfully will depend on two major conditions, 1) there must be some theoretical advantage over population improvement (outcrossing and mass selection), and 2) purging deleterious alleles must be effective and significant heterosis must be demonstrated. In this paper, theoretical grounds to support an inbreeding and cross-breeding strategy will be presented, together with some preliminary evidence from radiata pine

Theoretical Advantage

There are three kinds of gene effects controlling phenotypic traits. These are additive gene effect, intra-locus interaction (dominance effect) and inter-locus interactions (epistatic effects).

Under a model with additive gene effects only, inbreeding does not produce any depression and hybridisation will not produce particular vigour. Aggregated gene effects can be modelled using a normal distribution under an infinitesimal gene effects model. Since genetic variance is a quadratic function of the inbreeding coefficient (Crow and Kimura 1970), for additive gene effects, inbreeding would increase genetic variance in a linear way as $V_g = V_0(1+F)$, where V_g is the genetic variance at inbreeding level F and V_0 is the genetic variance in the base population (F=0). If inbreeding results in a subdivision of the population, this genetic variance would be distributed between lines ($2FV_0$) and within lines ($(1-F)V_0$). Since selection efficiency is a function of the additive genetic variance, any increase in additive variance will naturally increase selection efficiency if environmental variance is constant.

Dominance gene effects will cause inbreeding depression and hybrid vigour. To take advantage of dominance effects while avoiding inbreeding depression in breeding programs, various breeding strategies have been proposed. Partial dominance, over-dominance and epistatic gene effects have been proposed as genetic mechanisms for inbreeding depression and hybrid vigour (Crow and Kimura 1970). Under the overdominance model, the heterozygote performs better than the better of the two homozygotes (Ziehe and Roberds 1989). Thus, if overdominant loci are the cause of inbreeding depression, average fitness will decline continuously over several generations of inbreeding. Crossing two inbred lines in which different alleles are fixed could

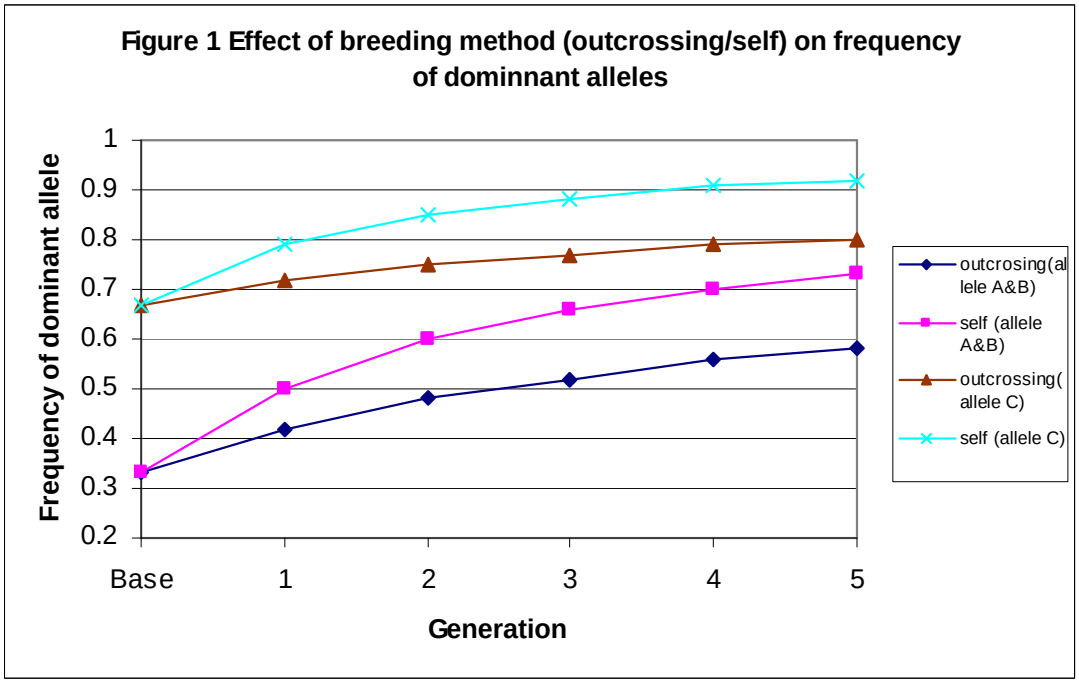
produce a superior F1 that is heterozygote at all loci. Since no more than half of the individuals can be heterozygotes for a particular pair of alleles in an outcrossing population at equilibrium, inbreeding-outcrossing will be a better means of improvement than the conventional selection and outcrossing breeding method, under the overdominance model (Falconer 1981). Therefore, when there is any overdominance for any desired character, the inbreeding and crossing breeding method can achieve more genetic gain than conventional selection and outcrossing breeding method. Although there is limited information on overdominance in forest trees (Barnes *et al*. 1987) it has been suggested that a general level of heterozygosity is required for vigorous growth of trees (Ledig et al. 1983).

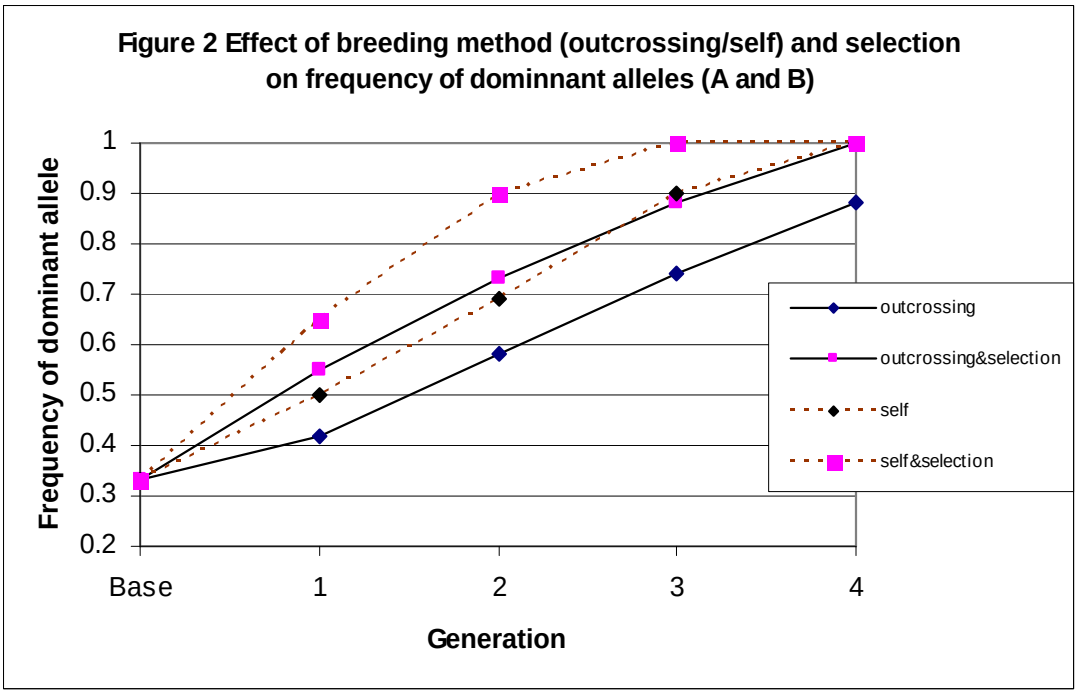
If partial dominance is the main cause of the inbreeding depression, deleterious recessive alleles would cause most of the inbreeding depression. It should be possible to eliminate or purge them by fixing the normal, dominant alleles. Thus, inbreeding depression will decline across generations as purging effectively removes deleterious alleles. Partial dominance is believed to be the most common case in trees since inbreeding depression does usually decline each generation. Under the partial dominance model, it is also possible to produce very good genotypes with most dominant alleles fixed from recombination in the early generations of inbreeding. Inbreeding and selection have produced highly inbred lines of mice that are better than the original population for the character selected (Falconer 1960). Although, under the partial dominance model, the best genotype is one of the homozygotes and all individuals can, in theory, be made homozygous either by outcrossing and mass selection or through inbreeding, selection, and hybridisation. The difference between inbreeding and outcrossing is that the selection intensity can be higher using inbreeding due to more effective natural purging and artificial selection in an inbreeding program. Selection in inbred populations is more effective due to greater genetic segregation. Hence it is quicker and more efficient to select the best homozygotes following inbreeding rather than outcrossing (for example random outcrossing).

Here we use a simplified example to demonstrate the advantage of a selfing breeding strategy relative to a conventional outcrossing breeding strategy when there is partial dominance. For simplicity, we assume
1. there are three loci with A, B.and C representing dominant alleles, and a, b, and c three recessive alleles and the frequencies of A, B and C are set as 1/3, 1/3 and 2/3 in a original population;
2. the three loci are unlinked;
3. the genotype value of any dominant homozygote (AA, BB, and CC) is set as 2, the value of any recessive homozygote (aa, bb, and cc) is set as 1, and the heterozygote (Aa, Ba, and Cc) is set as 1.8;
4. any genotype in which two or more of the loci are homozygous for the recessive allele will not survive to field planting;
5. all surviving trees will be tested in the field after natural purging;
6. same field test size and artificial selection intensity (50%) are assumed for trees planted in the field trial.
Under these assumptions, allele frequencies are calculated for two selection scenarios for the two breeding strategies: self versus outcrossing. In the first selection scenario, no artificial selection is imposed at any stage. Figure 1 indicates that after five generations of natural selection, the frequencies of dominant alleles A and B increased to 0.73 in the selfing strategy relative to 0.58

under random outcrossing. Dominant allele C increased its frequency to 0.92 through selfing relative to 0.80 through random outcrossing. This is because the selfing strategy produces higher natural selection intensity. After five generations of mating and natural selection, 25% of individuals will be homozygotes for the dominant alleles A, B, and C under the selfing strategy compared with only 7% under the random outcrossing strategy. Hence selfing is more than three time more efficient than random outcrossing at generating desirable homozygotes. In the second selection scenario, an additional artificial selection (50% selection intensity) was applied in the field trials for the two breeding strategies. The breeding cycle for the selfing strategy is selfing followed by field testing, selection and then selfing again. The cycle for outcrossing is outcrossing followed by field testing, selection and then outcrossing again. In this scenario, dominant alleles A and B were fixed in the third generation under the selfing strategy while same alleles were fixed a generation later using outcrossing strategy. Hence selfing reduced by 25% the time taken to fix dominant alleles A and B, and was more efficient at producing the best genotype. These results have been obtained using very simple assumptions, but the simulation can be applied to more realistic situations such as many more loci, multiple alleles with linkage, and less severe effect of recessive alleles.

Epistatic effects also cause inbreeding depression and hybrid vigour, but only in the presence of directional dominance. Epistatic effects with partial dominance at several loci could produce similar effect as overdominance, generating apparent heterotic effects under inbreeding and subsequent outcrossing (Crow and Kimura 1970). To demonstrate comparative efficiencies under epistatic interaction, a similar, but more complicated simulation package will be used.





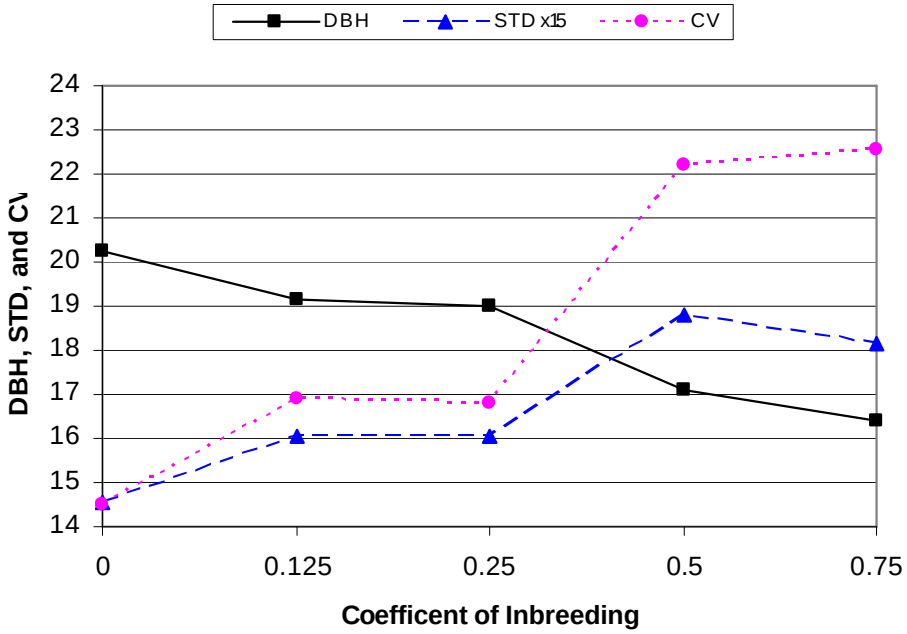
Experimental Results from a Radiata Pine Inbreeding Trial

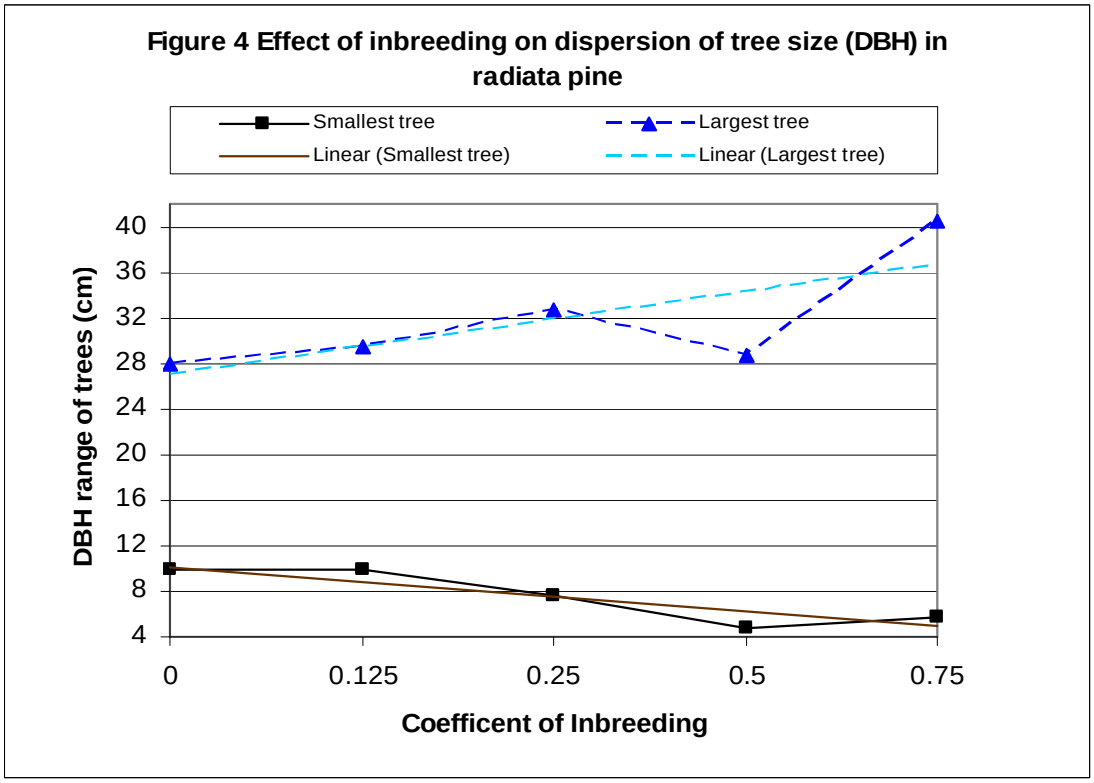
In the 1970s, Dr A.R. Griffin, working at the Gippsland Regional Station of the then Forest Research Institute, established an experiment to investigate the effects of inbreeding in radiata pine in which breeding values were balanced across the different inbreeding levels. He used existing crosses growing in inbreeding trials to create 5 populations involving 8 founder clones; outcrossed (F=0), half-sib matings and backcrosses (F=0.125), full-sib matings (F=0.25), selfs (S_1, F= 0.5) and double selfs (S_2, F=0.75). In addition to the five main populations, three other populations were created to investigate heterosis from crossbreeding selfs (S_1 x S_1, F=0, referred as hybrid F_1 below) and the performance of open-pollinated progeny from both selfed (F=0, referred as $S_{1\text{-}O}$) and full-sib mated individuals (F=0). The experiment was planted at two sites near Mount Gambier, South Australia in 1982 and 1981, respectively. Results from the proportions of filled seeds at different inbreeding levels were published by Griffin and Lindgren (1985).

The experiment was thinned in 1993 and the opportunity was taken to make use of the thinnings to measure the whole trial and to collect wood disks from thinned trees. Thinning was mechanical, ie not selective, and so there should be no bias in using results from thinnings compared with those from unthinned trees. Results from this measurement and other studies involving wood density and fecundity have been published (Wu *et al*. 1998a, 1998b, Matheson *et al.* 2002, Wu *et al.* 2002, Wu *et al.* 2004). Several observed results from the trial are summarised here.

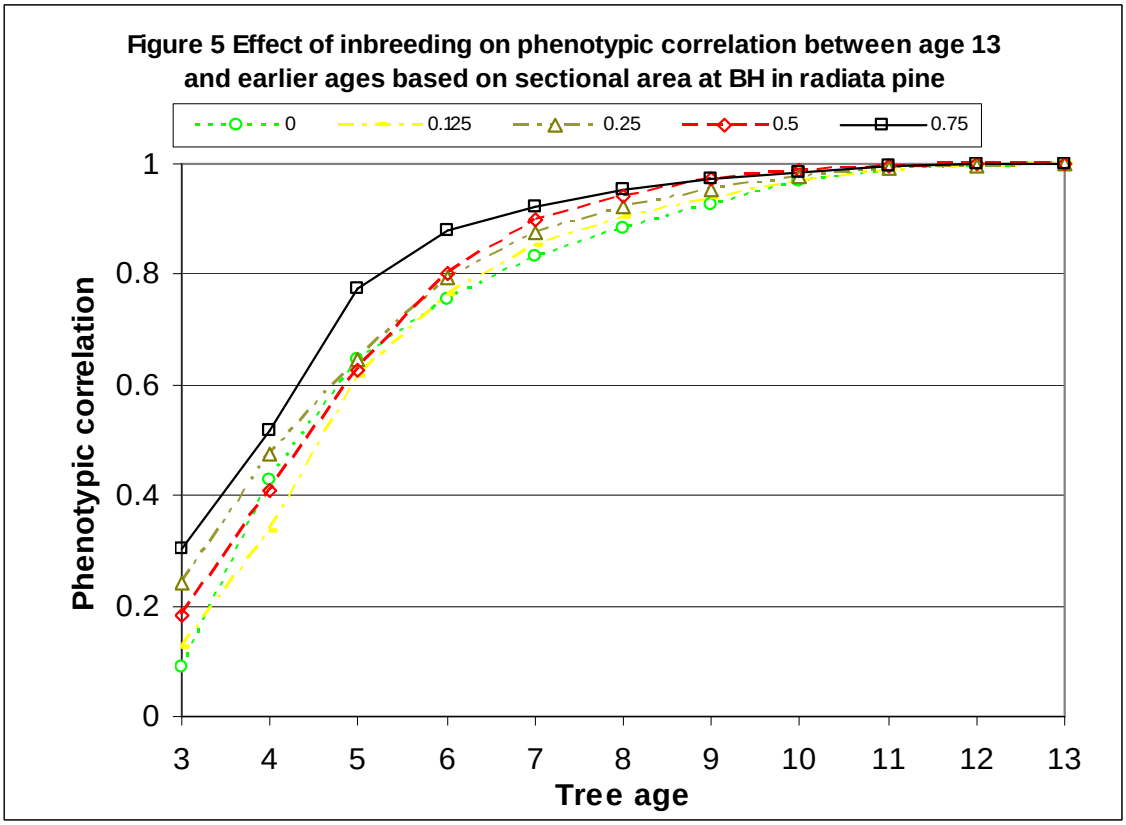
1. Inbreeding decreased growth, but increase dispersion of growth rate. The progeny of the first generation selfing (S_1) significantly depressed stem diameter growth (DBH -15% on average, Wu *et al*. 1998a), continued selfing from the first (S_1) to the second generation (S_2) reduced DBH by only 4% on average, statistically not significant (Figure 3). Within-plot standard deviation and coefficient of variation for diameter increased by 30% and 53% respectively after the first generation of selfing and did not change significantly from the first (S_1) to the second generation (S_2). Inbreeding reduced the size of the smallest trees and increased the size of the largest trees (Figure 4). DBH of the smallest trees was from 9.8cm for S_0 population to 4.7 and 5.8cm for the S_1 and S_2 populations, respectively. The largest trees were from 27.9cm for S_0 population to 28.7 and 40.4cm for the S_1 and S_2 populations, respectively. Data from a pre-thinning measurement showed that the four largest trees in the trial were all from S_2 families (mainly from three S_2 families). The average difference between these superior trees and mean DBH of the outcrossed population (S_0) was about 100%. So it seems likely that highly-quality inbred lines could be derived from inbreeding in radiata pine.

Figure 3 Effect of inbreeding on DBH, standard deviation (STD) and coefficient of variation (CV) of DBH in radiata pine

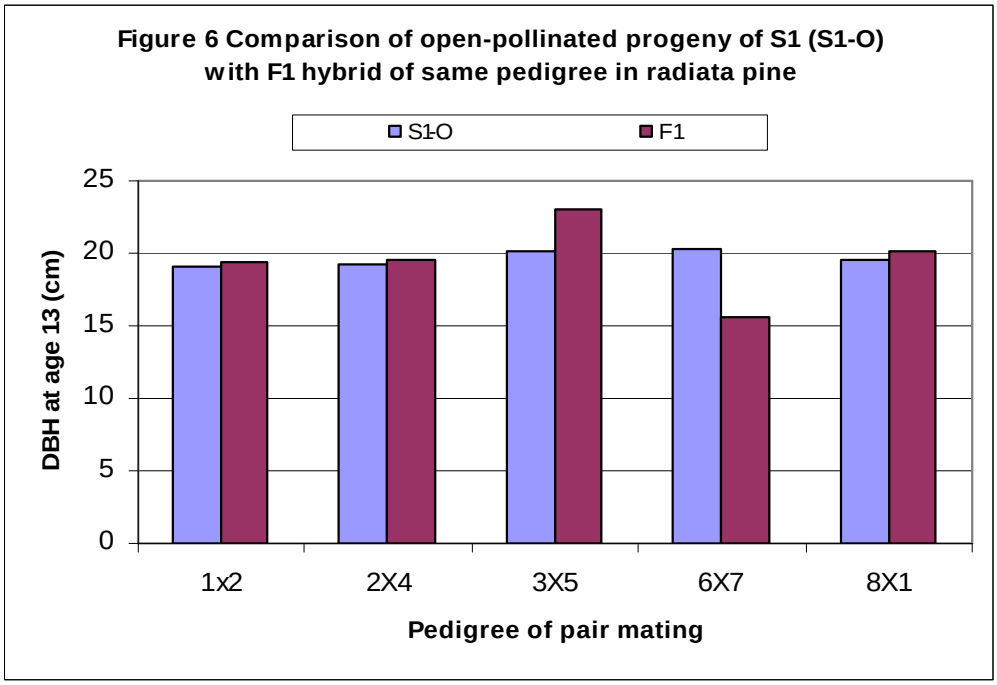




2. The superior trees can be identified early from their growth curve and study of age-age correlation in the five populations with different inbreeding levels. Inbreeding affected maximum growth rate and the time to reach maximum growth. Inbreeding started to show a significant effect on sectional area at breast height from age 3 (two years after planting, Wu *et al*. 1998b) and age-age family correlations (age 13 as reference age) were significant from age 6 for S_1 and age 2 for S_2 populations (Matheson *et al*. 2002). Individual tree correlations between early age and age 13 started to show significance from age 4 for the S_1 population and age 2 for the S_2 population. An interesting observation is that age-age correlation for cross-sectional area in the S_2 population (the highest inbreeding level) was the highest among all five populations for both individual and family mean correlations. There is a trend towards higher age-age correlations at higher inbreeding levels after age 5 (Figure 5). For example, at age 6, individual correlations were 0.75 for S_0 and 0.88 for the S_2, rising to 0.88 for S_0 and 0.95 for S_2 by age 8. This indicates that early selection would be more efficient among inbreds than among outcrosses in radiata pine.



3. We also observed apparent heterosis from four comparisons between the F_1 hybrid and the other four type of progeny (selfed progeny (S_1), grandparental cross (S_0 xS_0), mean GCA estimate of grandparents, and open-pollinated progeny of selfed trees ($S_{1\text{-}O}$)) of the same pedigrees planted on one site (Symonds). All comparisons revealed significant heterosis. Six pair-matings among the eight S_1 selections were made and planted. Among the eight S_1 clones used for the six pair matings, six S_1 had open-pollinated progeny ($S_{1\text{-}O}$) planted in the same trial. Thus, the performance of the F_1 and the average of open-pollinated families of the corresponding two S_1 used in the six pair matings were compared. Analysis of variance indicated significant differences between $S_{1\text{-}O}$ and F_1. These differences are illustrated in Figure 6. Heterosis (defined as percentage superiority of F_1 relative to $S_{1\text{-}O}$) was estimated as 2%, 2%, 15%, -23% and 3% for the five pair crosses (1x2, 2x4, 3x5, 6x7, and 8x1, respectively). Four of these exhibited positive heterosis and only one showed negative heterosis. Data from the sixth pair mating (4x3) is not presented as it had only six trees planted in one replicate, even though the average of these six trees was 45% better than $S_{1\text{-}O}$ trees.



4. The effects of inbreeding on basic wood density at age 17-years were studied using the same five populations. Although inbreeding resulted in slightly depressed wood density (inbreeding depression was 1.47%, 2.50%, 1.65%, 0.02%, respectively at F=0.125, 0.25, 0.50 and 0.75), the effects were not significant (Figure 7, Wu *et al*. 2002). However, the variation of wood density among trees was increased by inbreeding. Among first- and the second-generation selfs, density variation increased by 15.7% and 29.0% respectively (Figure 8). The lack of significant inbreeding depression for wood density at the population level combined with increased variation in wood density in the inbred populations suggests that it will be possible to quickly develop inbred lines with high wood density.

Figure 7 Effect of inbreeding on wood density in radiata pine

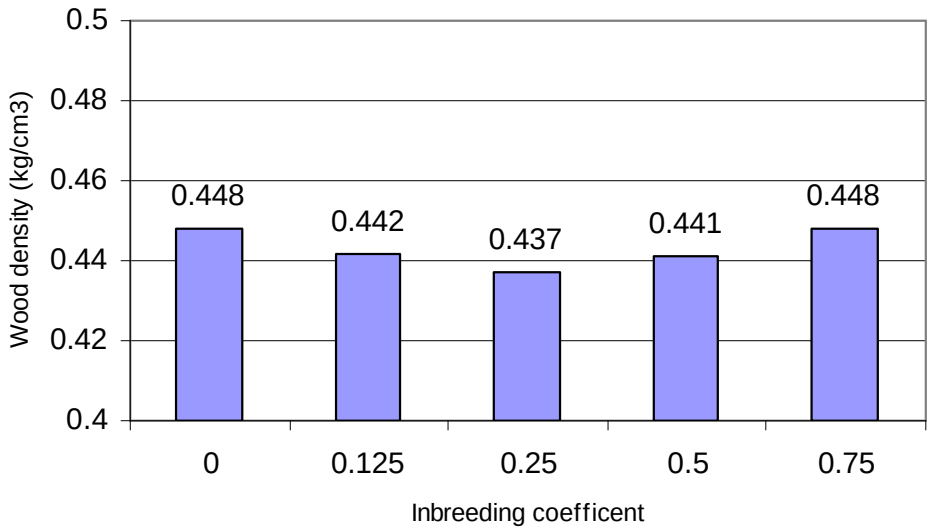
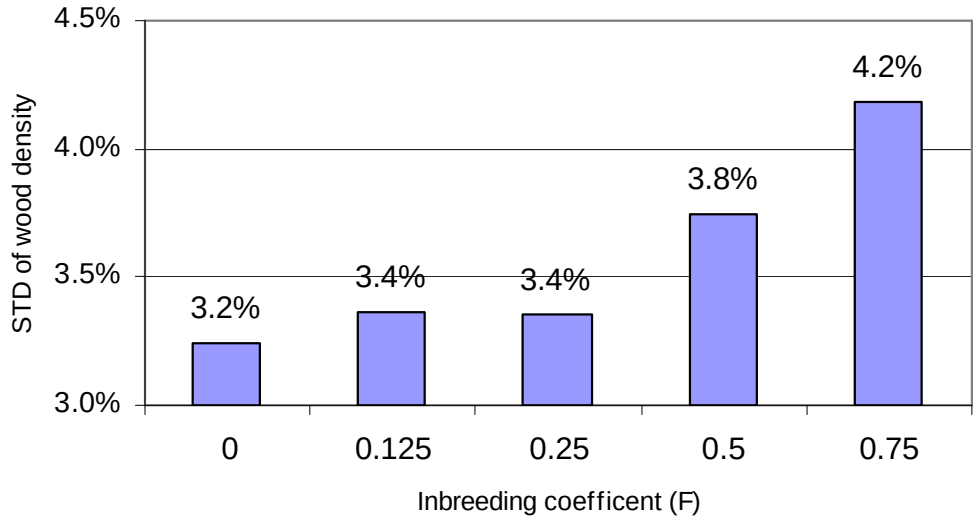
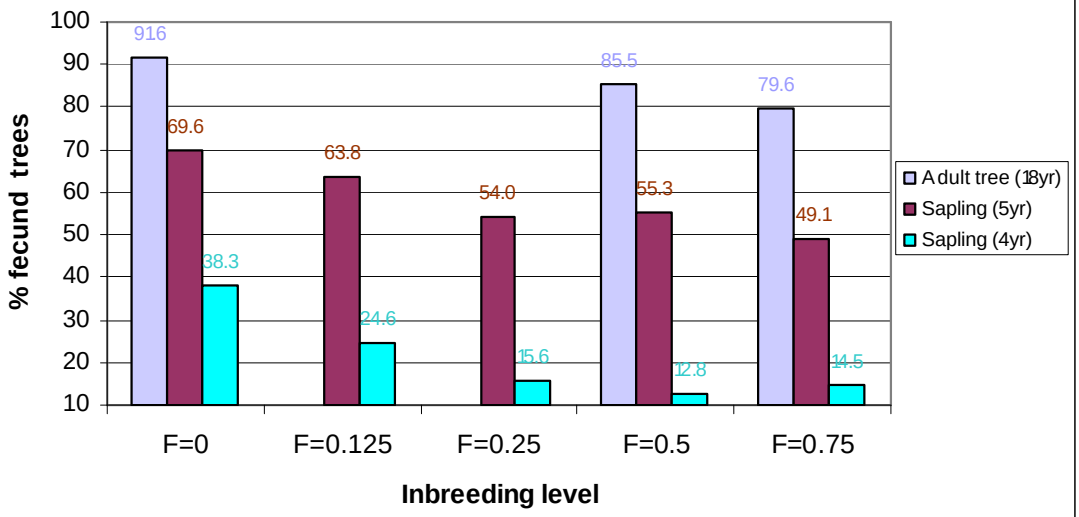


Figure 8 Effect of inbreeding on variation of wood density among trees in radiata pine



5. The effect of inbreeding on the reproductive ability in young and adult trees of radiata pine has been quantified from five populations of varied inbreeding levels.

Figure 9 Effect of inbreeding on fecundity (% trees producing female flowers) in young and adult radiata pine trees



The effect of inbreeding depression on fecundity was higher in young trees than when they were older (Wu *et al*. 2004). Inbreeding depression at a young age is due to two factors: 1) delay of reproductive age (about 8.3%, of trees delayed for F=0.5 and 8.5% for F=0.75 populations) and 2) true reduction in the number of trees flowering (compared with F=0, 6.7% more trees not producing flowers for F=0.5 and 13.1% for F=0.75 populations, Figure 9). Despite significant inbreeding depression effects on the percentage of female reproductive trees and on the number of cones on adult trees, overall inbreeding effects on fecundity were low in radiata pine. Only one of the eight founder clones contributed most of the significant inbreeding depression observed. It was also observed that fecundity varied more widely among the eight founder parents than among the selfs and outcrosses.

Based on our theoretical simulation analysis and the observations in the radiata pine inbreeding experiment, we believe there is great potential to develop high-quality inbred lines and produce highly productive crosses among lines in radiata pine. One of the critical issues with the existing radiata pine inbred experiment is that the founder population was too small, there were only 8 found clones (parents) used. There were also too few trees in the hybrid F_1 progeny, it was not systematically designed and only six pair matings were made. To study the inbreeding-crossbreeding method systematically in radiata pine, we have begun to develop an experimental inbred population, also using early selection (accelerated breeding technology) developed for radiata pine, as a pilot-scale experiment to explore this new tree breeding method. Besides being potentially more effective for breeding radiata pine, there will be other benefits from the inbred population. These include information on the genetic basis of inbreeding depression, estimates of the number and distribution of lethal alleles in an elite radiata pine population, information on the effectiveness of purging recessive and lethal alleles for growth from selected populations and information on the level of dominance in the genetic control of early growth.

Experimental Inbred Population in Radiata Pine

There are three components for the experimental inbred population in radiata pine:
1) creating a large inbred population;
2) continuing selfing from S_2 to S_3, S_3 to S_4;
3) crossing to produce hybrids among S_0, S_1 and S_2.

The ultimate worth of the final inbred lines depends greatly on the initial distribution of useful alleles in the progenitors. Hence the selection of a superior base population would increase the probability of generating high quality inbred lines (Namkoong et al. 1989). For this reason we used the best clones in a commercial breeding population (51 selections) for a large inbreeding (selfing) program. These selections have been selfed and polycrossed. First-stage nursery selection will be conducted for selfed families. We have also continued selfing from selected clones among the S_2 of the original eight founder clones in Griffin's inbreeding trial and so three generations of selfs plus S_0 will be created to study effects of purging under multiple generations of selfing.

Three half diallel crosses have been created for the three populations under different inbreeding levels (S_0, S_1 and S_2). The first half diallel was created using the 8 original founder clones. The second half diallel was produced using the best selections among the S_1 progeny of the 8 founder parents and similarly, the third half diallel was created using the best trees among the eight S_2 progenies of the 8 founder parents. Data will also be used to study the effects of inbreeding on genetic variance.

Acknowledgments

The inbreeding experiment reported here was planted by K.Walters on land now owned by Green Triangle Forest Products Ltd. We thank the Southern Tree Breeding Association for its support and for access to its nucleus population for inbreeding.

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Genetic Basis of Hybrid Performance in Wood Density and Stem Volume in *Pinus elliottii* var. *elliottii* ´ *Pinus caribaea* var. *hondurensis*

D. Kain[1,2], B. Li[3], M.J. Dieters[2,4,5] and K.J. Harding[2,5]

[1]School of Resources, Environment and Society, Australian National University, Canberra, ACT, Australia, [2]CRC for Sustainable Production Forestry, University of Tasmania, Hobart, TAS, Australia, [3]Department of Forestry, North Carolina State University, Raleigh, NC, USA, [4]School of Land and Food Sciences, University of Queensland, St Lucia, QLD, Australia, [5]Agency for Food and Fibre Sciences - Forestry Research, Indooroopilly, QLD, Australia.

The genetic basis of *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* (PEE×PCH) hybrid performance in stem volume and wood density was investigated using data from an 11-year-old progeny test at two sites in southeast Queensland, Australia. Progeny originated from four NCII mating designs: 6×6-parent designs in *Pinus elliottii* var. *elliottii* (PEE) and *Pinus caribaea* var. *hondurensis* (PCH), a 12×12-parent design in the F_1 hybrid using the same set of PEE and PCH parents, and an unrelated 6×6-parent design in the F_2 hybrid. The hierarchical family-within-taxon structure allowed the application of a novel quantitative genetic model specifically for hybrids of outcrossing species (Li and Wu 1996), to investigate the modes of gene action responsible for F_1 hybrid heterosis. Epistasis was assumed absent. Alleles contributed by PEE conferred increased wood density, and alleles contributed by PCH conferred increased stem volume. Overdominance or pseudo-overdominance did not contribute to better-parent heterosis in stem volume or to negative mid-parent heterosis in wood density. Partial dominance gene action was of minimal importance in wood density, and of moderate importance in stem volume. Most critically, in each trait, the superior genotype was an intraspecific homozygote, indicating the primary importance of additive gene action.

These findings were supported by results from conventional analyses of the data. The correlation between parental breeding values for pure species and hybrid progeny (r_{ph}) in stem volume was high in PCH but low in PEE, consistent with the effect of partial dominance. In wood density, r_{ph} was very high in both PEE and PCH, consistent with additive gene action. Correspondingly, variance due to SCA effects in the F_1 hybrid was high in stem volume, but very low in wood density. A comparison of the F_1 and F_2 hybrid populations revealed little difference in the mean and phenotypic standard deviation in either trait, consistent with the absence of overdominance.

The results support the hypothesis that PEE×PCH hybrid superiority results from an additive combination of alleles from the parental species, with partial dominance between species more important in some traits than in others. Resource-intensive breeding strategies designed to allow recurrent selection for overdominance or pseudo-overdominance in F_1 hybrid maize may not be justified. Recurrent selection for additive and additive × additive gene effects in a genetically diverse synthetic hybrid population, with careful control of inbreeding, is likely to be a highly cost-effective breeding strategy for PEE×PCH, and deserves further investigation in other 'complementary' forest tree hybrids.

Breeding Strategy for *Pinus radiata* in Australia.

M.B. Powell[1], T.A. McRae[2], H.X. Wu[3], G.W. Dutkowski[4] and D.J. Pilbeam[5]

Abstract: The Southern Tree Breeding Association Incorporated (STBA) breeding and selection program for *Pinus radiata* in Australia was developed after the amalgamation of independent member programs in 1983. The previous program documented by Boomsma (1997) and White *et al.* (1992) has been revised and changes implemented over recent years.

The STBA has modified its tree improvement strategy for *Pinus radiata* as a result of significant developments in four main areas – (i) economic breeding objectives, (ii) management of data and information, (iii) TREEPLAN® genetic evaluation and (iv) wood quality assessment. These developments have enabled the use of more flexible breeding and testing strategies which will lead to faster rates of genetic gain.

Significant developments include:

- adoption of a rolling front strategy with overlapping generations;
- increased selection pressure through larger population sizes;
- more crosses per cycle of breeding;
- greater focus on forward selection of progeny for breeding and deployment;
- a dynamic breeding population without a clearly defined nucleus and main population;
- more focus on pedigree control using full sib crosses and limited use of polycrosses;
- changes in breeding objective functions with greater emphasis on those growth, form and wood quality traits that impact significantly on profit; and
- production of genetic values targeting different production regions to account for genotype x environment interaction (GxE).

Research activities supporting the program are currently focusing on the continued development of: TREEPLAN® genetic evaluation software; the definition of economically derived breeding objective functions; an enhanced understanding of, and ability to use, GxE interaction in genetic evaluation and breeding and studies of the juvenile wood properties in the *Pinus radiata* breeding population.

[1] Pine Breeder, STBA, Mount Gambier, SA, Australia
[2] General Manager, STBA, Mount Gambier, SA, Australia
[3] Geneticist, CSIRO, Kingston, ACT, Australia
[4] Forest Geneticist, School of Plant Science, University of Tasmania, Hobart, TAS, Australia
[5] Eucalypt Breeder, STBA, Mount Gambier, SA, Australia

INTRODUCTION

The Southern Tree Breeding Association (STBA) manages the Australian cooperative genetic improvement program (breeding and selection) for *Pinus radiata*. This program was developed after the formal amalgamation of independent member programs in 1983.

The early history of the STBA program was documented by Boomsma (1997), who reported on the strategy revisions done through the 1980s and the early 1990s (White *et al*. 1992a, 1992b). The basic elements of the program and significant dates are listed in Table 1.

Significant events in the implementation of the STBA radiata pine breeding program, up until 1997 included: 1983 - 2 stage selection adopted; 1984/1985 - sublining of the breeding population;. 1986/1987 - single pair mating with combined index selection; 1987/1988 – proposed single-cross nucleus with open-pollinated main; 1988/1989 - expanded membership (other traits) and adopted nucleus strategy; 1990 - definition of breeding objectives and allocation of material to sublines and the nucleus; 1991/1992 - multiple population subline strategy and breeding value calculation; 1992 - breeding work commenced; 1993 - New Zealand implications caused changes (two sublines to manage inbreeding, deployment crosses between lines); 1993/1994 - crossing work completed; - 1996/1997 - third generation progeny trials planted (460+ families, ~30 progeny tests).

The essential elements of the past strategy as developed and eventually adopted for the crossing effort from 1992 to 1994 were (Figure 1):

- Nucleus and Main breeding populations (open nucleus of 40, main of 300)
- Two unrelated sub-lines each comprised of three trait-based sub-populations (MP – Multi Purpose; DG – Density and Growth; GP – Growth and *Phytopthora*).
- Two-way transfer between the Nucleus and the Main.
- Crossing for deployment between unrelated sub-lines.

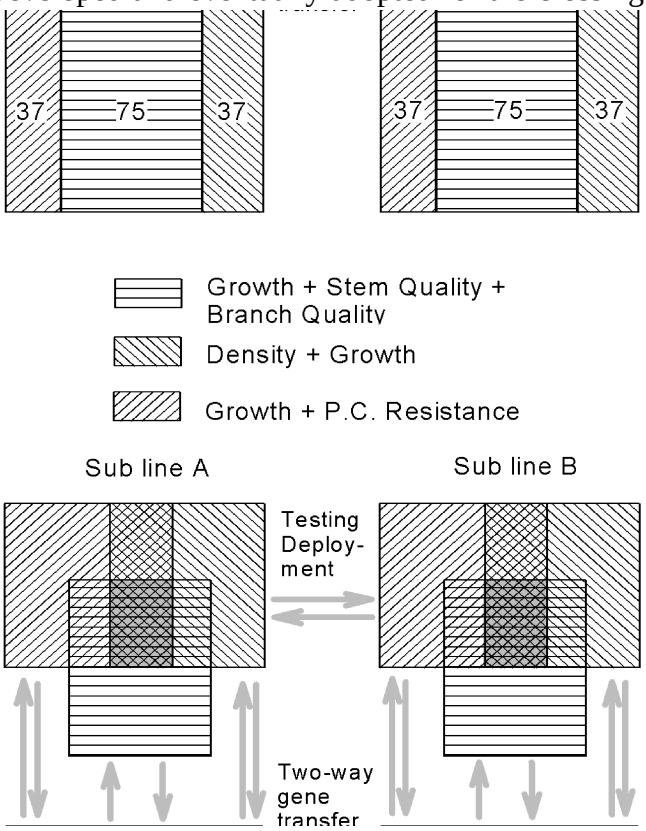

Figure 1. The 1993 STBA radiata pine breeding population structure

Over recent years, and particularly in relation to the development of data management and analytical tools, and the assessment of the 1996 and 1997 series of progeny trials, it was recognised that changes were needed to ensure rapid genetic progress and delivery of benefits to the STBA Membership. The STBA felt there was little value in continuing to

breed and collect data without the tools to process and analyse the information (Kerr *et al*. 2001, 2002, McRae *et al.* 2003, 2004 this conference). As will be discussed below some of these have now been implemented.

The STBA is currently modifying its tree improvement strategy for *Pinus radiata* as a result of significant developments in four main areas - (i) economic breeding objectives, (ii) management of data and information, (iii) TREEPLAN® genetic evaluation and (iv) wood quality assessment. These developments have enabled the use of more flexible breeding and testing strategies which will lead to faster rates of genetic gain.

ECONOMIC BREEDING OBJECTIVES

Clearly defined breeding objectives are the first and most important stage in a tree improvement program as traits in the breeding objective are the traits that determine profit. Selection criteria, which are the traits measured in the breeding and selection programs, may or may not influence profitability of an enterprise. The definition of an objective is basically an economic, not a genetic issue.

In the past, breeding objectives for *P. radiata* were loosely defined and usually developed without the use of economic information. The selection criteria used historically by the STBA in the different selection indices for *P. radiata* (multi-purpose; high growth and density; growth and *Phytophthora* resistance) included stem diameter, branch quality, stem straightness, *Phytophthora* resistance, *Dothistroma* resistance and wood density.

After preliminary work done in 2000 the STBA in partnership with The Forest and Wood Products Research and Development Corporation (FWPRDC), and CSIRO Forest and Forest Products (CSIRO) initiated a research project to more clearly define the objective in economic terms for solid-wood products in *Pinus radiata*.

There are two main components in this project: (i) deriving economic weights for breeding objective traits and using them to develop selection indices and, (ii) estimating genetic parameters and their variance and covariance matrices for key breeding objective (rotation age) and selection traits (younger age).

To date two interim reports (Wu *et al*. 2004, Ivkovich *et al.* 2004) have been produced that provide data for use in the STBA *P. radiata* breeding program.

Wu *et al*. (2004) reported estimates of genetic variance and covariance matrices between breeding objective and early selection criterion traits. This work was based on the results from a significant number of wood disks, bark-to-pith wood strips, and bark-to-bark through pith billets from genetic trials nearing rotation-age. Wood strip samples were processed through Silviscan® and the data were used for estimating genetic parameters for growth and wood property traits from early to rotation ages. The other wood samples were processed in various ways to help in determining correlations between traits and to also develop more effective methods of field

sampling for wood properties. Growth (DBH), stem straightness and branch size were also jointly studied with wood quality traits for the three sites.

Based on this work, genetic correlations, heritabilities, and genetic and phenotypic variance-covariance matrices were constructed by combining information for genetic parameters across the sites involved, and using information reported in the literature. These matrices will be used for deriving selection indices and for genetic evaluation purposes. The findings are currently being incorporated into the national breeding program for *P. radiata* by the STBA.

Ivkovich *et al.* (2004) reported results for a detailed study of the production systems of a number of STBA members involved in the growing and processing of radiata pine for solid wood products. They developed bio-economic models linking four breeding objective traits (MAI - mean annual increment, SWE - stem straightness or sweep, BRS - branch size, and MOE - modulus of elasticity) with the different production systems. Both industry confidential and published data were used. Economic impacts of the four breeding objective traits on the production systems were examined through the bio-economic model. Economic weights for the breeding objective traits were determined by estimating the improvement in overall profitability of various production systems as a result of changing trait values.

The economic weights developed in this study are now being incorporated into the STBA radiata pine breeding strategy through the development of selection indices. This will help industry to maximise profitability via increased genetic gain targeting solid wood production.

In addition to using the information in the breeding program, it will also be used by the STBA to develop deployment objectives specific for each members' business. This will permit members to maximise their individual benefit from tree improvement by making optimal use of the genetic information and genetic material available through the STBA.

MANAGEMENT OF DATA AND INFORMATION

The STBA currently has access to tree breeding data for about 500,000 trees and associated genetic material that has been developed over a sixty year period by members and the STBA. Data is also being captured on thousands of trees growing in current *P. radiata* trials planted by the STBA. This data is a valuable and a strategic asset that needs to be securely stored and yet made accessible for use by STBA staff and members alike. Prior to 2000 much of the data held by the STBA and/or its members was held in numerous *ad hoc* files. This made it difficult for staff to source and compile information for many purposes - statistical analysis, prediction of breeding values, management of germplasm and delivery of information to members.

The design for a data management system was developed over a period from the mid-1990s resulting in a document in 1998 that described in detail the specifications of an integrated software system to satisfy the STBA's requirements for data management.

Over the last five years the STBA has developed this system – the STBA-DMS™. This employs a web-based interface that makes access from multiple and diverse sites simple and flexible. The

system has allowed the rapid update of genetic values and their dissemination to users. Use of these values within the operational programs to ensure best information is used as soon as possible after it becomes available.

The STBA-DMS™ not only stores performance data but also manages the pedigree for genetic evaluation purposes and is fully integrated with the STBA's TREEPLAN® genetic evaluation system.

Once information is stored in the STBA-DMS™ it continues to be available for future genetic evaluations (TREEPLAN®) ensuring that the STBA maximises the use of all information at hand. All trees that are stored in the system are allocated a unique genotype identity. This gives the ability to track pedigree both for use in genetic evaluation but also for general purpose use in crossing programs, trial measure and assessment *etc*.

TREEPLAN® GENETIC EVALUATION

The accurate prediction of genetic (breeding) values for trees in the breeding population is fundamental for the success of the STBA tree improvement program for *Pinus radiata*. The optimal statistical method for breeding value prediction is Best Linear Unbiased Prediction (BLUP). BLUP was first introduced to tree improvement in the STBA by Jarvis *et al*., (1995) and is the preferred analytical method. In the past, consultants contracted by the STBA have used other methods, such as Best Linear Prediction (BLP), for the evaluation of *P. radiata* data and prediction of breeding values (White *et al*., 1992ab). Although appropriate at the time, given the capabilities of computer hardware and software for processing the large amounts of data available, the use of BLUP will advance capabilities in genetic evaluation and help to maximise the use of genetic material within the STBA cooperative.

The STBA, in partnership with The Animal Genetics and Breeding Unit (AGBU) at the University of New England, has developed the TREEPLAN® genetic evaluation system that is customised for forest tree improvement and has been using it routinely in STBA breeding programs for over three years (Kerr *et al.* 2001, 2002; McRae *et al.* 2003). The software allows the STBA to meet its obligations to members and is a vital tool in helping to deliver maximum genetic gain in plantations per unit of time and cost.

Powell *et al*. (2004) summarise the procedures and methods used in estimating genetic values for trees in the national *P. radiata* database. They also list Breeding Objective (BO) breeding values for each genotype (tree) in the database for traits of commercial interest. The breeding values for volume (VOLUME), density (DENSITY), branching (BRANCH) and stem straightness (STEMST) are combined into an index with different weights applied to each trait. As discussed, there is currently limited economic information that allows a reliable set of economic weights to be used in the construction of selection indices. As an interim position an alternative approach was used, based on maximising the correlation with an earlier BLP-based index. The current breeding objectives project (Ivkovich 2004) has substantially improved our position in this area. The estimated breeding values for individual traits along with the combined index are important for choosing elite parents for population improvement in the breeding program and for

propagation. The results are of commercial importance in choosing elite parents for grafting into seed orchards aimed at commercial deployment or crossing to produce smaller amounts of seed for use in the development of cuttings for deployment. The TREEPLAN® results can also be used to cull orchards and/or differentiate among seedlots that are currently available in the marketplace.

The TREEPLAN® system updates breeding values for genotypes in the national database on a regular basis as new data accumulates. Continually updated genetic values are readily available via the internet through the STBA-DMS™.

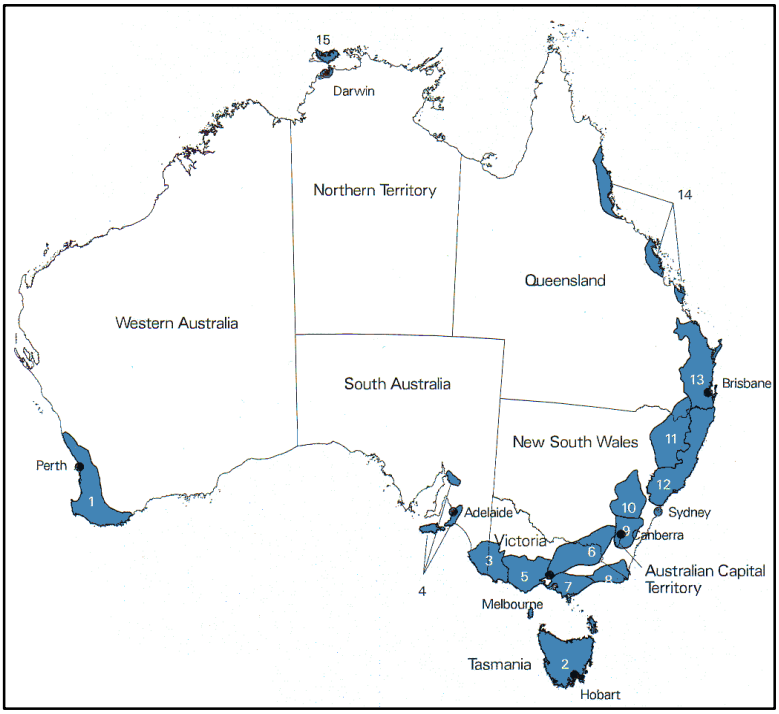

Figure 3. Regions used in defining *P. radiata* Selection Criteria used in TREEPLAN®

Genetic (breeding) values have been predicted for some 134,767 genotypes (different trees) in the population in 2004 and 21 selection criteria which are based on regionalised target production zones in Australia (Figure 3.). This included genetic values for genetic groups, first-generation (progeny of founder trees), second-generation and third-generation trees (progeny of crosses among selected parents).

Data was included from 78 trials including eleven STBA third-generation fullsib/polymix progeny trials. The flexibility of TREEPLAN® will enable genetic values to be region specific and to take account of any GxE interactions.

As larger volumes of data accumulate from progeny trials over the coming years these numbers are expected to increase rapidly. This will greatly improve the accuracy and precision of breeding values for genotypes in the population. The opportunity will be available for STBA members to realise greater genetic gain and increased profits in their commercial plantations.

The TREEPLAN® and STBA-DMS™ systems in combination will result in an ability to deliver marked improvements in the efficiencies of the STBA's breeding programs. Rapidly assessing the genetic merit of larger numbers of trees and then using the information to enhance crossing and progeny testing programs will enable real increases in selection intensity.

WOOD QUALITY ASSESSMENT

In the past, STBA breeding methods for radiata pine have concentrated on tree volume and form on the assumption that this would lead to more fibre and more profits. The first two cycles of selection in the STBA breeding population have increased growth significantly, while plantation owners have reduced rotation age from 40-45 years to about 30 years for commercial reasons. As a consequence, the proportion of juvenile wood in the harvested tree has increased to about 1/3 to 1/2 of total volume, a trend that is likely to continue. Hence the improvement of juvenile wood in terms of both quantity and quality is critical for the future of the softwood industry in Australia.

The STBA in conjunction with FWPRDC and CSIRO are funding activities which are focused on improving juvenile wood in radiata pine by combining information from broad-scale approaches in both quantitative and molecular genetics. Through acoustic testing of young standing trees and assessment of axial clearwood specimens together with collection and analysis of data from Silviscan® and WinDENDRO® X-ray scanning systems the aim is to develop methods to accurately and economically predict MoE or wood stiffness on young trees.

Quantitative genetic analysis of the data being collected will estimate heritabilities, genetic correlations and breeding values for juvenile wood traits (density, microfibril angle (MfA), etc.) and the transition point between juvenile and mature wood. This information will be used to derive a juvenile wood stiffness index to be compared with acoustic results for prediction of MoE of young trees.

Molecular information is also being collected as part of the project (identification of candidate genes and quantitative trait loci (QTLs) for various juvenile wood traits). The aim is to combine quantitative and molecular information to predict breeding values and then select advanced material for breeding and deployment from STBA breeding trials planted widely across south-eastern Australia.

This recognition of the important role of wood properties is integral to the future success of the STBA radiata pine breeding program.

RADIATA BREEDING STRATEGY

The current breeding strategy for *P. radiata* (White *et al*., 1999) was developed during the 1980s and implemented in the early 1990s. The strategy is continually under review. Although appropriate for the time, the focus was on theoretical aspects of the breeding strategy rather than the effective and efficient implementation of the program. New strategies will focus on total tree improvement, rather than just breeding, by integrating deployment with the breeding program. More effort is being directed towards the delivery of genetic gain per unit time. To achieve this, the generation interval must be reduced and selection pressure increased. Despite its strengths, there are several areas where the current strategy could be improved.

Revising the strategy

The STBA decided to review the strategy as part of an ongoing process. Strategically the STBA felt it was necessary to build some fundamental tools for data management and genetic evaluation as this would facilitate flexibility in the strategy and give an ability to quickly adapt to changes and enhancements.

Breeding objectives must be defined clearly and derived with the use of economic information. The selection criteria used in the different selection indices for *P. radiata* include stem diameter, branch quality, stem straightness, Phytophthora resistance, Dothistroma resistance and wood density. Clearly defined breeding objectives will ultimately determine the optimal suite of selection criteria for use in assessment and selection. In future, there will be increased emphasis on wood quality traits (juvenile wood, density, stiffness and spiral grain) and strategies for resistance to *Cyclaneusma* spring needlecast, Pitch Canker and Essigella aphid may be needed. The STBA is involved in projects that are starting to provide information on the breeding objective traits that will be crucial to the future success and relevance of the breeding effort.

BLUP is now the preferred analytical method for prediction of breeding values in trees and must be routinely applied. BLUP technology can overcome many of the statistical problems usually faced with 'rolling front' breeding and selection strategies and traditional methods of analysis. The STBA developed TREEPLAN® to ensure it has the analytical capability necessary for the effective use of BLUP.

A feature of the past strategy was a nucleus breeding system (Cotterill *et al.* 1988 and Cotterill 1989). A discrete nucleus and main breeding population is not retained in the new strategy. Nucleus breeding was largely adopted in animal breeding for situations (limited reproductive rates in females) not found in *P. radiata*. Although nucleus breeding has other advantages in management of inbreeding, there are now better and more efficient selection and mate allocation methods available.

Discrete generation breeding, a major component of the past strategy, leads to an inefficient use of resources. Annual budgets and work programs fluctuate widely with 'peaks and troughs' of activity. A 'rolling front' strategy with overlapping generations has been adopted. The advantages include: operational efficiencies, more stable budgets across years, increased selection pressure with a larger program, earlier adoption and use of elite genotypes for breeding, ease of infusing new material, reduced risk, better account of GxE and enhanced gains. A rolling front strategy also facilitates a more rapid response to changes in breeding objectives. The development of TREEPLAN® has been essential for the successful adoption of a 'rolling front'. Better selection and mate allocation tools will be needed for management of pedigree and inbreeding.

The use of pollen mix crosses for general combining ability (GCA) testing is not essential. In the revised strategy, there is no longer a discrete 'main' population. Parents will be used in several crosses (minimum 3 to 4) and more effective use of pedigree is made using BLUP evaluation.

The current practice of using independent breeding sub-lines will continue, at least in the short-term. This aids in managing inbreeding and ensures deployment populations are outcrossed.

The size of the breeding population (340) can be reduced and effective population size managed with selection and mate allocation genetic algorithms.

Where required (subject to the findings of the breeding objectives project) there may be multiple breeding sub-populations with 30-40 parents targeting different breeding objectives. Separate deployment 'breeds' will be developed with two sub-lines per breed. In the interim, three breeds (Multi-purpose, High-Growth and Phytophthora resistant) will continue to be used for breeding.

The revised strategy will review the importance of clonal propagation in progeny testing and its role in deployment. Presently in Australia, clones or cuttings are used as a propagation method largely to facilitate family forestry. Technologies (tissue culture and somatic embryogenesis) are now available to facilitate the use of clonal systems. Selection pressure can be greatly enhanced and both additive and non-additive genetic variance exploited with the deployment of tested clones.

Integrated breeding facilities must also be established by the STBA to better manage the breeding population. The population of parent trees must be available on a central location to facilitate cross-pollination activities on an annual basis. Flowering success and synchrony are necessary to increase selection pressure and optimise parental combinations, and ensure that the STBA pursues gain per unit time.

The Future

The STBA has access to a highly valuable genetic resource and is capitalising on this member investment by focusing on genetic gain per year and delivery of profit to its stakeholders. Third generation progeny trials are currently being assessed and consolidation of the program is critical. The strategy review process will be dynamic with changes adopted as opportunities are identified. Projects which target key areas have been developed and are now largely in place (breeding objectives, genetic evaluation, data management, integrated breeding facilities, clonal deployment, and selection and mate allocation). These will strengthen the breeding strategy and result in enhanced genetic gain. Future revision of the tree improvement strategy for *P. radiata* will focus on implementation issues and optimising gain via an appropriate and relevant breeding objective.

The breeding strategy is being revised in stages. The STBA had to first develop the tools needed to overcome well known technical difficulties that have plagued many breeding programs in the past. There is little point in having an optimal strategy with inherent weaknesses. The most important of these is the management of pedigree and performance information. The development of the STBA-DMS™ has overcome this limitation and the development of TREEPLAN® has given the STBA the analytical tool it needs to produce accurate breeding values efficiently and on a regular basis. Multi-site, multi-trait, multi-age and multi-generation analyses can be done, thus allowing the adoption of more efficient rolling front strategies. The last major deficiency (or opportunity) is then efficient selection and mate allocation. The STBA has proposals in train that will address this issue and enable the management of inbreeding more efficiently than by the use of unrelated sub-lines.

Research activities supporting the program are currently focusing on the continued development of: TREEPLAN® genetic evaluation software; the definition of economically derived breeding objective functions; an enhanced understanding of, and ability to use, GxE interaction in genetic evaluation; studies of the juvenile wood properties in the *Pinus radiata* breeding population.

CONCLUSION

The STBA is putting in place the key tools needed to effectively manage large scale breeding programs on behalf of its members. In relation to the radiata pine breeding program the switch to an annualised, rolling front program of crossing and progeny trial establishment, in combination with better ways to manage key genetic and resource information will see major benefits coming to fruition over the next few years. The result will be the deployment of highly genetically improved material in members plantations and the ability to advance the breeding population with a speed and a simplicity not contemplated a few years ago.

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History of Cloning Woody Plants

W. J. Libby[1]

Abstract

Many advantages of cloning were apparent to Mediterranean-region growers of trees such as figs, dates, pomegranates and olives 5,000 to 6,000 years ago. These were all propagated by cuttings or, in the case of olives, by rooting basal knobs. Citrus varieties that were apomictic also produced reliable seed-origin clones.

Detached-scion grafting technology came later, probably originating in China about 3,000 years ago. This led to cloning of such species as almonds, pears, apples, plums and cherries. These species could not be reliably propagated by rooting cuttings and, being outcrossers, did not generally produce healthy and consistent inbred lines.

Poplars and willows have long been cloned in Asia and near the Mediterranean because their propagation by sticking cuttings is easier and more reliable than starting with seeds or seedlings. Many of their earlier clones were developed for agricultural purposes such as erosion control, basketry, fodder and bedding for animals.

The first recorded example of purposeful forest-tree improvement and clonal forestry was in China, with Chinese-fir about 800 years ago. Local farmers established new plantings by taking both rooted basal shoots and unrooted sprouts from stumps of the best trees, thus developing excellent locally-adapted clones.

Although it was known 12 centuries ago that sugi and other Japanese conifers could be propagated using cuttings, plantations were not established with rooted cuttings until about 1400. This was soon followed by clonal selection and characterization, and there are now over 200 characterized "cultivars" of sugi just in southwestern Japan.

During the last two or three centuries, arborists and horticulturists have found and, using grafts or cuttings, propagated clones of many species for urban and amenity purposes. Cultivars such as weeping willow, London plane and Lombardy poplar have been named and used for over 300 years. By 1983, 941 validated cultivars of angiosperm trees were listed just for North America and Europe.

In the latter half of the 20th Century, the ability to clonally propagate trees advanced through a better understanding of maturation, and through the development of tissue-culture and somatic embryogenesis. The list of forest-tree species that could be effectively propagated clonally expanded from a very few in 1950 to hundreds by 2004.

Some more events

Most of the main historical events are covered in the Abstract, and I won't repeat them here. I will touch on a few additional landmark events, and on the initiation of cloning with a few

species, but the following is hardly a comprehensive list of either species or events. It is meant to merely help obtain a time sense of how the clonal propagation of trees has progressed.

S*ugi* stecklings have been used in Japan for about 600 years, first in addition to seedlings in general plantations, and later in pure clonal plantations as clonal characterizations were developed. Later, in the 1600s, methods and timing of collecting cuttings to be set directly in the planting site were developed. It has recently been shown by isozyme analyses that some of the described cultivars were actually mixtures of several similar clones, while others were a single clone.

Poplars have long been cloned, with particular clones selected and propagated for specific purposes. Clonal forestry with poplars accelerated in the early and mid 20th Century as breeding and interspecific hybridization made some wonderful new genotypes available. Because poplars are among the taxa most susceptible to disease and insect damage and epidemics, the strategy of prescribed multiclonal plantations was proposed and adopted with this genus first, in mid-20th Century.

Norway spruce stecklings were first reported produced by human intervention in 1828. This technology lay essentially dormant for a century until stecklings were used in some of the earliest Norway spruce seed-orchards. Development of the nursery techniques for establishing clonal plantations of Norway spruce was initiated in Germany in 1948, a decade later in Norway, and the research and development soon spread to adjacent Sweden, Denmark and Finland.

Cutting-donor chronological age was first demonstrated to be of crucial importance for successful rooting of cuttings of forest trees in 1929.

Phytohormones were detected, synthesized and used to enhance rooting of cuttings beginning in the 1930s.

Monterey pine (radiata pine) was used in early research on rooting in the second quarter of the 20th Century. Use of radiata pine stecklings in large plantations became operational in New Zealand in the 1980s, and was soon joined by operational use of plantlings in the late 1980s.

Tissue culture of a conifer was first successfully accomplished with coast redwood, in 1949. Coast redwood is one of the few conifers that clones naturally. Redwood stecklings have been used in research and for urban and amenity plantings since the 1960s. Research in France and California advanced tissue-culture of redwoods; by the 1980s tissue-culture was being used to store clones, and plantlings served as stock plants to produce stecklings for outplanting. By the 1990s, plantlings of several redwood clones were being operationally deployed into the forest.

Somatic embryogenesis was first demonstrated in carrots in the late 1950s. By the 1980s, it had been achieved in tree species, including several spruces, yellow-poplar, a poplar hybrid, and European larch. By the 1990s, the species list was expanding, and field trials of emblings were in place.

Eucalypt cloning operationally began in tropical and subtropical areas of Africa in the 1960s; in the 1970s the center of activity shifted to South America, particularly near the coast of Brazil.

Yellow-cedar became, in a British Columbia program, the first conifer species with more than half its total annual planting using stecklings rather than seedlings. Since 1975, between 500,000 and 750,000 stecklings were planted annually, comprising over 70% of planting stock.

Organ culture had, by the 1980s, largely replaced trying to regenerate most species of trees from callus in *in-vitro* cultures.

Maturation state and physiological condition

There is some use of terminology that I think has slowed progress and may still be causing some confusion. In particular, the terms "aging" and "physiological age" are variously defined and interpreted. I here support, instead, the terms "maturation state" and "physiological condition" in hopes of better focusing our attention on both the causes of differences among propagules of the same clone, and on research to better understand and deal with these problems.

In addition, it is worth being clear on several terms that are associated with the concepts and effects of physiological condition and maturation state. These terms are most applicable to the scion portion of grafted plants, to cuttings and stecklings, and to a lesser extent to tissue cultures and plantlings. "Cyclophysis" refers to the maturation state of the terminal meristem(s), and to the effects of that maturation state on propagation and subsequent development. "Topophysis" has elements of cyclophysis, but adds an effect of the additional differentiation that occurs after meristems are produced that grow into a branch hierarchy. It also includes the effects of the different physiology and morphology associated with branches of different order and location, as compared to each other and to terminal meristems. "Periphysis", less used, refers to the effects of the operational environment of the donor plant on the physiological condition of the plant parts collected from the donor, as expressed during their propagation and subsequent development.

Seedlings normally are juvenile when they germinate and their subsequent development is considered to be the norm. While the maturation state of most germinating seeds is early-juvenile, it is clear that the maturation state of graft scions, cuttings, and tissue-culture explants of many forest-tree species varies substantially and importantly. For most species, this variation is reasonably predictable by the chronological age of the donor plant and the part of the plant used as a scion, cutting or explant donor; most important is the current maturation state of the scion, cutting or explant when it is donated.

Maturation can be understood as a developmental-genetic process, proceeding more-or-less continuously from embryonic through various juvenile, adolescent and mature stages, to a late-mature stage when systems are likely to fail. Different parts of a large tree are typically at different maturation states, with the more-juvenile (usually early-adolescent) states being found in meristems low in the tree or in the roots, and the most mature occurring in the terminal meristem(s) of the uppermost stem(s). One useful indicator of likely maturation state of the donated part is its cumulative distance along the stem from the position of the seedling's

cotyledons. Thus, a propagator can influence the maturation state of propagules not only by choosing the chronological age of the donor plant or culture, but also by choosing the location on the donor plant from which to take the tissue or organ.

While the maturation of a clonal line can be slowed by techniques such as low-hedging of donor plants or rapid serial propagation, it is less clear whether the maturation state of tissue or other propagule material can be reversed. It is clear that elements of maturation affecting vegetative propagation proceed rapidly in some species (such as sugar pine) and are long persistent in the juvenile state in others (such as willows).

The effects of maturation state on the performance of propagules can be anticipated by considering the performance of the part of the tree whose terminal meristem is or was at that maturation state. Thus, in an example typical of many conifer species, a propagule produced at a mature maturation state will develop the lower-bole form, branch architecture and thinner bark typical of the upper bole; the wood of its lower bole will have, for a given ring number from the pith, the longer tracheids, lower (steeper) microfibril angle and lower specific-gravity typical of upper-bole wood; and its growing shoots will have greater resistance to juvenile diseases, greater susceptibility to browsing, and will more-quickly exhibit sexual competence.

A propagule's physiological condition depends on its supply of photosynthates and mineral nutrients, and on the interaction of its growth regulators (such as hormones and inhibitors) with its recent and current environment. The propagule's current physiological condition strongly affects its performance during its propagation and subsequent growth and development. Physiological condition can be influenced to produce more juvenile-appearing or more mature-appearing morphology of the propagules by the husbandry of the donor plants and during propagation. Such differences in morphology can also influence the short-term subsequent development of the propagule.

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[1] William Libby is a senior associate with Zobel Forestry Associates, Cary, N.C.

CLONAL FOREST PLANTATIONS IN THE WORLD

Bob Kellison, President
Institute of Forest Biotechnology
PO Box 13399
Research Triangle Park, NC 27709

EXTENDED ABSTRACT

Asexual reproduction of some forest trees is a natural phenomenon. Any species that reproduces from stump and root sprouts belongs in that category regardless of whether sexual reproduction is the dominant method of stand replacement. The classic example rests with *Populus* spp. and *Salix* spp. All members of those circumpolar genera can be vegetatively propagated by use of stem cuttings, but cuttings from some species, when set in a suitable environment, root more readily than others. Humans have, for centuries, taken advantage of that phenomenon. It was no accident that the Apian Way leading from Rome was lined with poplars, all equidistant apart and all having the same phenotype. The Romans or their forbearers had, in flood-prone areas, either observed that members of the *Populus (*and *Salix)* genus would develop roots on tree trunks at the water line, or that a broken branch would take root when its basal portion was fortuitously deposited in freshly deposited sediments. Thus, was borne clonal forestry.

Additional credence was ascribed to naturally occurring clonal forests when it was realized that aspens (section *Populus*) commonly reproduce from root sprouts. Following catastrophic site disturbance, such as from fire or wind, root sprouts of some trees dominate over the sprouts of other trees. A common observation in aspen country is to identify the confines of a clone by the different tints of green in spring and by the different tints of yellow in autumn. Research has quantified that one clone in the Rocky Mountains of North America occupies 40 hectares.

Taking advantage of the natural propensity of poplars to establish themselves by stump and root sprouts forest landowners began to purposefully employ the phenomenon for wood production, erosion control and amenity plantings. In localized areas, *Salix* spp. were and are clonally established, but they lack the broad applicability of poplars on a worldwide scale; they are primarily propagated for biomass production.

In a recent unofficial tally of poplar plantations in the world, the best estimate was that there are about 1.6 million hectares (Table 1). The further estimate was that the area had not fluctuated greatly in the last 50 years. In effect, the poplar plantations being established were largely a replacement of plantations that have been harvested. The one practice that could change the plantation area greatly would be a significant change in row-crop farming. The region that would be most impacted by releasing the protective measures on farmland preservation would be Europe. Significant areas would likely be forested with poplar clonal plantations if and when that eventuality occurs.

Table 1. Clonal plantations of *Populus* spp., by region/country.

Country/region	Area (000 hectares)
Europe	949
Asia/Pacific	484
South America	84
North America	40
Africa	10
Total	*1,567*

Seeing the advantages of clonal forestry from poplar plantations, forest industry began an all-out effort about 25 years ago to use vegetative propagation in the establishment of fast growing plantations. Significant progress was made, especially on *Eucalyptus* spp., that are extensively planted in the tropics and subtropics. During that time, it is estimated that upwards of 1.3 million hectares of that genera (inclusive of several species) have been planted (Table 2). The science for asexually propagating eucalypt species has been refined over the last two decades so that plantlet production is largely from micropropagation rather than macaropropagation. A major reason for the switch from use of seedlings for plantation establishment to propagules is the gain in growth rate, wood property uniformity, pest tolerance and adaptability.

Table 2. Clonal plantations of *Eucalyptus* spp., by region/country.

Country/region	Area (000 hectares)
Latin America	1,040
Africa	100
Asia/Pacific	46
Iberia	31
Total	*1,217*

About 15 years ago, a major effort was initiated in Asia to establish clonal plantations of *Acacia* spp. Species of that genus occur throughout the tropics, subtropics and warm temperate zones of the world, but the ones that are best suited for plantation establishment and, specifically, for pulping (*A. mangium, A. crassicarpa)* are from subtropical Australia and the adjacent northern islands. The greatest area of industrial plantations is on the island of Sumatra, Indonesia where more than 200,000 hectares exist. Additional areas of the species are being established in southern China, and elsewhere in Southeast Asia.

Clonal forests of a number of other species exist throughout the world (Table 3). They involve both gymnosperms and angiosperms, identified in Table 3 as conifers and hardwoods. The reminder to the reader is that the table is incomplete; the intent is to show that clonal forestry is practiced with many species and in many parts of the world.

Table 3. Clonal plantations of 'other' species, by region/country.

	Country/region	Area (000 hectares)
Conifers (Species)		
Cryptomeria Japonica	Japan	5,000
Cuppressus spp.	World	40
Pinus spp.	World	30
Cunninghamia lanceolata	China	5
Other	World	5
Total		*5,080*
Hardwoods		
Salix spp.	Europe	20
Grade (Walnut, cherry, etc.)	World	1
Other (Locust, *Paulownia* spp., etc.)	World	1
Total		*22*

These best estimates show that there are upwards of three million hectares of clonal plantations in the world and, at least half of those plantations- -the ones of *Populus* spp.- -had their origin hundreds of years ago. That forestry practice was accepted without question until the mid-1990s when a singular event raised the scepter about cloning. That event was the arrival of Dolly the Sheep. Never mind that Dolly arose from placing a mature cell in an oocyte and letting it develop (one out of many hundreds that came to fruition) as opposed to propagating living tissue from a tree and allowing it to form a tree of like kind, the result was cloning. Today clonal forestry is under attack in some societies because the distinction is not made between the two forms of cloning: cell transformation, multiplication and maturation versus cell multiplication and maturation.

Unless we do a better job of communicating the results we stand to lose the benefit of hundreds of years of vegetative plant progagation.

Methods of Cloning Forest Trees

Yousry A. El-Kassaby[1] and Jodie Krakowski[1]

Abstract: Foresters in developed and developing countries have begun to adopt cloning as a viable option to multiply superior genotypes for increased yield and product uniformity in planted forests. Cloning, or vegetative propagation, can be achieved through organogenesis (including macropropagation and micropropagation) or through gametic and somatic embryogenesis. Macropropagation (e.g., rooted cuttings) is widely used for a variety of hardwoods and softwoods; success depends on the physiological juvenility of the donor plant. Micropropagation produces fully developed plants from juvenile tissues including embryos, cotyledons and hypocotyl segments. Excised tissues must be treated with growth regulators to form buds or roots. Development of tissues into complete plants, and long-term storage of tissues to maintain viability and juvenility are the major hurdles for successful micropropagation. Somatic embryogenesis has shown great promise for producing infinite copies of embryos *in vitro* derived from plant tissues without sexual reproduction, which can be stored frozen indefinitely. Converting somatic embryos into plantable seedlings at the industrial scale is also a challenge. A production model combining various propagation methods is being utilized by many organizations to capitalize on the benefits of each technique while overcoming their respective difficulties. The utility, methods and challenges of cloning techniques will be reviewed.

Keywords: Cloning, macropropagation, micropropagation, organogenesis, somatic embrogenesis, tissue culture.

INTRODUCTION

Forest trees can be propagated sexually, via pollination and seed production, and asexually, where offspring carry the same genetic make up (genotype) as a single, unfertilized parent. Wild and production populations (seed orchards) generate seed by fertilization to produce seedlings for natural and planted regeneration. The traditional tree improvement system employs seed orchards to deliver seedlings for reforestation by cross-pollinating the best selected trees. The next generation then performs better than their parents for desirable traits such as growth rate and form. This difference in trait means between generations, or between wild and selected populations, is called genetic gain. Asexual propagation can achieve much higher and more rapid gains than traditional tree breeding. Asexual propagation avoids the random mixing of the parental genes during fertilization, so superior genotypes can be preserved and duplicated. Cloning is creation of multiple copies of the same genotype. Cloning can also capture gains for traits which cannot be reliably bred for sexually due to their genetic mode of inheritance, such as pest resistance. Clonal forestry is a management system featuring single clone plantations implemented in some high-yield forestry regimes.

[1] Professor and Research Scientist, respectively; Department of Forest Sciences, Faculty of Forestry, the University of British Columbia, 3616-2424 Main Mall, Vancouver, BC, Canada V6T 1Z4.

Forest trees can be cloned several ways. There two major categories are organogenesis and embryogenesis (Figure 1). The former refers to the replication of a complete individual from rooted stem cuttings or from tissue culture of small portions of juvenile tissue. The latter involves making multiple copies of an embryo, which itself may or may not have arisen from sexual reproduction. Producing rooted cuttings is the most common form of cloning practiced in forest trees. Not all individuals are amenable to vegetative propagation; success is species- and genotype-specific.

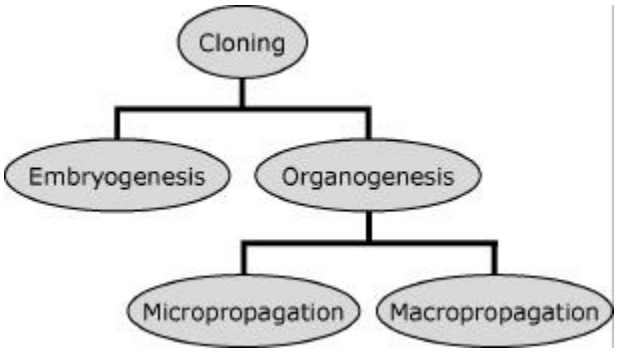

Figure 1. Conceptual schematic of cloning techniques in conifers.

ORGANOGENESIS

For successful organogenesis, juvenility of the tissue is critical (Bonga and von Aderkas 1992). As plants, and the tissues within them age, there is a lower chance of capturing the pluripotency (the ability of each cell to develop into any other type of cell) inherent in plant cells and regenerating an accurate copy of the genotype. To circumvent this, juvenile tissues such as embryos, cotyledons and hypocotyl segments (Figure 2) are typically used (Bonga and von Aderkas 1992; Minocha and Jain 2000). Since plants grow from meristems, tissues from terminal shoots, root tips, and epicormic shoots (vigorous sprouts from the main stem) of mature trees represent additional regions where physiological tissue juvenility is maintained, even though the plant itself may mature.

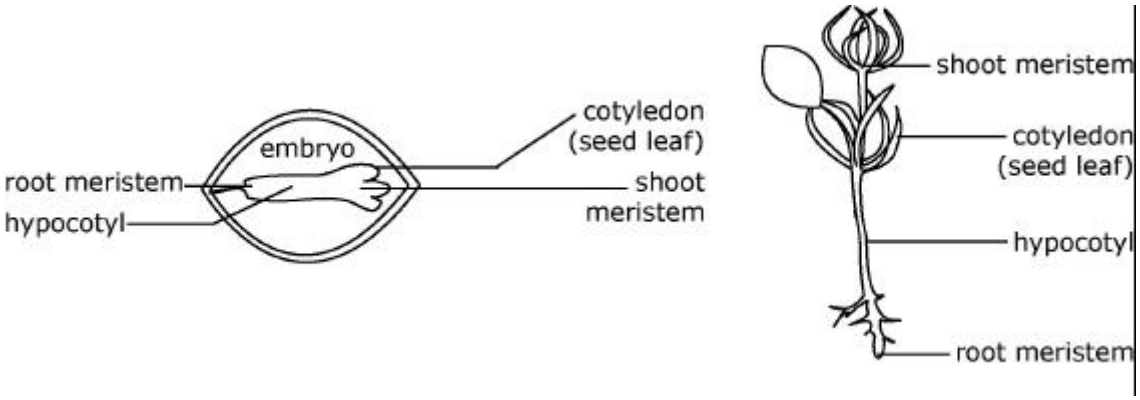

Figure 2. Some plant tissues used for organogenesis.

Organogenesis can be subdivided into macropropagation and micropropagation (Figure 1). Macropropagation uses large portions of a plant to reproduce whole plants. Examples of this include rooted cuttings, grafting and layering (the formation of adventitious roots from a piece of stem tissue). Since there are many types of meristems in plants, it is critical to select the most suitable meristem for macropropagation. For instance, cuttings taken off the shoot apical meristem, located at the tip of the shoot leader of a mature tree (Figure 2), may actually be too juvenile and perform inconsistently with some techniques (Bonga and von Aderkas 1992). Meristems from the lower portions of the plant, such as bottom branches, originated years before the shoot apical meristem, and often exhibit poor growth when cloned due to the combination of accumulated mutations and the changes in plant hormone concentrations with age. A typical problem is topophysis, or the tendency of tissue taken from a branch to retain the growth form of a branch and not grow in an upright or symmetrical fashion (Pierik 1987). Rooted cuttings are the primary means of propagating radiata pine in New Zealand and Chile and yellow-cedar in British Columbia. Other conifer species such as Douglas-fir, sequoia, spruces, western hemlock, western redcedar, loblolly pine and yews all were successfully rooted with varying results (Ritchie 1991; Gupta et al. 1996).

Micropropagation features a more sophisticated approach, using tissue culture techniques to induce formation of roots and shoots from meristems, eventually developing into a whole plant (Pierik 1987; Bonga and von Aderkas 1992). This technique requires rigorous scientific conditions at each stage and the application of plant hormones and other chemicals. There are four steps: (1) tissue culture initiation, (2) proliferation of shoots, (3) developing roots, and (4) acclimatization of plants in a nursery environment (Bonga and von Aderkas 1992). Micropropagation has been used to produce radiata pine in New Zealand and Chile and sequoia in the United States, but the high cost and necessity for highly skilled technicians impose limits to more widespread application.

EMBRYOGENESIS

To circumvent problems associated with aging of both tissue and the selected tree, somatic embryogenesis (SE) was developed in the mid-1980's (reviewed in El-Kassaby 2001). SE replicates embryos without fertilization or meiosis, and can produce vast quantities of a single genotype (Gupta et al. 1996; Minocha and Jain 2000). While most of the technical requirements are in the public domain, several processes relevant to its commercial application are proprietary or patented. The common steps are: (1) induction to obtain a mass of embryonic tissue which can replicate indefinitely; (2) cryopreservation enabling maintenance of long-term juvenility and viability; (3) proliferation of selected genotypes into undifferentiated masses; (4) maturation of embryos from the undifferentiated tissue; (5) dessicating embryos to mimic zygotic embryo development; (6) sowing and germinating embryos; and (7) acclimatizing germinants to a nursery environment (El-Kassaby 2001). The potential economic returns of SE have driven extensive public and private research programs to overcome biological and logistical challenges. Commercial scale applications of SE are just emerging.

CHALLENGES OF CLONING

Each cloning technique has inherent challenges and is best applied in particular circumstances. Cloning methods have dramatically improved over the past decade; however, much of the technology is patented due to high research and development costs. To avoid patent infringement, each organization must develop new approaches or create licensing agreements. The genotype-specific facility of cloning can also be problematic, particularly if the most desirable individuals are difficult to clone (Bonga and von Aderkas 1992).

The major difficulty of macropropagation is maintaining the appropriate level of juvenility in tissues. Hormones and environmental controls (e.g., moisture, temperature, pH) are widely used to correct this.

The primary challenges of micropropagation are (1) maintaining juvenility in the tissue culture and storage, (2) the ability to simultaneously induce shoot and root formation, (3) maintaining consistency from the lab to the nursery, and (4) transferring results successfully from research to operational scope. Cryopreservation has not proven effective for maintaining juvenility in these tissue types. Continuous transfer of culture is used to rejuvenate tissues, a costly and labour-intensive technique.

Cryopreservation is highly successful for embryonic tissue (Hargreaves and Smith 1994). Special techniques and equipment such as bioreactures and liquid media have enabled mass embryo production (Attree et al. 1987). Challenges still preclude more widespread application of SE: (1) low induction rates, (2) asynchronous embryo development, (3) long germination time, (4) difficulty germinating naked or encapsulated embryos (i.e., using synthetic seed coats), (5) low rates of seedling development from embryos, and (6) problems acclimatizing to commercial nursery environments.

Combinations of the various cloning methods are being developed to overcome some of these technical, logistical, and legal challenges, and facilitate mass production of the best genotypes (Figure 3). These processes capitalize on combining relatively inexpensive proven effective methods (e.g., rooted cuttings), while minimizing technical limitations associated with scale differences between research and practice, and avoiding legal issues arising from patents. The initial common step is to produce embryos using the imperfect SE methods in the public domain, followed by micropropagation to bulk up tissue or to create mother plants and/or hedges for rooted cutting production in a serial cutting propagation program (Figure 3). Both approaches can produce plantable seedlings through either micropropagation or rooted cuttings from a mother plant. These combination approaches are being implemented successfully at the experimental or modest production scales in Canada, Chile and New Zealand.

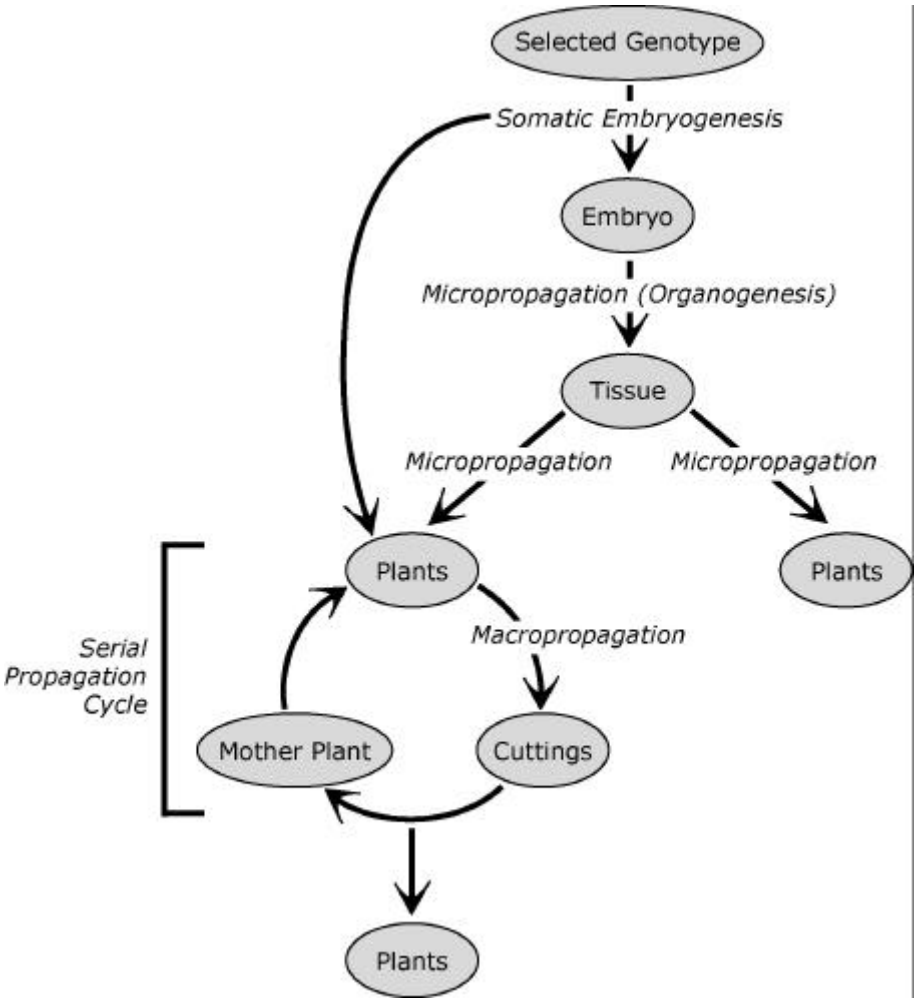

Figure 3. Combinations of methods used to overcome limitations of cloning techniques in conifers.

CONCLUSION

Cloning is an effective tool to increase the genetic gains attained through traditional tree improvement programs. Successful mass production of top performing genotypes is expected to yield higher and more quickly realized investment returns than conventional tree improvement systems, and may lead to more widespread adoption of high-yield clonal forestry on appropriate sites. Although major strides have been made in cloning forest trees, additional investments in research and development are needed to overcome the limitations discussed above.

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Benefits of Clonal Forestry

John Pait

Vice President, Business Development
CellFor LLC
Vancouver, BC, CANADA

Deployment of tested clones for plantation forestry can result in the highest genetic, productivity, product quality and financial gains possible from conventional tree breeding. At the stand level, planting clonal propagules in numerous species around the world is generating extraordinary growth rates of uniform, clonal trees yielding shorter rotations, higher revenues, and more consistent wood quality than conventional seedling stock. At the forest level, managers and land owners have more flexibility in their management and portfolio options with clonal stands as part or all of their regeneration stock types. By concentrating production on a smaller area, non-timber benefits can accrue to landowners and society such as retention of habitat for wildlife, and conservation of areas of special ecologic or historic value. With cryopreservation of somatic embryonic tissue, long term gene conservation is also possible. Novel applications such as carbon sequestration may also be contemplated and uniquely designed with targeted, clonal genotypes. Key requirements for operational clonal reforestation include elite germplasm, well-designed genetic tests, a deployment strategy and perhaps most important, an efficient, high-volume propagation system. The choice of propagation system is a function of species, scaling technology, costs, and the need for genetic transformation capability. The high-volume somatic embryogenesis system designed by CellFor and which is now available for conifers is described.

Clonal Forestry with Radiata Pine

M.J. Carson[1], S.D. Carson[1], J.D. Hayes[1], and C. Te Riini[1]
Carson Associates Ltd.[1], 34 Parawai Rd., Rotorua, New Zealand.

True clonal forestry with radiata pine, and with pines in general, is only just becoming an operational reality. Although cloning of pines has been practiced for many years, the high costs of propagation, coupled with problems associated with retaining juvenility during the clonal testing period, have inhibited progress in the application of full-scale clonal forestry. Recent large investments in both somatic embryogenesis (SE) and cryopreservation technologies have led to the development of reliable clonal storage systems, capable of holding clones in juvenile condition almost indefinitely. Also, SE either alone, or in combination with other forms of vegetative propagation, can now be used to reliably propagate and multiply selected, performance-tested clones from a full range of elite families, at a cost that is increasingly competitive with other, seed-based options.

The potential benefits of clonal forestry of pines have been recognized for many years, including: direct selection gains in yield, log and wood quality and health traits, indirect selection gains from optimal matching of clonal attributes to site and silviculture, and perceived gains from added uniformity of clones when grown in pure stands. However, the requirement to justify marginal investment in clonal forestry at an estate and stand level has necessitated a much greater emphasis both on researching the impacts of genetic change on regime profitability and wood value, and using the traditional tools of investment and risk analysis to determine and quantify the impacts of clonal gains. In general, these analyses have confirmed the feasibility of large-scale clonal forestry.

Clonal forestry poses some interesting challenges for radiata pine growers. Maintenance of genetic diversity against pests and diseases will trade off against the pursuit of selection gains in deployment decisions concerning numbers of clones, and whether to deploy them in sets or as pure stands. Models for predicting growth rate and quality will need to be validated and/or adjusted for clones, and for clonal uniformity, but there should be rewards in increased repeatability and predictability of clonal performance, particularly for high-heritability log and wood quality traits. In addition to delivering higher genetic gains from conventional breeding, clonal forestry will provide an ideal platform for applications of new biotechnologies, including marker-aided-selection and gene transfer. There is potential for clones to greatly enhance the profitability of pine plantations, with the cautionary note that this can only be achieved with excellent establishment and management practices, and well-defined breeding objectives.

Finding New Strategies for the Genetic Improvement of *Populus* Wood Quality

R. B. Hall[1], I. Peszlen[2], and M. Doungpet[2]

Plantation research with poplars has focused on improving biomass growth rates. Shifting attention to improved wood quality brings both opportunities and challenges for achieving joint improvement. Our group has begun taking on those challenges and is seeking to exploit the opportunities. In this talk, we focus on the first of three areas we are investigating:

1)The relationship between stem diameter growth rate and wood specific gravity. While reviews such as the one by Zobel and van Buijtenen (1989) suggest there is little relationship between these two traits in diffuse porous woods, our experience with *Populus deltoides* was suggesting otherwise. Many of our fastest growing selections had lower specific gravity. Other programs have also noted this negative correlation. For example, Brian Stanton (personal communication) evaluated the wood of 48 of Reini Stettler's improved poplar clones from ages 1 to 3 and 4 to 6. He found significant correlations of -0.2 to -0.3 respectively between diameter growth rate and specific gravity. We hypothesized that variation in vessel content and dimensions are involved in this negative correlation.

2) The joint impacts of insect and pathogen defoliation on tree growth and wood quality.

3) The competition response mediated by the phytochrome pigment system and its impact on growth and wood quality.

For discussion of areas 2) and 3), please see our earlier papers (Hall et al. 2001, Coyle et al. 2002, Bae et al. 2004).

MATERIALS AND METHODS

Stem disks were cut at breast height from 75 clones in an eight-year-old regional test plantation (Riemenscheider et al., 2001) and from 72 progeny from 21 families in a four-yr-old trial, both growing at Ames, IA. Specific gravity was determined by the water displacement method and correlations were computed with diameter at breast height. Wood samples from trees representing the range in variation in these two traits and their interaction are being analyzed in more detail. Microscopic slides are prepared and studied with an image analyzer system to determine the diameter and distribution of vessels, cell lumen area, and other parameters such as ray area and fiber length.

[1]Professor, Department of Natural Resources Ecology and Management, Iowa State University, Ames, IA, 50010 USA
[2]Associate Professor and Graduate Student, Dept. of Wood and Paper Science, North Carolina State University, Raleigh, NC, 27695, USA

RESULTS AND DISCUSSION

Specific gravity varied from about 0.29 to 0.43 g/cm^3 in both the clonal and progeny tests. In both tests, faster growing selections tended to have lower specific gravity (r = -0.21 in the clonal test and -0.37 on a family mean basis for the progeny test). However, individual selections with all combinations of the extremes have been observed. So far, our microscopic studies reveal that the typical pattern in *Populus deltoides* is for vessel diameter to be smaller and the number of vessels per unit stem cross-sectional area to be higher for the first growth ring. Furthermore, average vessel diameter by growth ring age is about the same for most selections. However, the typical low specific gravity tree has less of a decrease in its vessel numbers in older rings and a high vessel lumen area per unit stem cross-sectional area compared to trees with higher specific gravities. We believe that this enhanced amount of water conducting tissue is a common, indirect product of selecting for rapid growth. However, we have begun finding alternative growth strategies that allow this relationship to be broken to obtain fast growing, high specific gravity clones. For example, one of our faster growing selections has a specific gravity 0.40; it is similar in vessel properties to our other high specific gravity trees, having a reduced vessel density and fewer aggregate vessels in older growth rings. However, it is also remarkable for having more than 10% greater ray area per field than any of the other trees. It also has longer fiber lengths. The functional significance of these observations will require further study.

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Rapid Assessments of Wood Density in Progeny Trials

Fikret Isik[1] and Bailian Li[2]

[1]Research Assistant Professor, [2]Professor
Department of Forestry, North Carolina State University, Campus Box 8002, Raleigh, NC 27695-8002 USA

We assessed the use of Resistograph for quick assessment of wood density in two loblolly pine (*Pinus taeda* L.) genetic tests. In the first study, fourteen full-sib families produced by a six-parent half-diallel mating design were sampled. For each family, about 120 increment cores (12 mm) were collected to determine wood density. The same trees were drilled with the Resistograph, which measures the drilling resistance by amplitude reading. The readings had weak (0.29) to moderate (0.65) phenotypic correlations with wood density on an individual-tree basis over the four sites. The family-mean correlation between the two measurements, however, was much stronger (0.92). The additive genetic correlation between the two measures was also high (0.95). The efficiency of using the Resistograph as a means of indirect selection for improvement of wood density was 87% at the family level. Individual-tree breeding values of two traits yielded more accurate rankings than phenotypic values. The rankings of parents were identical for general combining abilities of density and amplitude.

In the second study, relationships between the Resistograph readings and wood density of 64 clones were studied. Two traits at the clone-means level had a correlation of 0.65. Correlation between clone genetic values was 0.82. When clones are ranked and classified into three density groups as high, medium and low, about 82 % of clones were correctly assigned into the low density group by Resistograph. None of clones in high density group was placed in low Resistograph group. Results from this study suggest that the Resistograph could be used efficiently to assess relative wood density for selection in tree improvement programs. A practical application of the tool would be to rank the genotypes and measure wood density of only the top group to save resources and time. The method is rapid, non-destructive and much cheaper than the traditional volumetric method.

Potential Value of Partially CAD-Deficient Loblolly Pine For The Pulp and Paper Industry

Qibin Yu[1], Ewellyn Capanema[2], C. Dana Nelson[3], Steven E. McKeand[1], Bailian Li[1], Hasan Jameel[2], Hou-min Chang[2], and T.J. "Tim" Mullin[1],

[1] Department of Forestry, NC State University, CB 8002, Raleigh, NC 27695-8002

[2] Department of Wood and Paper Science, NC State University, CB 8002, Raleigh, NC 27695-8005

[3] Southern Institute of Forest Genetics, USDA – Forest Service, 23332 MS Hwy 67, Saucier, MS 39574-9344

The southern region of the United States produces 58% of the nation's timber supply, much of it grown in intensively managed plantations of genetically improved loblolly pine (*Pinus taeda* L.). One of the most valuable loblolly pine selections made by the NCSU-Industry Cooperative Tree Improvement Program is also the only known natural carrier of a rare null mutation, *cad-n1*. The *cad-n1* allele causes a deficiency of an enzyme catalyzing a final step in the biosynthesis of lignin precursors, cinnamyl alcohol dehydrogenase (CAD). While large areas of partially CAD-deficient pine have been deployed in the Southern US, the impact of *cad-n1* has never been well characterized across environments or genetic backgrounds.

The effect of the CAD null mutation on pulping, wood density, lignin structure and growth was studied in a open-pollinated family segregating for *cad-n1*, and growing at a 10-year-old genotype-by-nutrition field trial in Scotland County, North Carolina. We found that the *cad*-nl allele was associated with a significant effect on wood density. The partially CAD-deficient *cad-nl* heterozygotes had significantly higher wood density (+2.6%) compared to the CAD-normal trees. The higher density was apparently due to the higher percentage of latewood in the heterozygotes. No CAD genotype x treatment interactions were found for any of the traits studied.

Analysis of the samples by Near Infra-Red (NIR) allowed rapid estimation of lignin and ? ?ellulose contents in each annual ring. Selected samples were also subjected to detailed structural analysis. A microtechnique was used to isolate lignin and analyze with correlation 2D NMR and quantitative ^{13}C NMR of non-acetylated and acetylated lignin followed by wet chemistry methods using only 200 mg of wood, the amount that can be obtained from each annual ring in an incremental core. In a preliminary study, ^{13}C NMR studies showed that the structure of the kraft lignin isolated from CAD-deficient pine pulp is rather similar to that of the CAD-normal control. However, increased amounts of COOH and phenolic OH were observed in the CAD-deficient lignin.

For pulping, as the H-factor (energy) was increased, the kappa (lignin content) difference decreased. The rates of pulping appeared to be similar between the partially CAD-deficient and normal trees, however, there were some indications that the *cad-nl* heterozygotes had higher pulping yield and were easier to pulp at lower H-factors then CAD-normal trees. The study indicates that the *cad-nl* allele could be a particular valuable gene to the pulp and paper industry by increasing wood density and decreasing the energy required for pulping.

Prediction of Wood Properties of Loblolly Pine Using Transmittance NIR Spectroscopy

Robert Sykes[1], Bailian Li[1], Gary Hodge[1], Barry Goldfarb[1], John Kadla[2], and H-m. Chang[2]

[1] Department of Forestry, Box 8002, and [2] Department of Wood and Paper Sciences, North Carolina State University, College of Natural Resources, Raleigh, NC 27695

Near infrared (NIR) spectroscopy is evaluated as a rapid non-destructive technique for characterization of wood chemical and physical properties in loblolly pine in this study. The transmittance measurements of NIR spectra from thin wood wafers cut from increment cores were used to develop calibration models for the rapid estimation of a-cellulose yield (ACY), fiber length (FLW), coarseness (COA), and lignin content (LIG) measured in the laboratory. Eleven-year-old trees from two sites were sampled using 12 mm increment cores. Early and late wood of ring 3 and 8 from these samples were analyzed in the lab using standard wet chemistry methods for ACY, FLW, COA, and LIG. Calibrations of NIR and lab measurements based on one site were generally reliable with coefficients of determination (R^2) ranging from 0.54 to 0.88 for FLW and ACY, respectively. Predicting ring 8 spectra using ring 3 calibration equations may be possible for ACY and COA with R^2 values of approximately 0.60. Predicting the wood properties between two sites may be possible with moderate success for ACY (R^2 = 0.60) and COA (R^2 = 0.68), but predictions for FLW were poor. LIG content proved difficult to predict using transmittance NIR, which was partially due to imprecision and low variation of laboratory data.

Estimation of Microfibril Angle in *Pinus taeda* L. by Near Infrared Spectroscopy

P.D. Jones[1], **L.R. Schimleck[2],** R.F. Daniels[3],G.F. Peter[4] and A. Clark III[5]
[1]Graduate student, [2]Assistant Professor, [3]Professor
Warnell School of Forest Resources, The University of Georgia, Athens, GA, USA
[4]Associate Professor
School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA.
[5]Wood Scientist
USDA Forest Service, Southern Research Station, Athens, GA, USA.

Microfibril angle (MFA) is defined as the angle that the helical windings of cellulose chains, within the fibre wall, make with the fibre axis. MFA is important in determining the mechanical properties of individual fibres and in combination with density largely determines the strength properties of solid wood. Several methods exist for the measurement of MFA but it was not until the development of SilviScan-2 that it was possible to measure MFA on a large scale.

Near infrared (NIR) spectroscopy can be used to estimate MFA but studies published in the literature have been based on sample sets of limited variation. If NIR is to be used operationally for estimating MFA then calibrations based on samples from a wide variety of sites must be developed. We have investigated the development of MFA calibrations based on 729 NIR spectra collected in 10 mm sections from the radial longitudinal face of 89 wooden strips. The strips were cut from *Pinus taeda* L. (loblolly pine) breast height increment cores that were obtained from trees growing in plantations located in Georgia, USA. For each of the three physiographic regions in Georgia (Lower and Upper Atlantic Costal Plain, and Piedmont) five plantations, ranging in age from 21 to 26 years, with a range of site indices were sampled. When applied to a separate set of 30 breast height increment cores (5 cores per plantation, 2 plantations per region) the MFA calibrations generally predicted the MFA of individual cores very well but results varied depending on the math treatment applied to the spectra. It was found that the predictive performance of the calibrations could be improved by adding a single randomly selected core from each site in the prediction set to the calibration set. For studies based on increment cores the resolution at which NIR measurements can be made is important and we will also discuss the development of an automated sampling stage which increases the resolution of the NIR measurements.

The Role Of Genetic Markers In Tree Improvement: An Industrial Perspective

M.W. Cunningham, J.L. Knoth, R.C. Purnell and J.Y. Yan

International Paper Company, P.O. Box 1391, Savannah, GA 31402

Genetic markers offer a number of opportunities for pine tree breeders to accelerate the achievement of genetic gain through early selection or to improve the efficiency of selecting parents for tree breeding. International Paper has had an in-house genetic marker program for nearly 10 years. We have developed SSR markers for a number of traits including growth, stem straightness, specific gravity and lignin content. In this talk we will discuss how genetic markers are being used or could be used for early selection in our clonal forestry programs, marker directed breeding and in the more basic activities of genetic finger printing for paternity analysis or genotype identification. While SSR markers serve as useful tools in many aspects of an operational tree improvement program there are also several limitations to their utility. We will discuss these limitations and alternative genomic technologies which may overcome the obstacles.

MICROARRAYS BEYOND GENE EXPRESSION: DISCOVERY OF CANDIDATE GENES FOR QUANTITATIVE TRAITS TO LARGE SCALE SNP GENOTYPING

Matias Kirst

Cornell University, US

We have demonstrated that transcript levels, estimated by cDNA microarray analysis of an *Eucalyptus* mapping population, can be analyzed as normal quantitative traits, identifying gene expression networks and expression QTLs (eQTL). eQTLs that co-localized with wood quality and growth QTLs identified candidate genes that may regulate quantitative variation for those traits. The limitation of this approach is that inferences are exclusive to the parental genotypes. An alternative strategy is to combine transcript levels and quantitative phenotypes measured in large sets of unrelated genotypes that encompass a broad range of the genetic and phenotypic variation of a target population. We are testing this method for identification of aluminum (Al) tolerance genes in maize. Transcript profiles of Al treated and control plants are being generated for a population of 27 inbred lines that represent most of the genetic variation in the species, using long oligonucleotide microarrays containing 60,000 features. This approach may more readily identify valuable genes to be tested by traditional association genetic tests, particularly for traits where poor physiological and biochemical information limits the selection of candidate genes. Another application of microarrays that we are pursuing is in genotyping single nucleotide polymorphisms. Random primed complementary DNA strands are being synthesized, incorporating fluorescently labeled nucleotides, and hybridized to short oligonucleotide probes. Preliminary evaluation of a set of maize inbred lines genotyped with DNA chips containing short oligonucleotides (25-mer) was able to detect and call the correct genotype for approximately 50 SNP loci in a set of ten genes. Preliminary results, and advantages and limitations of these strategies will be discussed with regards to woody species with large-genomes.

Functional Genomics of Wood Formation In *Acacia Mangium* Willd. X *Acacia Auriculiformis* Cunn. Ex Benth. Hybrid: Analysis of A Set of Expressed Sequence Tags From Inner Bark

Wickneswari Ratnam.[1], Choong CheeYen[1], Cheong Peck Lei[1], Pang Shek Ling[1], Jennifer Ann Harikrishna[2], Robert C. Elliott[3] and Renë E. Vaillancourt[3,4]

[1]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia; [2] Department of Biotechnology, Malaysian University of Science and Technology, GL33, Ground Floor, Block C, Dataran Usahawan Kelana, 17, Jalan SS7/26, Kelana Jaya, 47301 Petaling Jaya, Selangor, Malaysia; [3]School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia; [4]Cooperative Research Centre for Sustainable Production Forestry, University of Tasmania, Hobart, Tasmania 7001, Australia.

Functional genomics greatly increases the understanding of wood formation for improvement of wood quality for two main end uses i.e. sawn timber and wood pulp Lignin is one of the important components of wood but, is an undesiarable component in the conversion of wood into pulp and paper. Inner bark tissues are a rich source of genes involved in lignin biosynthesis. A cDNA library of 7000 clones was constructed using pooled RNA from different stages of inner bark development along a developing branch. From this library, 4032 cDNAs were single pass sequenced from 5' end, generating 3246 high quality ESTs which represented 2298 assembled sequences or unique transcripts with an average size of 498 bp. In the analysis, 982 ESTs (30%) were found to significantly match (E-value = 10^{-10}) proteins with known function in the public databases using BLASTX. In addition, 219 ESTs (9%) significantly matched proteins with unknown function and 337 ESTs (13%) matched to proteins described as putative. 993 ESTs (31%) failed to match with significance to any protein sequence found in the public databases. The remaining 713 ESTs yielded no hits and are likely to represent genes specific to *Acacia*. 18 ESTs (0.4%) matched to six enzymes involved in lignin biosynthesis.

Lignin Modification in Silver Birch – Characterisation of Transgenic Lines Containing *PtCOMT*- or as*Pt4CL1* and Their Ecological Interactions

T. Aronen[1], H. Tiimonen[1], T. Ylioja[1,2], K. Niemi[3], C.-J. Tsai[4], T. Laakso[2], P. Saranpää[2], H. Roininen[2], V. Chiang[5], and H. Häggman[6]

Silver birch (*Betula pendula*) is economically the most important deciduous tree species in Nordic countries. In Finland, approximately 15 % of growing stock (311 mill. m^3) is birch, and the birch roundwood is raw material in the chemical pulp industry. Silver birch is the main broad-leaf species of conventional tree breeding, and it is also one of the key species in Nordic forest ecosystems.

Lignin is a phenolic heteropolymer that is deposited together with polysaccharides in plant cell walls, and has the strengthening and water transportation function. Biosynthesis and properties of wood lignin have been studied extensively, and during the last decade studies on GM-trees have gained more information on the lignin biosynthetic pathway. The lignin-modified wood may turn out to be a potential raw material for the pulp industry, if the modified lignin properties can be related to improved delignification (pulping) characteristics. Wood lignin modifications have, however, evoked concern about the potential effects on tree fitness and also of potential unintended side-effects and pleiotrophic effects of the transgenes having e.g. ecological impacts.

The aim of the present work was to study the lignin biosynthesis in silver birch (*Betula pendula* Roth) focusing on the role of caffeate/5-hydroxyferulate *O*-methyltransferase (COMT) and 4-coumarate:coenzyme A ligase (4CL). Furthermore, the potential effects of lignin modification on the ecological interactions of silver birch are studied in transgenic birch lines under *in vitro* conditions.

MATERIAL AND METHODS

The *PtCOMT* gene in sense-orientation driven either by CaMV 35S promoter or sunflower ubiquitin promoter and the *Pt4CL1* gene in antisense orientation driven by 35S promoter, both the genes originating in aspen (*Populus tremuloides* L.) were introduced into silver birch by particle bombardment. Two 35S-PtCOMT lines and two UbB1-PtCOMT lines were regenerated and grown for two years in greenhouse together with the non-modified control plants, and their lignin characteristics were examined. A similar two-year greenhouse experiment was performed with four regenerated 35S-asPt4CL1 lines.

[1]Finnish Forest Research Institute, Punkaharju Research Station, FIN-58450 Punkaharju, Finland
[2]Finnish Forest Research Institute, Vantaa Research Centre, FIN-01301 Vantaa, Finland
[3]Dept. of Applied Biology, P.O. Box 27, FIN-00014 University of Helsinki, Finland
[4]Plant Biotechnology Research Centre, MTU, Houghton, MI49931, USA
[5]Dept. of Forestry, NCSU, Raleigh, NC 27695-7247, USA
[6]Dept. of Biology, University of Oulu, PO Box 3000, FIN-90014 Oulu, Finland

To test potential ecological effects of lignin modification the interactions of the COMT-modified birches with insect herbivores, i.e larvae of geometrids *Aethalura punctulata*, *Cleora cinctaria* and *Trichopteryx carpinata* as well as the adults of birch leaf-feeding beetles *Agelastica alni* and *Phyllobius* spp. were studied by controlled feeding experiments including tests for both feeding preference and growth performance. In addition, the ability of the COMT-modified birch lines to form symbiosis with an ectomycorrhizal fungus *Paxillus involutus* was studied under *in vitro* conditions.

RESULTS AND DISCUSSION

Characterisation of the birch lines carrying *PtCOMT*

Southern blot analysis confirmed the presence of *PtCOMT* and *nptII* transgenes in the regenerated four birch lines with the copy number of five in all of them. The gene expression studies by Northern blot analysis established the functioning of the *PtCOMT* gene in leaves, phloem, and developing xylem when driven either by the 35S or UbB1 promoter. In the UbB1-PtCOMT lines, however, the *PtCOMT* mRNAs seem to be slightly bigger in size than in the 35S-PtCOMT lines. Stem height growth and stem diameter measurements showed that the majority of the transgenic lines grew like the controls and were also morphologically similar to the control plants. The only exception was the UbB1-PtCOMT line 130 that showed dwarf genotype in the first growing season and belated growth rhythm also later on.

Characterisation of the birch lines transformed with *PtCOMT* revealed no considerable alterations in the Klason lignin content of the stem wood, leaves, or roots in transgenic lines. The thioacidolysis showed changes in the monomeric composition of lignin, the syringyl/guaiacyl (S/G) ratio in the two 35S-PtCOMT lines being remarkably reduced when compared to controls. In addition, abnormal 5-OH G units were discovered in the lignin. Apparently, the expression of the *PtCOMT* in birch resulted in suppression of the COMT activity and in the reduction of the S/G ratio, most probably due to high degree of homology between the sense transgene and endogenous gene.

Characterisation of the birch lines carrying *asPt4CL1*

Southern blot analysis verified the integration of the *asPt4CL1* into the genome of the regenerated birch lines, transgene copy number varying from one to five in different lines. Northern blot analysis for examining the expression of both transgene and endogenous gene are currently under way. The height growth of the transgenic lines differed from that of the controls in two-year greenhouse experiment. In the first growing season, some transgenic lines grew less than the control, but in the second growing season the majority of the transgenic lines grew significantly better than the control clones. There were no differences in the morphology and growth rhythm among the lines.

Lignin analysis of the two-year-old 35S-asPt4CL1 birch lines revealed no changes in the Klason lignin content. When total lignin content was examined, a clonal difference could be seen, as well as a slight increase in transgenic lines compared to controls. Determination of lignin quality, i.e. S/G ratio, is still in progress.

Ecological interactions of the COMT-modified birches

In the controlled feeding experiments, the feeding preferences of the tested insect herbivores differed in a few cases among the tested birch lines, but these differences could not be directly associated to lignin composition i.e. the reduced S/G ratio. The found differences between lines could more likely be explained by other than lignin related leaf characteristics, either natural or induced as transgene site effects. The growth performances of lepidopteran larvae *Aethalura punctulata*, *Cleora cinctaria* and *Trichopteryx carpinata* on transgenic leaves did not differ significantly from the ones on control leaves, but there was an indication that the leaf material with the reduced S/G ratio could in some species be less supportive for larval growth.

The ability of the COMT-modified birch lines to form symbiosis with an ectomycorrhizal fungus *Paxillus involutus* was studied under *in vitro* conditions. The outcome of these experiments showed that all the tested birch lines, both transgenic and control ones can form mycorrhiza when grown as rooted plantlets on tissue culture medium. More detailed analysis of the data are in progress.

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Improving Wood Quality in the Western Gulf Forest Tree Improvement Program: the Problem of Multiple Breeding Objectives

T.D. Byram[1], J.H. Myszewski[2], D.P. Gwaze[3], and W.J. Lowe[4]

Abstract: Both industry and private landowners in the Western Gulf Forest Tree Improvement Program (WGFTIP) area are currently compensated for their timber primarily by weight or volume. Despite the immediate economic focus that this places on adaptability and volume growth, most WGFTIP members also consider wood quality an important selection criterion. Wood quality, as evaluated by stem straightness, wood specific gravity, and microfibril angle, has either neutral or slightly negative genetic correlations with volume growth in loblolly pine (*Pinus taeda* L.) progeny tests evaluated by the WGFTIP. Selection for volume growth alone can thus be expected to result in decreased wood quality over time. Therefore, some form of restricted selection index will be necessary to simultaneously improve both growth rates and wood quality. As a result, the WGFTIP must define the best possible set of selection criteria given that 1) no single set of breeding objectives can be considered optimal for multiple products and 2) improvement in wood quality has no readily recognized economic importance in the existing market. To meet this challenge, the WGFTIP has implemented different strategies for the mainline breeding populations and the various deployment populations tailored to meet the needs of specific members. An elite wood quality population (WQEPop) based on backwards selection of parents is also under development. The WQEPop will include a within-family selection population that will be produced as either seedlings or clonal lines. Clonal lines will be primarily intended to support the evaluation of the breeding population by providing multiple observations on each genotype.

Keywords: Breeding strategy, wood quality, microfibril angle, specific gravity, clonal testing.

INTRODUCTION

The Western Gulf Forest Tree Improvement Program (WGFTIP) consists of five state forestry agencies and nine industrial members operating in the Western Gulf Region of the USA. Collectively, these programs are responsible for planting approximately 300 million trees per

[1] Director, Western Gulf Forest Tree Improvement Program, Texas Forest Service and Assistant Professor, Forest Science Dept., TAMU. College Station, TX, USA.
[2] Research Geneticist, USDA Forest Service, Saucier, MS, USA.
[3] Resource Scientist, Missouri Department of Conservation, Columbia, MO, USA.
[4] Former Director, Western Gulf Forest Tree Improvement Program, Texas Forest Service (Retired) and currently Senior Associate with Zobel Forestry Associates.

year on both industrial and private forestland (roughly 180,000 ha). Forest industries within the region operate multiple manufacturing facilities producing a range of both solid wood and pulp/paper products. To do this, they depend heavily on gate wood, rarely growing more than 50 percent of their own raw material. In fact, many large wood users within the region maintain no forestland of their own and rely entirely on external supplies. Several categories of property owners, representing the majority of commercial quality forestland in the region, own no mills and grow timber for the open market. As a result of these factors, timber is traded as a commodity. Assuming that it meets minimum standards for straightness and size, both industry and private landowners are currently compensated for harvested timber solely according to the weight or volume of green wood. Therefore, the immediate economic focus for the breeding program is on improved adaptability and volume growth. Despite these facts, WGFTIP members also consider wood quality an important selection criterion. Thus, the WGFTIP must define the best possible set of selection criteria given that 1) no single set of breeding objectives can be considered optimal for multiple products and 2) improvement in wood quality has no readily recognized economic importance in the existing market.

DISCUSSION

Structure of the Western Gulf Forest Tree Improvement Mainline Breeding Population

The WGFTIP operates in the five states of Texas, Louisiana, Mississippi, Arkansas and Oklahoma. This region encompasses environments that range from the maritime gulf coastal plains to the more continental uplands that have traditionally been mixed pine-hardwood forests. Soils range from poorly drained clay-dominated flatwoods, through sandy and silt loams, to rock/gravel dominated upland soils. The region includes four of the USDA plant hardiness zones on the north-south transect with annual average minimum temperatures varying from –18° C to a –7° C (USDA 1990). Average rainfall during the March to August growing season varies from a low of 457 mm in the north and west to a high of 965 mm in the south and east (USDA 1969). Because of these diverse climatic conditions, the USDA Forest Service has divided this region into three east-west deployment zones (Schmidtling 2001). This is in contrast to the eastern population of loblolly pine that consists of a single such zone (the WGFTIP area overlaps the eastern loblolly pine population in Mississippi). In both the eastern and western populations, Schmidtling (2001) recommends that seedlings be moved no further north than to sites experiencing 5.6° C lower minimum winter temperatures (generally 240-300 km).

WGFTIP members are attempting to improve growth rates of loblolly pine within its natural range and to extend its utility both to the north where its introduction has been limited by cold and drought and to the west where rainfall totals and distribution during the year are the limiting factors. In response to both environmental variables and the political and organizational boundaries of its members, the WGFTIP has divided its breeding population into eight seed zones with overlapping deployment and procurement recommendations (Byram et al 1988, p. 16-17). This has resulted in effectively dividing the population into four breeding zones within

which cooperative members are collaborating to progeny test advanced-generation selections (Figure 1).

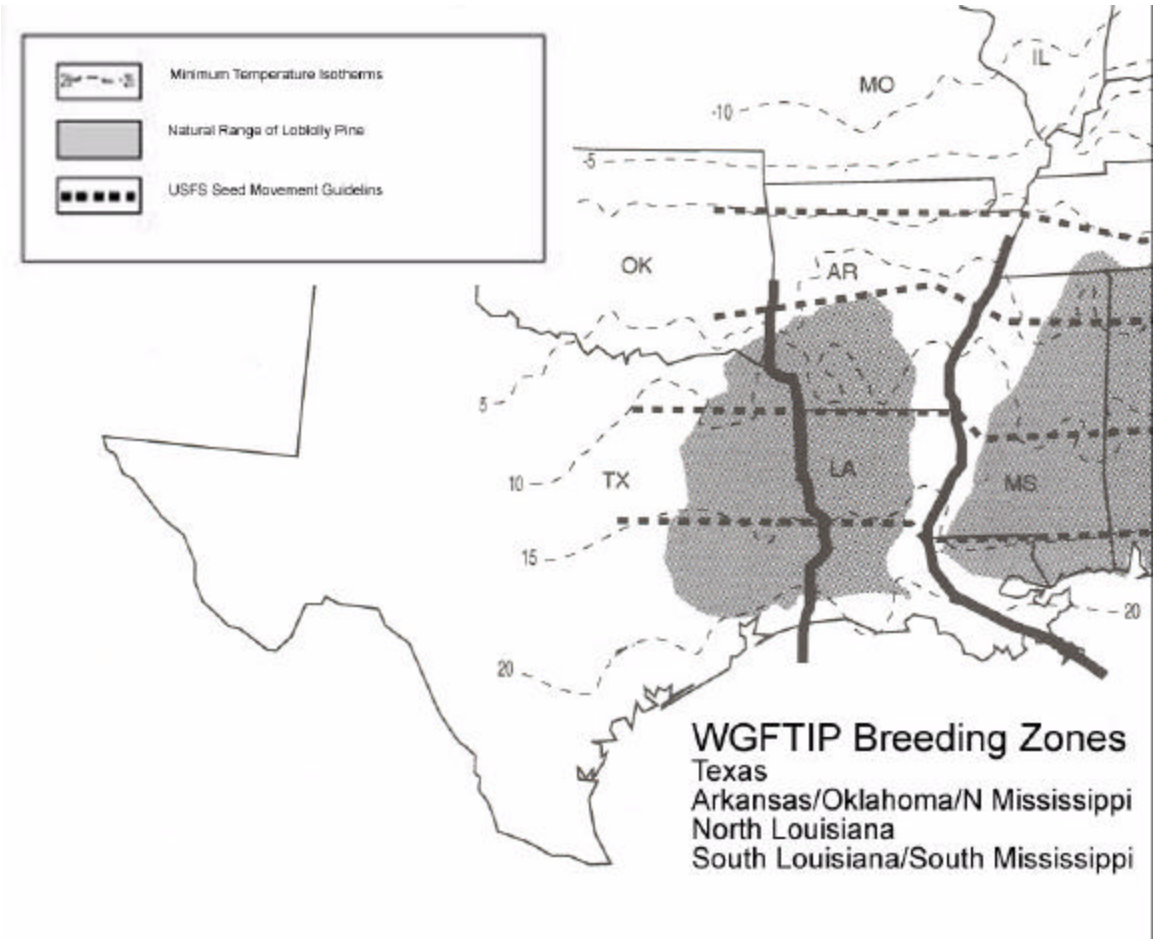

Figure 1. The natural range of loblolly pine, minimum temperature isotherms and USDA seed movement guidelines from Schmidtling (2001). WGFTIP breeding zones in which members are collaboratively testing advanced-generation selections are noted.

Within each breeding zone, the population is further subdivided into breeding groups of 18 to 25 individuals. The number of breeding groups within breeding zones varies from 13 to 44 for a total of 113 loblolly pine breeding groups. Advanced-generation breeding relies on a complementary mating system that uses distinct evaluation and selection populations. The evaluation population is comprised of seedlings produced with a zone-appropriate polymix pollen selected from average parents and is used to rank parents based on their general combining ability. Selections are then made from block plots of pedigreed crosses restricted to crosses among members of the same breeding group (Lowe and van Buijtenen 1986).

Breeding objectives and selection criteria for the mainline population are consistent across all members of the cooperative and are focused on producing an all-purpose tree with emphasis on improved value for the average landowner. Because of the immediate economic focus is on green weight at the mill gate, this means that the primary emphasis is on volume growth with lesser emphasis on quality. Primary selection criteria include traits related to vigor (height and

diameter growth) and adaptability (survival and disease resistance). Wood quality receives secondary emphasis with selection to improve stem straightness and an attempt to hold specific gravity near regional averages. Measurement schedules call for evaluation of survival, height, diameter, disease incidence, stem straightness, forking, and specific gravity at age 5. Growth rates are reevaluated at age 10 with a sub-sample of tests measured again at ages 15 and 20. The current population consists of 3,223 progeny-tested first-generation parents and a total of 1,554 second-generation parents, 247of which have been evaluated in polymix tests that are five years of age or older.

Genetic Variation in Wood Quality

Wood quality in loblolly pine, as evaluated by stem straightness, wood specific gravity, and microfibril angle, has either neutral or slightly negative genetic correlations with volume growth in the loblolly pine progeny tests evaluated by WGFTIP. Stem straightness is evaluated in all progeny tests within a planting series at age 5 while specific gravity is evaluated on all parents, however, because of its strong inheritance and lack of genotype by environment interaction it is only assessed in a single progeny test within each series. The correlation between the selection criteria for volume and specific gravity is slightly negative over all first-generation parents evaluated by the cooperative. Correlations between volume and form and between form and specific gravity are negligible (Table 1).

Table 1. Pearson rank correlations among parental breeding values for volume growth, specific gravity, and bole straightness score. (from Byram et al. 1998)

	Specific Gravity	Bole Straightness
Growth		
Correlation	-0.13	0.01
Prob >|R|	0.0001	0.75
Number of Obs.	878	781
Specific Gravity		
Correlation		0.02
Prob > |R|		0.66
Number of Obs.		781

Microfibril angle (MFA), the angle at which the cellulose bundles within the secondary cell wall deviate from the cell axis, is also very important in determining the quality of wood and fiber products. High MFA are associated with lower strength characteristics (Ifju and Kennedy 1962, Cave and Walker 1994, Evans and Ilic 2001) and increased longitudinal shrinkage of lumber (Megraw et al. 1998). Ninety-three percent of the variation in modulus of elasticity (MOE) of loblolly pine is accounted for by the combined variation of specific gravity and MFA (Megraw et al. 1999). MFA may also have an impact on paper as core wood pulp produces paper with increased stretch and distortion (Watson and Dadswell 1964).

MFA exhibits a complex pattern of variation from the base to the crown, the pith to the bark, and from the earlywood to latewood within each ring (Megraw et al 1999). Core samples taken from

two mature control-pollinated loblolly pine progeny tests grown in South Arkansas revealed heritable differences in MFA in both the core and outer wood and within early and latewood samples from the same growth rings (Myszewski 2003, and Myszewski *et al* In press). Individual-tree heritabilities for MFA were moderate ranging from 0.17 ± 0.13 to 0.51 ± 0.21 depending on the progeny test and the specific location of the wood sample used for MFA determination. This study was based on a relatively small number of trees (approximately 20 trees per each of 17 control-pollinated crosses in one test and 25 trees per 12 control-pollinated crosses in the second test for a total of 632 samples) so large standard errors were associated with the estimates of most genetic parameters. Within this limited sample, however, genetic correlations between corewood MFA and outer wood MFA were generally positive, indicating that mature MFA could be improved by indirectly selecting for more desirable MFA at juvenile ages. Genetic correlations between MFA and specific gravity were generally nonsignificant because of the large standard errors, but tended to be positive at one location and negative at the other. Genetic correlations between MFA and volume growth were also nonsignificant, but were almost entirely positive indicating that improvements in growth may well result in an undesirable increase in MFA (Table 2). This may well be the case as previous studies have shown that as diameter growth increases, tracheid length decreases (Megraw 1985, p. 38) and as tracheid length decreases, MFA increases (Megraw 1985, p. 49).

Table 2. Estimates of genetic correlation (standard errors) between early- and latewood microfibril angle and volume growth at ages 4 and 20 for two progeny tests. (taken from Myszewski *et al*. In press)

	EW 4[1]	LW 4	EW 20	LW 20
GP065	0.15	0.60		-0.43
	(3.06)	(1.40)		(2.78)
GP258	0.68	0.45	0.42	0.81
	(3.34)	(2.81)	(3.36)	(2.83)

[1] EW = earlywood, LW= latewood, 4 = fourth ring from the core, 20 = 20th ring from the core

Given the likely trends in the inheritance patterns of volume growth and wood quality, selection for volume growth alone can be expected to result in decreased wood quality. To meet this challenge the WGFTIP is taking two steps. First, it is using the large number of selections available in the mainline breeding population to tailor the design of the deployment population to meet the objectives of individual members. Secondly, the WGFTIP is developing an elite wood quality population.

Incorporation of Wood Quality in the Mainline Breeding Program and Deployment Populations

While breeding objectives and selection criteria for the mainline population are consistent across all members of the cooperative, the decision was made to maintain a larger base population than that needed for a program focused on a single trait. The number of progeny tested first-generation selections evaluated in balanced control-pollinated progeny tests currently available for inclusion in a deployment population (the total from within the breeding zone plus selections from all the appropriate procurement zones) varies from 902 to 1853 per zone. Census numbers

of second-generation selections will be approximately 75% of this total. This gives the WGFTIP considerable flexibility and allows additional traits to be incorporated into selection schemes should overall breeding objectives change.

Deployment populations are managed independently by each of the WGFTIP members and can be tailored to meet the unique needs of each cooperator. WGFTIP members use advancing-front orchards to expand and replace their seed orchard populations. On average, members are grafting a new block of orchard every five years while phasing out the oldest, most genetically obsolete block of orchard in their program. With an expected economic lifespan of 20 years, this results in each orchard complex being made up of four or five orchard blocks of different ages and genetic quality. This allows gain from the breeding population to be quickly captured once it is identified in the breeding population. One chief advantage of this strategy is that it allows each WGFTIP member the opportunity to review their organization's objectives and to adjust the selection criteria for their deployment population on a five-year cycle.

To illustrate the impact that these decisions can have, consider a twenty-clone orchard appropriate for Arkansas. If the clones for this orchard are selected for volume alone, they have an expected breeding value of 37.8 percent for growth expressed as percent improvement in mean annual increment at age 20 compared to an unimproved source, a slight improvement in stem straightness, and lower specific gravity than the unimproved checklot. Selection of a twenty-clone orchard for the same deployment area using the pulp index method derived by Lowe *et al* (1999) which gives specific gravity an economic weight seven times larger than volume growth would result in an expected breeding value of 14.9 for growth, a similar score for stem straightness and an improvement of 0.03 for specific gravity compared to the unimproved checklot (Table 3). Actual orchard design is frequently a compromise between these two extremes.

Table 3. Average gains for two 20-clone orchards designed for Arkansas compared to an unimproved checklot.

Selection criteria	Volume[1] (% MAI)	Pulp Index[2] ($/ton of kraft pulp)	Specific Gravity[3]	Straightness[4] (standard dev.)
Volume	37.8	0.13	-0.019	0.45
Pulp Index	14.9	12.90	0.030	0.52

[1] Volume is expressed as breeding value for change in mean annual increment projected at age 20 compared to unimproved material.
[2] Pulp Index is after Lowe *et al*. (1999) and is expressed in dollars saved per ton of pulp produced.
[3] Specific gravity is change in absolute units compared to an unimproved seed source.
[4] Straightness is change in a standardized score where the mean of improved material = 0.0 and positive scores indicate better stem straightness.

Development of a Wood Quality Elite Population

In addition to the mainline breeding population, the WGFTIP is developing a wood quality elite population (WQEPop). This population will ultimately be comprised of 30 backwards selections from each of four breeding zones identified by the pulp index developed by Lowe *et al* (1999). This index, derived after Borralho *et al* (1993), expresses improvements in volume and specific gravity in terms of dollars saved per ton of pulp produced and is therefore specific for each set of production assumptions. As implemented by the WGFTIP, specific gravity receives an economic weight seven times larger than volume growth. MFA is currently not evaluated within the breeding population despite the fact that it appears to be genetically controlled and has obvious importance to wood quality because it is difficult and expensive to assess. The development of a wood quality elite program with a more limited population size makes it more feasible that this trait will be added in the future. Values for the currently selected WQEPop are given in Table 4. Most of the WQEPop selections are not currently represented in seed orchards because they do not have sufficient gain in volume production.

Table 4. Current composition of the parents in the Wood Quality Elite Population and their average performance.

Zone	Selections (No.)	Volume[1] (% MAI)	Pulp Index[2] ($/ton of pulp)	Specific Gravity[3]
Texas	18	19.8	16.33	0.030
Arkansas	11	19.5	13.69	0.023
N. Louisiana	5	25.4	13.57	0.018
S. Mississippi	4	22.8	17.40	0.034

[1] Volume is expressed as breeding value for change in mean annual increment projected at age 20 compared to unimproved material.
[2] Pulp Index is after Lowe *et al*. 1999 and is expressed in dollars saved per ton of pulp produced.
[3] Specific gravity is change in absolute units compared to an unimproved seed source.

The WQEPop selections will be bred together in small groups comprised of five parents nested within breeding zones to form a selection population from which the next cycle will be reconstituted. By combining selections from the appropriate procurement zones, it should always be possible to select the minimum number of unrelated individuals needed for seed orchard establishment or to produce outcrossed families for vegetative propagation. The first block plots that will form the selection population for the WQEPop were planted in 2004. The WQEPop will not be a closed population as infusions can be made from the mainline population. The entire breeding population, regardless of breeding group or selection cycle is screened annually for additional candidates. Selections from the WQEPop, however, will not be fed back into the mainline breeding program if they cross breeding group lines, which will most likely be the case.

The original breeding plan anticipated that the selection population would be created by crossing progeny-tested parents with known breeding values for volume growth and wood specific gravity. Seedlings from these crosses would then be planted in block plots of 100 trees from which the next cycle of selections would be identified. It is anticipated that within-family

selection will be somewhat inefficient because we are attempting to simultaneously improve a low heritability trait (volume production) and wood quality traits (specific gravity) that may have negligible genetic correlations with volume. To insure that these trees actually have the desired characteristics, it will be necessary progeny test these forward selections before they are used in seed orchards or for further breeding (Figure 2). Essentially, the polymix tests are used to inform the within-family selection decision.

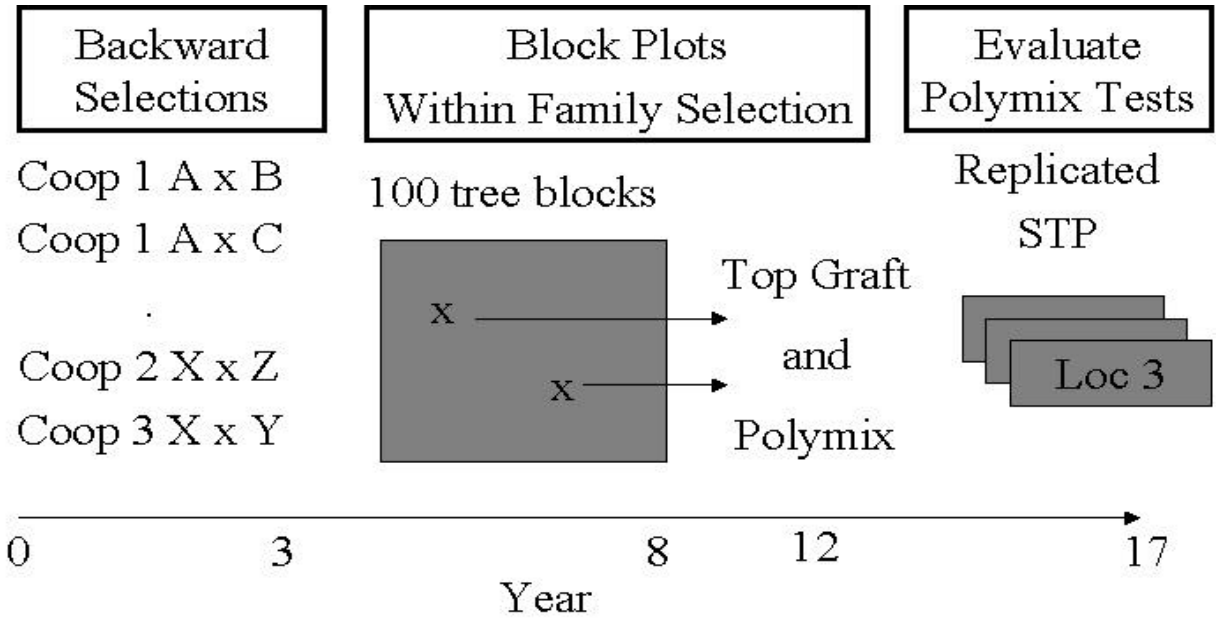

Figure 2. Timeline showing implementation of the elite breeding program consisting of crosses among progeny tested parents, block plots for selection at age 5, and polymix testing for estimation of breeding values with replicated single-tree-plot (STP) progeny tests.

Clonal Testing in the Selection Population.

Recent developments in the vegetative propagation of loblolly pine offer an alternative to selection within seedling families. It is now possible to use rooted cuttings and somatic embryos to produce clonal replicates of seedlings within full-sib families and to clonally test these lines (Isik et al 2004). Clonal testing the selection population has a number of advantages. Multiple observations on each genotype improve the estimate of phenotypes and greatly improves within-family heritability making it possible to more accurately identify individuals that combine desired traits. This is especially true when traits are poorly expressed or negatively correlated. Better within-family selection leads directly to a higher probability of truly assortative matings and improved gain per cycle. Because the mid-parent values for the crosses are known in advance, the sole objective of clonal testing in the WQEPop selection population would be to improve within-family selection (Figure 3). Therefore, the program need not commit to a single forward selection strategy. The WGFTIP will use seedling selection plots for crosses that are recalcitrant to vegetative propagation and clonal tests for identifying lines in those families that

are easily propagated by this method. Furthermore, as selection efforts are nested within full-sib families, these tests can contain vegetative propagules produced by any number of different methods as long as all of the lines within a full-sib cross were produced by the same procedure. Of course, breeding values can only be estimated by making crosses and going through the intermediate step of progeny testing. Previous research, however, has shown that a large part of the genetic variation for volume production in this population is additive (Byram and Lowe 1986). Clonal testing further offers the option of completing multiple generations of breeding and selection before reevaluating the selections for breeding value. This approach is only possible because complementary breeding systems separate the estimation of breeding values from the creation of the selection population.

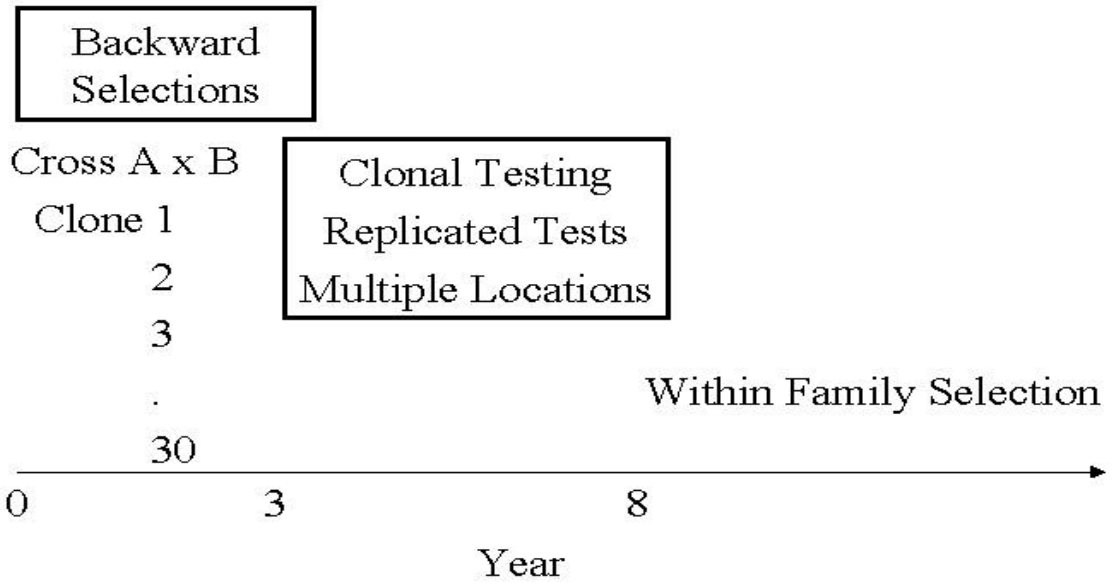

Figure 3. Timeline showing implementation of clonal testing for within family selection.

CONCLUSIONS

With rare exception, the tree improvement community has taken the lead in balancing the need to improve growth while maintaining or improving wood quality in loblolly pine. The tree improvement community has been forced to make these choices because the forest industry produces a range of solid wood and pulp/paper products, depends heavily on gate wood, and compensates growers primarily on the basis of green weight or volume. No single set of breeding objectives can be considered optimal for multiple products, and while most managers recognize the need to improve quality, inventory and valuation systems continue to reward production of standing volume. Therefore, the immediate economic focus for the breeding program will continue to be on improved adaptability and volume growth.

The WGFTIP is meeting the challenge by maintaining a large base population with sufficient variation to allow individual members to tailor their deployment population to their individual

needs. In addition, we are attempting to develop a specialized wood quality elite population that will increase the diversity of selections available for the deployment population.

A number of issues, however, need to be resolved before a wood quality elite breeding program can be successful. First, a consensus needs to be reached as to the economic importance of wood quality. This will entail recognition by land mangers that increased gain in value can be made with improvements in wood quality even though this may result in smaller gains in volume production. The timberland owner will also have to be compensated for this improvement in quality or he will have no incentive to participate. Secondly, better and less expensive ways to evaluate wood quality need to be developed. This is necessary so that the genetic control of the constituent characteristics of wood quality can be elucidated and efficient selection indexes developed. It is also necessary so that the large numbers of individuals in the progeny testing and selection populations can be quickly screened. These methods need to be capable of quickly and accurately evaluating the 2,000 to 3,000 trees in a typical progeny test. Thirdly, better methods of within-family selection need to be developed to deal with low heritabilities and negatively correlated traits. These methods may include genetic markers and clonal testing as well as other methods not yet envisioned. Genetic engineering to put the wood quality traits directly into proven planting stock has not been discussed, but also holds promise. Finally, the acceptance of clonal forestry to deploy individuals that have both combinations of traits and a level of uniformity that may be difficult to achieve with sexually propagated material will add greatly to the value of the deployment population. The ultimate breeding objective remains increased value whether it is achieved solely with improvements in volume or a combination of improvement in volume growth and wood quality.

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Improving Wood Quality in a Hybrid Pine Clonal Forestry Program in Queensland, Australia.

K.J Harding[1], T.R. Copley[1], P.G.Toon[2] and M.J. Dieters[3]

Abstract: Research into the vegetative propagation of *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* (slash × Caribbean pine) F_1 hybrid started in Queensland in 1982. The first clonal test was established in 1986 to evaluate 250 clones planted on two sites, followed by a second series of tests containing 480 clones planted over 20 sites in 1994-97. Wood studies were initiated in 1993 and the first large screening of clones was carried out in 1999-2000 on the best 50 clones for growth and form traits in the series II tests. Screening of the best 170 clones from 1180 in the Series III tests was undertaken in 2004.

Wood quality screening has been incorporated into DPI Forestry's clonal forestry programme and the nature of the activities and potential for improvement has increased as the programme has matured. Incremental improvements in the amount of wood property information available on pedigrees and parents can be seen over time. There is a minimum lead-time of 8 years from when the crosses are made for each series of clonal tests. By overlapping the activities for each cycle of clonal tests there is approximately 5 years age difference between each clonal test Series.

The R&D resources available are tailored to support the size of the resource and its planting programme, providing challenges to maximise gains for the R&D dollars invested. There are very encouraging signs from studies on clonal tests and operational plantings that the advancements in acoustic technologies, particularly for standing tree assessments, offer very useful low-cost options for clonal screening and studies of variation. Clonal forestry is by nature very expensive due to the level of screening for growth, wood and propagation traits needed to identify and then bring in to commercial production the very best genetic material to maximise gains. Easy to use, low cost, non-destructive evaluation tools are needed to provide opportunities to make clonal forestry more economical by delivering higher gains at reduced assessment and screening costs.

Two elite Series I clones were planted on adjacent sites of 2 and 0.5 ha that ran 400 m along an environmental gradient from a ridge down into a poorly drained gully. Variation in basic density across this environmental gradient had a range of 83 and 102 kg/m^3 for individual ramets of each clone. The range in mean basic density of the

[1] Horticulture and Forestry Science, Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, 4068, Queensland, Australia
[2] DPI Forestry, Department of Primary Industries and Fisheries, Fraser Road, Gympie, 4570, Queensland, Australia
[3] School of Land and Food Sciences, Faculty of Natural Resources, Agriculture and Veterinary Science, University of Queensland, Queensland, Australia

50 series II clones screened in 1999 was 76 kg/m^3 at age 5 years and was 102 kg/m^3 for the 170 series III clones assessed in 2004 at age 4-5 years. This variation provides many opportunities to match clones to product requirements. However, within and between clone variation across and between sites is of great practical significance to wood processors and requires management by both tree improvement researchers and forest managers to optimise gains from clonal forestry.

Keywords: Clone, wood properties, *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* hybrids.

INTRODUCTION

Research into the vegetative propagation of *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* (slash × Caribbean pine) F_1 hybrid started in Queensland in 1982 (Walker *et al*., 1996). The first clonal test was established in 1986 to evaluate 250 clones planted on two sites. Following an intensive review of early results from this test (DPI Forest Service and QFRI, 1993), DPI Forestry decided in 1993 to invest in the nursery and propagation infra-structure and a research and development programme to implement a clonal forestry programme. A second series of clonal tests containing 480 clones was planted on two sites in 1994 and selected sub-sets of these clones were planted out on 14 sites in Queensland and northern New South Wales over 4 years. Small scale operational plantings of some of the best Series I clones were planted in 1995 and the whole annual planting program of approximately 4,500ha for south-east Queensland was fully clonal in 2002 using the best series II clones.

This paper summarises results from some key studies to demonstrate how wood quality screening has been incorporated into DPI Forestry's clonal programme. It highlights the incremental nature of the activities and potential for improvement as the programme matured from the initial Series I tests through to the Series III tests sampled this year, to the crosses made in 2003 and 2004 for the Series V tests. Incremental improvements in the amount of wood property information available on pedigrees and parents are obtained over time. It is not practical or cost-effective to delay selection and crossing programmes to obtain all the wood property screening information that might be desirable. The lead time to make crosses (1-2 years), collect and sow seed (2 years), plant out trials (1 year) and then allow 4 years to attain a minimum screening age and size, means that there is a minimum lead time of 8 years for each series of clones. By overlapping the activities for each cycle of clonal tests there is approximately 5 years age difference between each series of tests and the tests themselves tend to be planted out over sites and with varying designs (single-tree plots, line plots, small plots) over about 3-4 years.

The programme in Queensland is based on a priority resource of approximately 100,000 ha in SE Queensland. With the available R&D resources tailored to support a resource of this size, and its planting programme of around 4,500 to 5,000 ha/annum, there are obvious challenges to maximise gains for the R&D dollars invested. A key element to address this challenge is the rapid adoption of non-destructive tools for the assessment of wood properties to provide more rapid, cost-effective screening opportunities so that selection intensities can be maximised. Priority traits must be identified and low-cost non-destructive evaluation sampling and

assessment strategies developed to target these. In Queensland, traits such as juvenile wood stiffness and stability are critical to the dominant local market of structural timber framing.

MATERIALS AND METHODS

The sources of clonal and parental material used in some wood studies undertaken as part of the clonal programme are briefly described below. The types of studies are also indicated but methods are not described in detail, as the main aim of this paper is to discuss the variation found and both the opportunities and the challenges this provides.

Series I clonal tests

This initial test Series of clonal tests included about 250 clones from 14 slash × Caribbean pine (PEE × PCH) F_1 hybrid families. It was planted in 1986 on two sites representing good (ridge) and poor (swamp) drainage sites. The swamp site was sampled for a sawing study at age 13 years. A selection of 46 clones was cored with a 12mm increment borer to obtain a broad sampling of the basic density variation in this trial (196 ramets in total). The clones for sawing were selected to provide subsets of clones representing clonal differences for density, straightness and branching traits. The sawing study produced structural framing dimension recovery that was machine stress graded.

Series I operational clonal plantings

The first operational plantings of two PEE × PCH pine F_1 hybrid clones were established in 1995 as two adjacent monoclonal blocks of 2.5 ha and 0.5 ha. The 400m long block changes gradually from a moderately drained “grey podzolic” to a poorly drained (problematic) “podzol” soil type. When it was sampled at age 6 years there were obvious growth differences along this environmental gradient. The blocks were increment core (12 mm) sampled to assess variability in wood density, followed by an intensive study of wood properties. A stratified sample of the ramets were then assessed for both standing tree and 3m butt log acoustic velocity before sawing to structural framing (70 x 35 mm). The recovered boards were machine stress graded on flat and then tested for strength and stiffness on edge.

Series II clonal test series

This second Series of clonal tests included 479 clones from 48 PEE × PCH pine F_1 and F_2 hybrid families (4 to 16 clones/family) and was planted in four stages during 1994 to 1997. The best 50 clones for growth and form were sampled at age 5 years in the Stage 1 tests on two sites (Landsborough and Tuan) to select 10 superior clones for operational deployment. Increment core samples (12 mm) were used to assess basic density and spiral grain patterns for growth rings 2, 3, 4 and 5.

In 2001 the most promising 12 clones in this Series were assessed in the Stage 2 tests on 4 sites to screen these clones across a broader range of sites. This screening included densitometric density and microfibril angle (MfA) assessment.

Stage 4 tests were planted out as 5-tree line plots in 1996 and 1997 and these were destructively sampled in 2003. Additionally, several large-scale block plantings of the top 6 deployment clones were also destructively sampled at this time. Each felled stem was crosscut to produce a 1.5 m butt log for stress wave velocity assessment with a WoodSpec accelerometer (used under a licence agreement from Industrial Research Limited, New Zealand). The felled stem sections were assessed with the WoodSpec to examine within and between site variability in acoustic stress-wave velocity.

Series III clonal test series

The third Series of clonal tests consisted of 1180 clones from 34 PEE × PCH F_1 and F_2 hybrid families, 6 (PEE × PCH) × PEE backcross families and 2 (PEE × PCH) × PCH backcross families. The tests were planted out in 1999 to 2001 and the best 170 clones for growth and form were assessed for density in mid-2004. A second stage screening of the best 34 clones selected after considering the density results was assessed for spiral grain and microfibril angle will be measured before final selections are made. The goal is to select the best 10-12 clones by early 2005, as deployment of the Series II clones will be phased out from 2005/06.

Caribbean pine parents

The majority of superior PCH parents used in the crossing program (2003 and 2004) to produce hybrid families for the Series V clonal tests have been screened for wood density, spiral grain and microfibril angle. Parents were sampled as ramets in seed orchards to provide a ranking without having to adjust for site and age differences between the ortets identified and selected in a range of genetic trials. Results will be used in 2006 to select the most promising families for inclusion in the Series V clonal tests.

RESULTS AND DISCUSSION

The results presented and discussed from some of the wood studies undertaken for the clonal program are used to highlight the variation in wood properties found. This variation provides very significant opportunities to select clones with highly desirable wood quality combined with good productivity and form. However, it also highlights some of the challenges for forest managers to consider in clonal deployment so that clonal variation across environments and among clones is monitored and managed to limit this variation. Timber processors are looking for uniformity and/or predictable conversion and processing characteristics from clonal blocks; knowledge of both sites and clonal properties and how they may interact is important to deliver this.

Series I Clones

Twenty clones were selected for destructive testing from around 50 sampled for density. The felled clones were stratified across the observed density range. Three ramets of each of the 20 selected clones were felled and sawn. The sawn recovery was machine stress graded and the proportion of recovery assigned to MOE classes (Figure 1). The ranking of clones by mean basic density was generally correlated to higher MOE recovery but with some standout inconsistencies

such as the clones ranked 11 and 3 for mean wood density but 5th and 15th out of 20 for stiffness respectively. Other factors such as the interaction of microfibril angle, grain spirality and/or knot size and frequency must account for these large departures from density ranking. Nevertheless, a large difference in average wood density of the first five growth rings from the pith was found between the best 20% of clones for stiffness and the worst 20% of these 20 clones; 414 kg/m^3 versus 359 kg/m^3. The best of these clones would produce approximately 50 to 60% structural grade recovery at age 13 years, raising the prospect of significantly improved structural grade recovery in rotation age (28-30 years) stems.

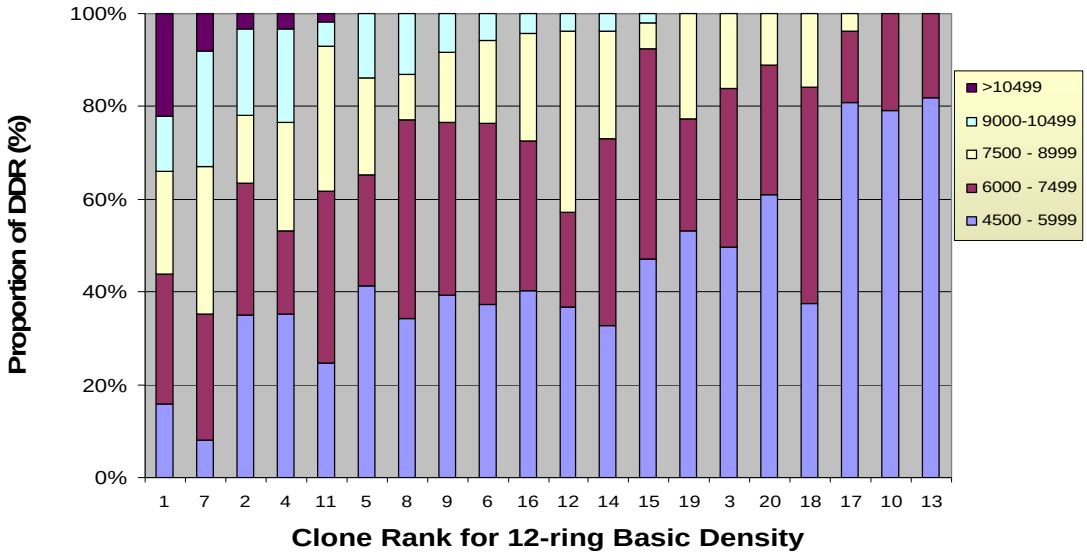


Figure 1. Proportion of dried dressed structural grade volume in MOE (MPa) classes for Series I clones. Clone ranking for 12-ring mean basic density indicated.

The spiral grain trends for these clones also displayed significant variability with several clones consistently producing grain angles in excess of 5° (Figure 2). It is clear from this sample, selected across the range in basic density, that there is considerable scope to select superior clones. It is also clear that screening is essential to avoid the inclusion of clones with sub-standard wood properties (i.e. angles > 4.5°) in the deployment population.

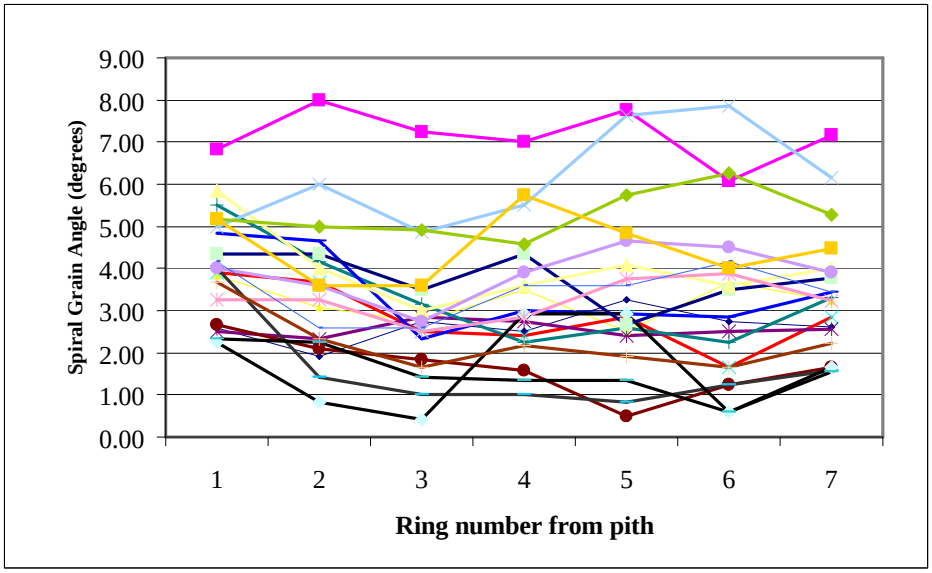


Figure 2. Mean spiral grain variation in twenty Series I clones from Experiment 221 GYM

Wood density variation within an operational planting of two Series I clones

One of the first operationally planted PEE × PCH F_1 hybrid clones from the Series I tests was established in southeast Queensland in 1995. This two-hectare planting was on a site that runs along an environmental gradient from a ridge down into a poorly drained gully. Variation in basic density across this environmental gradient has been examined by Toon (2004) who established 18 plots of 6 trees (2 rows x 3 trees) across and along the gradient. Density was relatively uniform in plots 1 to 6 on the well-drained ridge-top (Figure 3) but variation increased in the plots located in the poorly drained lower-slope (plots 13 to 18). It is clear that site variation can impact significantly on clonal uniformity even across relatively small distances in routine plantations.

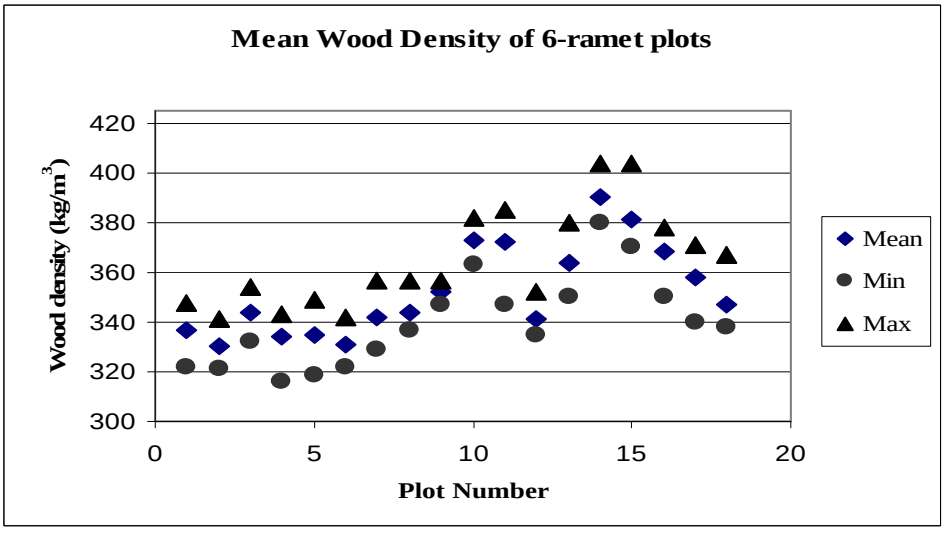


Figure 3. Mean wood density and range for 18 plots established along and across an environmental gradient in the first 2ha operational planting of a series I clone.

Following the 2001 sampling for the Toon (2004) study, core samples from the clone above (25 trees) and an adjacent 0.5ha planting of a second Series I clone (19 trees) were assessed using CSIRO's Silviscan (Evans 1998). This sample of ramets was stratified to encompass the density range and was part of a study to investigate non-destructive evaluation techniques for early screening of juvenile wood. The Silviscan results (Table 1) indicate superior density and MfA in clone TWO. These results are of particular interest as these two clones are sibs from the same full-sib family, and provide a good example of the within-family variation that can be exploited in a clonal forestry programme.

A Fakopp tool was used to assess acoustic velocity in the 44 ramets of these two clones while standing and then they were felled and a 3m-butt log was sawn into 70 x 35mm structural framing dimensions. Prior to sawing acoustic velocities were obtained on each log with the WoodSpec accelerometer. The boards recovered were then tested for MOE and MOR on a Shimadzu timber tester.

Table 1. Silviscan comparison of two superior Series I clones for estimated basal area, air-dry density, microfibril angle and predicted MOE at age 6 years.

Clone		Basal Area (mm^2)	Air-dry Density (kg/m^3)	Microfibril Angle	Predicted Modulus of Elasticity (MPa)
	Mean	14875	495	18.8	10.0
ONE	Maximum	25762	599	23.8	12.7
	Minimum	7074	410	13.5	7.1
	Mean	16987	519	15.4	12.4
TWO	Maximum	28208	602	20.4	15.1
	Minimum	10137	446	11.8	9.5

WoodSpec predicted MOE of log was very highly significant and moderately to strongly correlated with Shimadzu MOE on flat (0.70), and standing tree Fakopp MOE (0.87). Given these relationships, predictive regression models were evaluated to predict WoodSpec log MOE. A regression using whole core basic density and Fakopp MOE prediction was very highly significant ($Pr>F = <0.0001$) in predicting 80 % of the variation in WoodSpec MOE ($r^2 = 0.81$). Adding Silviscan variables (weighted density, MfA and predicted MOE) improved this coefficient of prediction slightly ($r^2 = 0.84$) but the model parameters were not significant except for Fakopp.

It therefore appears that we have a strong prediction of log MOE from the gravimetric assessment of basic density using a 12 mm increment core combined with a standing tree prediction of MOE using a time of flight acoustic tool. As log MOE is also linearly associated with the average MOE of sawn boards ($r = 0.70$) we have some reliable capacity to rank trees

into broad quality classes for juvenile wood stiffness, which is a primary focus for juvenile wood quality improvement. Additionally, the much more expensive information obtained from Silviscan analysis of cores did not appear to add significant value to this prediction once a mean density and a standing tree acoustic velocity was obtained. This suggests a greatly improved capacity to screen larger numbers of progeny or ramets with low cost tools before identifying the most superior part of the population for more intensive evaluation with Silviscan and for grain spirality. The impact of MfA and spiral grain on warp in solid timber means that this final screening is required to ensure that all selected clones are stable during drying and in use, as well as having high stiffness properties.

A further regression analysis was undertaken using 12 variables derived from the 120 boards recovered. These variables included density, microfibril angle, spiral grain, twist, spring, bow and their standard deviations from Silviscan and direct measurements on boards. The best regression (Stiffness index) for board stiffness (on flat) was:

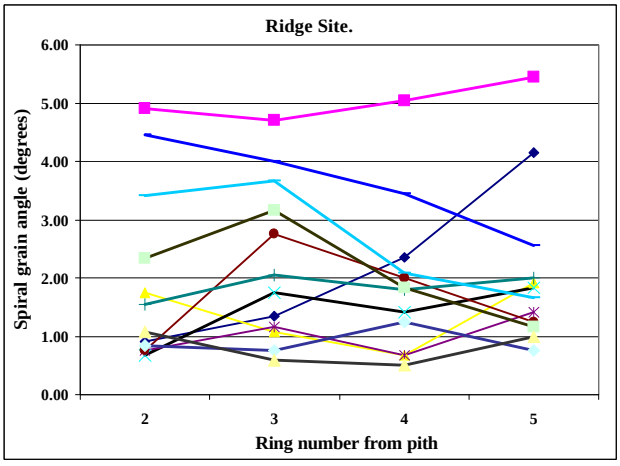
MOE_board = 6.8975-0.11045*Spring-0.03440*Bow-0.16363* S_W_MFA
+ 0.00962* S_W_DEN

where S_W_MFA and, S_W_DEN are Silviscan microfibril angle and density weighted by area. All four variables are statistically significantly at 5% probability level and the R^2 is 0.492.

Series II clonal screening

To select a superior set of 10 clones for deployment, the best 50 clones for growth and form were sampled on two sites, a ridge site at Landsborough and a poorly drained site at Tuan, at age 5 years. The clones ranged in unextracted basic density from 295 to 385 kg/m^3 on the ridge site and 329 to 400 kg/m^3 on the swamp site with a highly linear trend in the mean densities of the 5 ramets sampled at each site (r = 0.79). This provided a pool of clones at the high end of these ranges that had relatively high juvenile wood basic density at both site types.

Spiral grain trends for the 10 select clones and two standby clones assessed are plotted in Figure 5 and indicate that most of these clones have acceptable to good grain angles. However, the clone with high (>5°) grain spirality on the ridge site is undesirable and the increasing angles with increasing ring number from the pith for some clones are very undesirable.



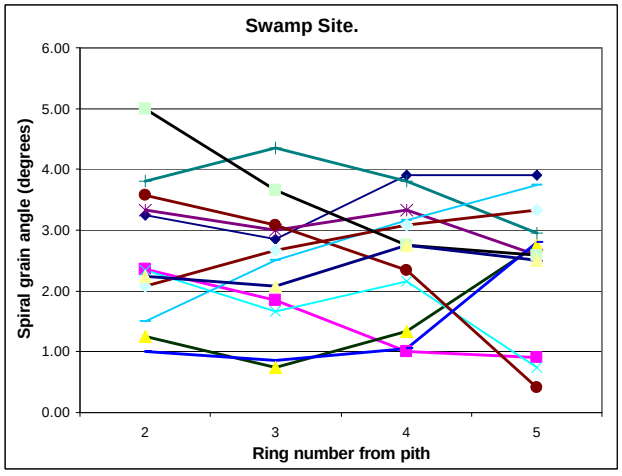


Figure 4. Mean spiral grain of ten selected Series II F_1 clones plus two standby clones sampled in clonal tests at Landsborough (ridge site) and Tuan (swamp site).

Series II deployment clones

The parents used to produce the progeny for the Series II tests were selected on growth and form criteria. Wood property assessments of these parents were not available. Therefore, there was little expectation that screening of the best performed of these clones would identify superior wood quality types. Nevertheless, a few clones approached the 400 kg/m^3 juvenile wood density threshold found in the best Series I clones for graded sawn recovery. However, some of the clones selected displayed relatively low density, which is likely to preclude them from consideration for reduced rotation length. A key goal of the research programme has been to screen early juvenile wood properties in growth rings 1 to 5 to select and/or develop clones with the potential for earlier harvest without reduced graded sawn recovery yield. To produce clones suitable for this flexible rotation age option requires superior juvenile wood properties capable of meeting structural grade requirements that the juvenile wood of the current seedling resource does not.

The best clone for basic density was not included in the original Series II deployment population as it fell marginally below the volume yield of the ten 'best' selected clones. However, when it was assessed for microfibril angle (MfA) using CSIRO's Silviscan, its MfA was significantly lower (by about 5 °) than the next best elite Series II clone and its predicted stiffness for the first 5 rings was 20% higher than the next best clone (Table 2). Although it ranked 4th of these 12 clones for density its very superior MfA marked it as a standout for stiffness, which is the primary selection goal for the largely structural framing market targeted by Queensland's large softwood sawmills.

Table 2. Density, microfibril angle and predicted stiffness (MOE) for superior Series II clones assessed for DPI Forestry's clonal forestry deployment population.

CLONE	% Density > 400 kg/m^3	Air Dry Density (kg/m^3)	Std Devn of pith to bark density	MfA (degrees)	Predicted MOE (MPa)	Predicted MOE Std Devn
1	47.92%	461	153	26.89	6.33	2.14
2	62.49%	470	115	25.13	6.80	1.51
3	59.02%	460	110	25.49	6.07	1.61
4	47.09%	443	114	24.44	6.48	1.78
5	64.57%	478	121	24.67	6.76	1.40
6	56.34%	478	137	29.12	5.96	1.68
7	65.20%	485	130	26.18	6.37	1.49
8	50.64%	468	149	25.19	7.31	1.89
9	57.99%	475	136	19.84	8.81	2.05
10	71.73%	491	111	25.25	7.14	1.49
11	53.48%	453	112	26.76	5.53	1.29
MEAN	57.84%	469	126	25.36	6.69	1.67
MAX	71.73%	491	153	29.12	8.81	2.14
MIN	47.09%	443	110	19.84	5.53	1.29

Series II Stage 3 acoustic testing

Five-tree line plots in 3 clonal experiments and large block plantings were sampled to provide 6 ramets per experiment and 15 ramets from the blocks. A 1.5 m long section of the butt log of each felled tree was assessed with a WoodSpec (IRL, New Zealand) acoustic velocity tool. The variation among clones and sites in acoustic velocity (Figure 5) demonstrates again the impact of site on wood quality; however, whether or not the observed rank changes are significant requires further investigation.

Series III clonal screening

The first wood property screening of the Series III clonal tests commenced in May 2004. The best 170 clones for volume, straightness, branch size and angle, and also with the lowest incidence of double-leaders and ramicorn branches, were selected from the 1200 clones established over 6 sites. The best clone from this initial screening had a mean resin-extracted basic density of 413 kg/m^3 and 50 of these clones exceeded 365 kg/m^3 in their first 3 to 4 rings from the pith. Further screening has been undertaken on 34 'top' clones selected on growth, form and density to assess their spiral grain and MfA trends before recommending a final 10 to 15 to form a new deployment population to produce plantlets for the 2005/06 planting programme.

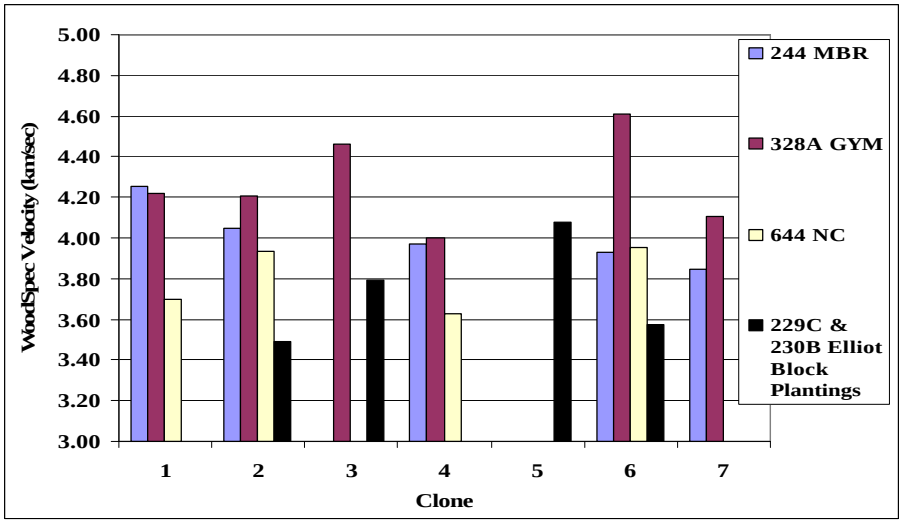


Figure 5. Average WoodSpec velocity of some well-performed Series II clones sampled at four sites in SE Queensland.

Parental ranking

Breeding values are available for volume and straightness of all parents used in the crossing programme to produce new clonal test series. However, it is difficult and very expensive to estimate accurate breeding values for wood properties as it is not practical to undertake large samplings and assessments of parents and their progeny. Even if the latter were affordable there

would still be difficulties in utilising the results without using adjustments for age and site affect. Both parents and progenies are spread throughout a range of different genetic trials, established across a wide range of sites/locations planted over the last 30 years.

To overcome some of these difficulties and provide an initial ranking of key parents, a sampling program of ramets in seed orchards has been commenced. Seed orchards provide multiple ramets of the same parent grafted at the same time and therefore the same age as other parents Sampling is undertaken about 0.5 to 1.0 m above the graft union (to minimise potential rootstock and grafting effects) from up to 10 ramets per parental clone. Density and microfibril angle results for 8 Caribbean pine parents sampled in North Queensland (Figure 6) demonstrate the considerable variation observed in this small sample of parents.

Series IV and Series V clonal screening

The Series IV clonal tests were planted out in late 2003 and early 2004. Based on the results of the parental rankings it will be possible to target the progeny of the best parents and best parental combinations when these tests are screened.

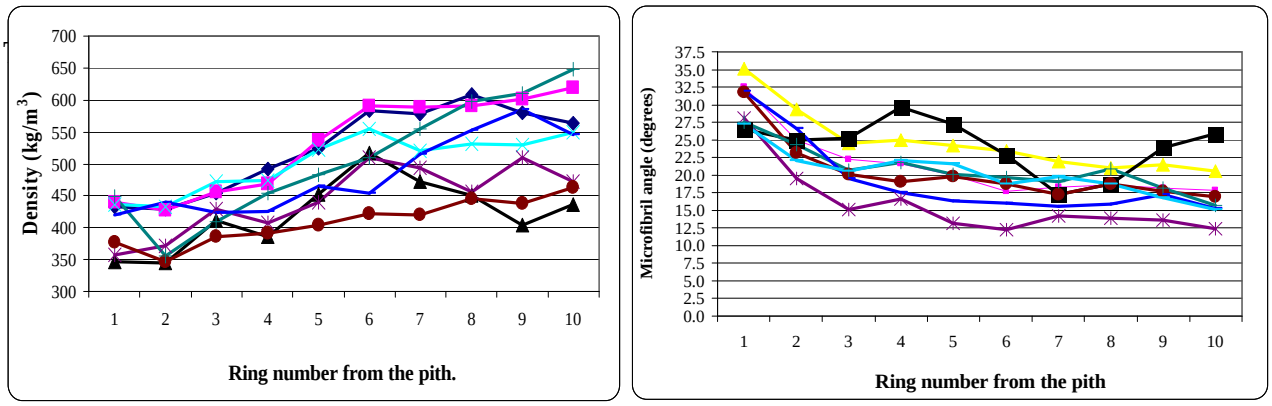


Figure 6. Trends for density and microfibril angle assessed using CSIRO's Silviscan for eight Caribbean pine parents.

The crosses to produce around 2000 clones for the Series V tests have been made in 2003 and 2004. All parents used in these crosses will have been ranked for wood quality by the time the seed is collected and sown from these crosses. This will provide the opportunity to prioritise the use of seed from the most promising families, i.e. those from parents with high quality juvenile wood. By establishing as many full-sib progeny as possible in these tests with families from parents highly ranked for wood quality, the expectation would be that a much larger pool of superior clones will be produced. The first four Series of clonal tests have relied on creaming the best wood quality clones from the pool of superior clones produced from crosses evaluated for volume, straightness and crown characteristics and relying on genes for superior wood quality to act independently of those for the growth and form traits. Given the variation observed in the wood traits assessed in Series I, II and III tests there is support to suggest that this proposition has occurred. Nevertheless, selecting material for the Series V tests based on all available

breeding values and wood quality rankings should greatly increase the potential to improve wood quality from clonal selection.

The Series V tests and future test series will be designed to include tests for early destructive sampling as well as replications that will be maintained for longer-term assessments. Commencing with the Series III assessments commenced this year a structured evaluation programme has been implemented to ensure that all growth, form, crown and wood quality information is available when needed to select the most superior genetic material from each test series to produce cutting hedges for operational deployment. The speedy adoption of non-destructive tools that provide more rapid, cost-effective screening opportunities so that selection intensities can be maximised will be important to achieving good gains in quality.

CONCLUSION

This presentation documents a potted history of wood quality research undertaken since 1993 as part of a clonal forestry research program for slash × Caribbean pine hybrids that commenced in 1982. It provides some insights into the incremental nature of improvements and progress that are possible over time whilst working within budget and resource constraints.

There are very encouraging signs that the advancements in acoustic technologies, particularly for standing tree assessments, offer very useful low-cost options for clonal screening. Clonal forestry is by nature very expensive due to the level of screening for growth, wood and propagation traits needed to identify and then bring into commercial production the very best genetic material to maximise gains. Easy to use, low cost, non-destructive evaluation tools provide very significant opportunities to make clonal forestry more economical by delivering higher gains at reduced assessment and screening costs.

Acknowledgements: DPI Forestry provided funding for the wood quality assessments and clonal tests used in this presentation and their ongoing support for this R&D programme is gratefully acknowledged. The work presented represents the collective efforts of a very large team from the Propagation, Genetics and Wood Quality Improvement groups within the Queensland Forestry Research Institute (now part of DPI&F Horticulture and Forestry Science) supported by staff of DPI Forestry in regional centres. Some of the work undertaken on the series I operational block plantings was part of FWPRDC project PN03.1916 - Juvenile Wood Initiative: Improving juvenile wood of radiata pine and slash × Caribbean hybrid pine through quantitative and molecular genetics. An earlier and similar version of this paper was presented at the Innovatek Wood Quality 2004 Workshop “Practical tools and new technologies to improve the segregation of logs and lumber for processing”, Albury, NSW, 5-6 August, 2004.

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Prediction Of Lignin And Cellulose Content In Tropical And Sub-Tropical Pines Using NIR Spectroscopy

G.R. Hodge and W.C. Woodbridge

Camcore, NC State University, Raleigh NC, 27695-7626

Near-infrared spectroscopy (NIR) was used to predict lignin content for trees from five species of tropical and sub-tropical pine (*Pinus caribaea, P. maximinoi, P. oocarpa, P. patula,* and *P. tecunumanii*) grown in Brazil and Colombia. Breast height disks were taken from 174 trees, and wedges from the disks were sectioned to sample juvenile and mature wood. The sections were ground into woodmeal and NIR reflectance spectra were measured on both unextracted woodmeal and woodmeal with extractives removed. Klason lignin content was measured on the woodmeal samples, and partial least squares were used to fit calibration equations to predict lignin content from the reflectance spectra. Good prediction models were obtained regardless of which dataset (i.e., combinations of species and regions) was used for model calibration. A model using reflectance spectra for woodmeal with extractives removed and combining data for all species across both regions had R^2 of 0.90 and standard error of cross-validation of 0.43% lignin for the calibration data set, and an $R^2 = 0.91$ and standard error of 0.40% for the validation data set. Calibration equations developed using only Brazil or Colombia data were tested on the other data set. Predictions were very good, with prediction R^2 ranging from 0.83 to 0.90, and standard errors of prediction from 0.43 to 0.54% lignin.

A subset of the same wood samples was used to develop a calibration to predict cellulose content using NIR reflectance spectra measured on unextracted woodmeal. Results for these calibrations will also be presented.

Silviculture and Genetic Impacts on Productivity of Loblolly Pine in the Southern United States

S.E. McKeand and H.L. Allen[1]

Deployment of improved loblolly pine (*Pinus taeda* L.) genotypes across the southern United States is standard silvicultural practice. Virtually every one of the 1.2 billion loblolly pine seedlings planted each year is the result of one of the tree improvement programs in the South (McKeand et al. 2003). Most of that planting is with open-pollinated (OP) families from first- and second-generation seed orchards. These OP families typically display remarkable rank stability for productivity and quality traits across a range of site characteristics, climates, and silvicultural systems. Open-pollinated families are genetically heterogeneous and appear to be well buffered to environmental extremes. Families that do well on one site usually will do well on all sites within a climatic zone with few exceptions. To date, management decisions have been fairly simple as to where to plant OP families and how to manage them in different silvicultural systems.

Intensive management of plantations of genetically improved loblolly pine in the southern United States is having a dramatic impact on forest productivity and financial benefits. Forest managers have recognized that intensive plantation silviculture is like agronomy; both the plant and the soil need to be actively managed to optimize production. When the best genetic material is planted and given the necessary resources to grow, mean annual increments of 20 $m^3 ha^{-1} yr^{-1}$ can be readily achieved on many sites (Allen et al. 2005). There are few other regions in the world where the use of integrated silvicultural systems that include the manipulation of site resources, management of stand density, management of pests, and use of genetically improved planting stock is having as positive an impact on plantation productivity. Today's plantations are growing more than twice as fast as plantations of the previous rotation.

Most planted hectares are receiving some form of chemical and/or mechanical site preparation to reduce competing vegetation and improve soil conditions to ensure excellent survival and early growth of the planted pine. In addition, most plantations are also receiving at least one and sometimes up to four nutrient applications to ameliorate widespread nitrogen and phosphorus limitations. Thinning is now commonly practiced to increase the production of sawtimber in rotations that typically range from 22 to 28 years.

Foresters have recognized that the largest responses to resource manipulation such as nitrogen fertilization usually occur on poorer sites (Allen, unpublished data). An example is given in Figure 1 where the most responsive site to fertilization was the site with the poorer inherent volume growth (low volume growth on control plots). In contrast, genetic gains typically have the greatest impacts on the best sites (e.g. McKeand et al. 1997). The percentage increase of a good family vs. an average or poor family may be the same across sites with different productivities, but the absolute increase in volume is highest on the best sites (Figure 2). These 2

[1] Professors, Department of Forestry, NC State University, Campus Box 8002, Raleigh, NC 27695-8002.

simple examples illustrate that productivity gains can only be optimized when tree improvement is an integral component of the silvicultural system for managing plantations, and when the forest manager understands where the greatest benefits arise from resource manipulation and / or genetic inputs.

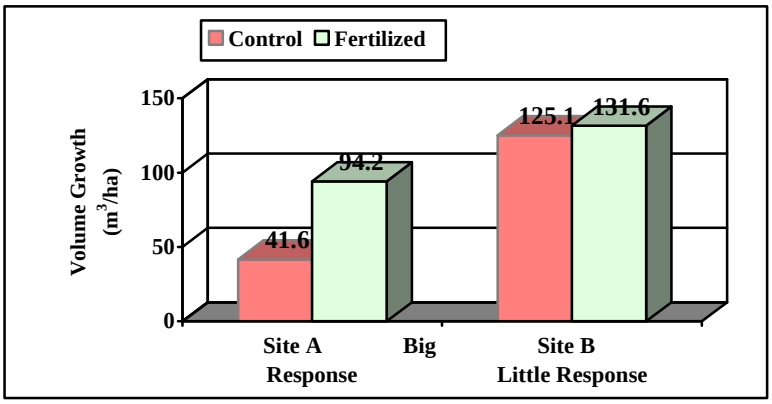

Figure 1. Comparison of responses to fertilization on a poor site (A) vs. a good site (B). Greater absolute and relative responses to fertilization are typical on poorer sites (unpublished data, H.L. Allen).

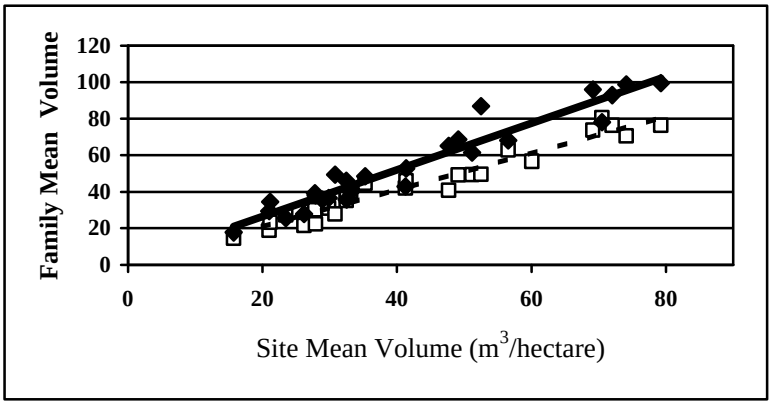

Figure 2. Response of highly productive family (solid line and diamonds) compared to average family (dashed line and open boxes) across a range of sites. While the relative volume differences are the same across sites, the absolute volume differences are higher on the most productive sites (from McKeand et al. 1997).

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Accounting For Genetic Gain in Growth Models: Bridging the Gap between Geneticists and Growth Modelers

G.T. Howe[1] and M.L. Cherry[2]

[1]Director and [2]Assistant Director, Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Science, Oregon State University, Corvallis, OR, USA

The main objective of tree improvement is to increase the value of tree crops at rotation age. Nonetheless, most selection decisions and predictions of genetic gain are based on measurements made on individual trees at young ages (often ¼ rotation age or less). From a geneticist's perspective, growth models (i.e., forest yield models, process/mechanistic models, and hybrid models) offer useful tools for estimating genetic gain at rotation age. Growth modelers typically use these same models to guide forest management decisions—but the models are mostly based on the performance of unimproved genotypes—whereas much of the reforestation stock is now genetically improved. From a modeler's perspective, it would be valuable to know how to modify existing models (or how to develop new models) that would better predict the performance of genetically improved stands.

The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and other organizations recently held a 3-day meeting involving both geneticists and growth modelers to address these questions. We will discuss the conclusions and recommendations resulting from this meeting. Recommendations that focus on tree improvement include the need to (1) incorporate genetic improvement into existing growth models, (2) use existing progeny test information to calculate growth multipliers for genetic improvement, (3) use operational planting programs to outplant and compare a large number of genetically improved vs unimproved stands, (4) standardize the calculation of breeding values, (5) create standard genetic reference populations for use throughout the region, and (6) develop a seedlot certification system for the Pacific Northwest. Recommendations that focus on improving growth models for the Pacific Northwest include the need to (1) expand the availability of young stand growth models, (2) investigate whether better site characterization or climate/weather data can be used to improve growth models, (3) make recommendations on the appropriate frequency and type of measurements for growth plots, and (4) develop the next generation of growth models for the Pacific Northwest—with an eye toward hybrid models.

Early Yield Prediction for Scots Pine (*Pinus sylvestris* L.) in North Sweden

Tore Ericsson[1] and Ola Rosvall[1]

Abstract: Old Scots pine (*Pinus sylvestris* L.) experiments were revisited in order to improve the accuracy of long-term production forecasts in breeding programs based on early evaluations of field trials. Using data from five mainly north Swedish provenance trials (in the 'Stefansson provenance series') we estimated the within-site relation between survival and mean tree height at 18 years, and the total yield at 51–52 years (V_T) as $V_T = c_i n^{0.42} h^{1.42}$, where c_i = a site-specific constant (i = 1, …, 5), n = number of surviving trees·ha^{-1}, and h = their mean height. The form $s^{0.42}h^{1.42}$ (s = survival) should be generally useful for comparative yield prognoses for different Scots pine stocks within the same site.

The field trials are situated mainly at about 63–66°N on sites up to 500 m in elevation. They are composed of open pollinated seeds collected from Scots pine in natural stands at about 59–68°N. The general applicability of the expression may be diminished by, among other things, the fact that only about half as many seedlings than were planted in the trials are usually planted per unit area in commercial forestry nowadays. That offers fewer selection opportunities at thinnings and may cause more remaining open patches, due to mortality, in the final forest.

In addition, our results elucidate once again the well-known benefit of a southward transfer of Scots pine to improve hardiness at harsh regeneration sites. The transfer distance for the highest production per unit area is shorter than that for maximizing the product of early survival and mean height (sh).

INTRODUCTION

The pine forest rotation time in north Sweden is in the region of 100 years, so it is important to make production forecasts based on results from younger trials (10–20 years). This is particularly important in tree breeding operations where selection is based on young progeny tests. It would be advantageous to have a tool for production prediction that is independent of the varied prerequisites associated with the environment and cultivated trees.

In 2002, five provenance experiments in the 'Stefansson provenance series' were reinvestigated with the aim of collecting data to construct such a tool. The experiments were mainly in north Sweden, and were 51–52 years old. All experiments in the series were thoroughly investigated in 1968, at the age of 18 years (Remröd 1976). These data, together with other results (e.g., Eiche 1966), have served, until now, as the basis for understanding pine transfer effects in north Sweden. The fundamental finding was that mortality on harsh sites can be partly counteracted by

[1] Skogforsk (The Forestry Research Institute of Sweden), Box 3, 918 21 Sävar, Sweden

using the progeny of trees that evolved in more northerly locations. The regular connection between growth and survival under varying climatic conditions has been studied in other works (Persson 1994a), but few data are available regarding the influence of provenance choice on the full rotation production.

Hardiness and growth expression are closely connected on the individual-tree level, but the genetic correlation is, basically, negative (Persson *et al*. 2004). Thus, appropriate southward transfer that maximizes the production per unit area depends on increased survival (stand density) compensating for the loss of growth resulting from the transfer. Over the period of rotation, the principal importance will shift gradually from survival ability in the young phase to the growth potential of older trees.

Our aim was to express the total wood production up to 2002, over 51–52 growing seasons, incorporating the data collected after 18 growing seasons. We thus hoped to create a general tool for total production forecasts based on early measurements.

MATERIAL AND METHODS

The investigated field trials were mainly planted in the spring of 1951 and are thoroughly described in earlier publications (Stefansson 1965, Stefansson & Sinko 1967, Remröd 1976). They are located at about 63–66°N (Fig. 1), except for a single site at 59° (116B Laxå), and all contain the progeny of Scots pine from native stands at about 59–68°N. All sites were planted with 4444 or 6944 seedlings·ha^{-1}, corresponding to 1.5 or 1.2 m square spacing, using standard (at that time) three-year-old transplanted seedlings. The experiments (either complete or balanced incomplete, randomized blocks) and the provenances of the Scots pine that were used are presented in Tables 1–2. A standard thinning (roughing) was carried out after the measurements in 1968 with the aim of equalizing the number of trees within each plot. This was not always successful due to patchiness as a result of early mortality.

The variables measured in 2002 included the breast height diameter ($d_{1.3}$) of every tree and height (h) of 1–4 sample trees per plot, selected randomly in advance. In trial 116 Brattfors, naturally regenerated trees in more or less empty plots were preserved and labeled.

Naturally regenerated Norway spruce (*Picea abies*) and birch (*Betula* spp.) were measured in most trials in order to estimate their additional contribution to the total production. Stump diameters (under bark) were measured on sites that had been thinned by the land-owner during the period 1970–2000 (trials 98, 116, and 116B). In addition, the stump-height diameter and bark thickness of sample trees were also measured. Not all stumps were recovered (most obviously at 116B), contributing to the number of missing trees in Table 1.

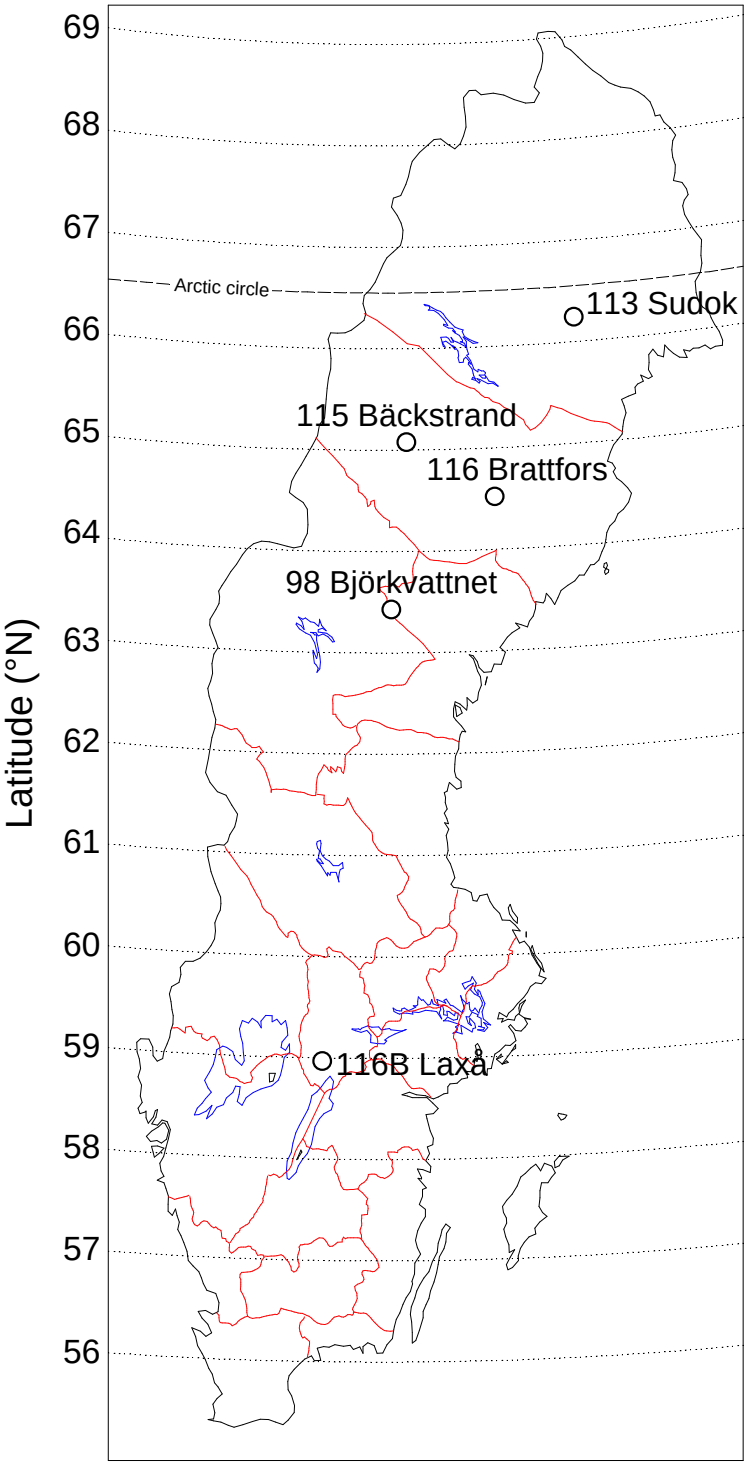

Fig. 1. Map of Sweden showing the field trials of the 'Stefansson provenance series', established in 1951 and investigated in this study.

Table 1. Details of the field trials

	98 Björkvattnet	113 Sudok	115 Bäckstrand	116 Brattfors	116B Laxå
Latitude (°N)	63.43	66.23	65.07	64.52	59.00
Elevation (m)	460	150	500	310	100
Temperature sum (day-degrees) [a]	772	888	639	851	1303
Tested provenances	21	21	16	21	21
Plots/provenance (replicates)	4	5	4	5	5
Plot shape	12×12	11×11	12×12	11×11	11×11
Spacing (m)	1.5×1.5	1.5×1.5	1.5×1.5	1.5×1.5	1.2×1.2
Missing trees [b]	1.2%	1.2%	0.9%	1.4%	17.7%
Sample trees	80	357	209	103	106
Sample trees/plot	1	4	4	1	1–3

[a] Threshold +5°C (Odin *et al*. 1983)
[b] Proportion of trees that disappeared between 1968 and 2002 for no known reason or recovered stumps (thus missing from the volume estimates)

Table 2. Survival, mean heights and stem wood production in five provenance trials after 18–52 growing seasons (subscript numbers): s_{18} = survival (%); h_{18}, h_{51}, h_{52} = mean tree heights (dm); V_T = total volume produced; v_x = naturally regenerated additional volume (not recorded in 116B). Volumes ($m^3 \cdot ha^{-1}$) are stem wood over bark after 51 or 52 seasons. The boldface entries that had produced at least 50 $m^3 \cdot ha^{-1}$ were used for the production index (PI).

Provenance label	Origin (°N)	98 Björkvattnet					113 Sudok					115 Bäckstrand					116 Brattfors					116B Laxå			
		s_{18}	h_{18}	h_{51}	V_T	v_x	s_{18}	h_{18}	h_{52}	V_T	v_x	s_{18}	h_{18}	h_{51}	V_T	v_x	s_{18}	h_{18}	h_{51}	V_T	v_x	s_{18}	h_{18}	h_{52}	V_T
BD115	68.07	**85**	**31**	145	**230**	4	**83**	**36**	137	**275**	6	–	–	–	–	–	–	–	–	–	–	83	17	104	8
BD116	67.68	**86**	**31**	147	**241**	3	**76**	**36**	141	**309**	0	–	–	–	–	–	**85**	**33**	166	**230**	2	85	20	116	7
BD117	67.05	**87**	**34**	151	**297**	2	**65**	**39**	145	**316**	7	**68**	**27**	109	**169**	1	**77**	**38**	171	**288**	1	87	24	112	31
BD118	65.92	**81**	**33**	151	**292**	2	**56**	**35**	143	**282**	8	**45**	**25**	108	**126**	14	**72**	**34**	171	**255**	5	81	24	119	44
BD121	65.87	**86**	**37**	154	**331**	6	**56**	**35**	145	**289**	2	**43**	**28**	111	**122**	10	**75**	**39**	175	**304**	1	**81**	**29**	118	**52**
BD119	65.77	**72**	**35**	156	**321**	6	**37**	**41**	154	**310**	0	**40**	**26**	111	**98**	7	**68**	**37**	176	**299**	9	**88**	**28**	120	**65**
BD120	65.75	**88**	**36**	151	**314**	6	**55**	**36**	145	**285**	1	**59**	**28**	109	**157**	6	**74**	**36**	170	**265**	2	**86**	**26**	118	**49**
AC316	65.6	**77**	**36**	153	**311**	7	**52**	**36**	146	**256**	4	**51**	**29**	110	**139**	5	**69**	**40**	174	**302**	5	90	27	120	46
BD122	65.57	**84**	**37**	153	**307**	3	**46**	**37**	149	**284**	1	**50**	**27**	110	**114**	16	**76**	**41**	173	**302**	4	**91**	**26**	120	**59**
AC319	64.63	**65**	**35**	153	**305**	2	**31**	**38**	148	**218**	17	**28**	**27**	111	**89**	9	**51**	**40**	176	**250**	4	**90**	**32**	125	**78**
AC318	64.62	**64**	**34**	152	**272**	8	**34**	**37**	151	**267**	2	–	–	–	–	–	**58**	**39**	174	**291**	3	**89**	**28**	118	**64**
AC317	64.37	**54**	**33**	156	**286**	16	**24**	**33**	143	**156**	4	**30**	**26**	111	**100**	24	**50**	**36**	175	**259**	2	**92**	**29**	120	**69**
AC327	64	–	–	–	–	–	**25**	**36**	154	**196**	0	–	–	–	–	–	–	–	–	–		–	–	–	–
Y46	63.78	**52**	**35**	154	**275**	3	**13**	**36**	154	**107**	8	15	26	107	41	10	**43**	**41**	179	**259**	7	**82**	**34**	126	**75**
Y22	63.53	**61**	**37**	156	**333**	7	**27**	**39**	148	**192**	3	**26**	**27**	109	**81**	8	**47**	**40**	178	**266**	13	**92**	**30**	125	**76**
Y48	62.53	**40**	**34**	155	**248**	5	4	32	137	43	0	5	20	99	4	0	**23**	**41**	180	**200**	22	**90**	**36**	128	**82**
Y47	62.5	**29**	**33**	154	**204**	7	3	37	152	25	9	2	31	107	7	0	**19**	**39**	180	**168**	24	**88**	**30**	131	**103**
Z271	62.5	–	–	–	–	–	–	–	–	–	–	**31**	**25**	107	**87**	14	**46**	**37**	172	**238**	4	–	–	–	–
Z270	62.42	**51**	**37**	155	**284**	2	**17**	**36**	150	**142**	2	19	27	111	48	5	**47**	**40**	178	**257**	7	**89**	**31**	125	**82**
Z272	62	–	–	–	–	–	**13**	**34**	146	**105**	2	**25**	**27**	103	**75**	10	**50**	**39**	176	**269**	4	**93**	**31**	124	**79**
Z273	62	–	–	–	–	–	**24**	**30**	145	**162**	4	–	–	–	–	–	**65**	**37**	175	**301**	1	–	–	–	–
Bg33	61.22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**17**	**37**	178	**146**	37	–	–	–	–
Bg29	61	**9**	**36**	159	**107**	45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**89**	**33**	130	**115**
Bg31	59.03	5	27	147	28	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**78**	**28**	135	**103**
Bg32	59	**8**	**29**	147	**62**	23	1	28	125	8	8	0	–	–	0	0	2	34	169	34	84	**89**	**29**	131	**91**
Bg30	58.9	1	27	137	7	39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 3. Functions used to estimate the height and volume of individual trees

Function	Source
Tree height from DBH (applied to trees with a DBH measure): $h = 13 + (d_{1.3})^2 (a + bd_{1.3})^{-2}$	Näslund (1936); applied to sample trees with *a* and *b* specific to each test site
Tree volume from DBH and height (applied to sample trees): $V = 10^a D^b (D + 20)^c H^d (H - 1.3)^e$	Brandel (1990); *a–e* specific to regions of Sweden
DBH from stump diameter (applied to removed trees with only stump measurements available): $d_{1.3} = (d_0 + 10)^2 (a + b(d_0 + 10))^{-2} - 10$	Basically, Näslund (1936); modified for this application and applied to sample trees with *a* and *b* specific to each test site
Tree volume from DBH (applied to all trees without a height measurement): $V = \exp(a + bd_{1.3} + cd_{1.3}^4)$	Hoffmann (1982); applied to sample trees with *a–c* specific to each test site; *a* corrected for logarithmic bias

$d_{1.3}$ = DBH = stem diameter at 1.3 m over bark (mm)
d_0 = stem diameter at stump height under bark (mm)
D = stem diameter at 1.3 m over bark (cm)
$H = h$ = tree height (dm)
V = tree volume over bark (dm^3)
a–e = constants specific to each function

The sample tree data were used for developing functional relationships for estimating tree heights and, more importantly, tree volumes for all trees found in each plot on each site. Each test site was treated separately. The volume functions of Brandel (1990; functions 100-01 for Scots pine) were applied to the sample trees to provide a starting point. The other functions are least square fits produced using the SAS procedures REG and NLIN (SAS Institute 1999). The functions are listed in Table 3, omitting details such as considerations regarding choice of function type and estimation principles in order to avoid as much as possible of extrapolation errors that may arise, for example, as a result of sample tree sparseness in some size classes. The validity was confirmed by residual examination.

All tree volumes, including those representing trees removed previously and recovered stumps, were summed to give plot values and converted into stem volumes per hectare ($m^3 \cdot ha^{-1}$) over bark. The approximate volume of measured Norway spruce and birch (if any; sometimes as

stumps) was also considered, using the same method and the same functions as for Scots pine. There was no practical way to obtain more accurate volume estimates from the limited data available.

Thus, the mean plot volume per hectare for each Scots pine provenance on each site represents the total volume production of the cultivated trees at 51–52 years. These figures should be compared with the mean height and number of living stems per hectare at 18 years (Table 2). Following preliminary studies, the equation $V_T = c_i n^a h^b$ was chosen for least-square fit to the data (condition: $V_T > 50$ m^3·ha^{-1}), which gives V_T (the total production in m^3·ha^{-1} at 51–52 years), in terms of: c_i, a site specific constant for site i; n, the number of stems·ha^{-1} at 18 years; and h, the mean height at 18 years. The a and b parameters were estimated using SAS NLIN, supported by examination of the residuals.

The number of stems per hectare can be expressed as s (survival) multiplied by the original number of stems. Therefore, for relative comparisons within a potential reforestation site, the expression $c_i n^a h^b$ may be converted to a production index PI = $s^a h^b$.

An application, using a relative index, was worked out. Overall estimates of expected mean tree height and survival for local and latitudinally transferred wild Scots pine, grown on sites with different latitudes and temperature regimes (following temperature sums mapped by Odin *et al*. 1983), were calculated using 'Persson's functions' for Scots pine in north Sweden (Persson & Ståhl 1993 pp. 39–40: functions **5A.II** and **5A.v**, Persson 1994b p. 278: functions **I** and **iv**). The functions are based on quite early measurements (less than 30 years after planting or about 2.5 m mean height), providing acceptable early growth and survival starting-points. Thus, for Scots pine at a given site, the relative production index for different wild seed sources with varying PI is $100(\text{PI})(\text{PI}_0)^{-1}$ with $\text{PI}_0 = s_0^a h_0^b$, where s_0 and h_0 represent expected survival and mean height of Scots pine from locally collected seed, respectively.

RESULTS

The equation $V_T = c_i n^a h^b$ provided the best fit to the five-site dataset at $a = 0.42$ and $b = 1.42$ (rounded). Besides the site-specific constants (c_i), a and b explained 85.5% of the remaining variation in volume production per unit area (Fig. 2). A relative-index application of 'Persson's functions' for growth and survival is presented in Fig. 3.

DISCUSSION

This work presents an attempt to construct a model for predicting long-term production based on early records of survival and growth. Such a tool is essential in reforestation situations and for forecasting breeding progress, since most knowledge about traits and performance originate from early records of young progeny tests. The study focuses on stem-wood production per unit area up to about half the rotation (51–52 years), based on measurements taken around a fifth of the way through the rotation (18 years).

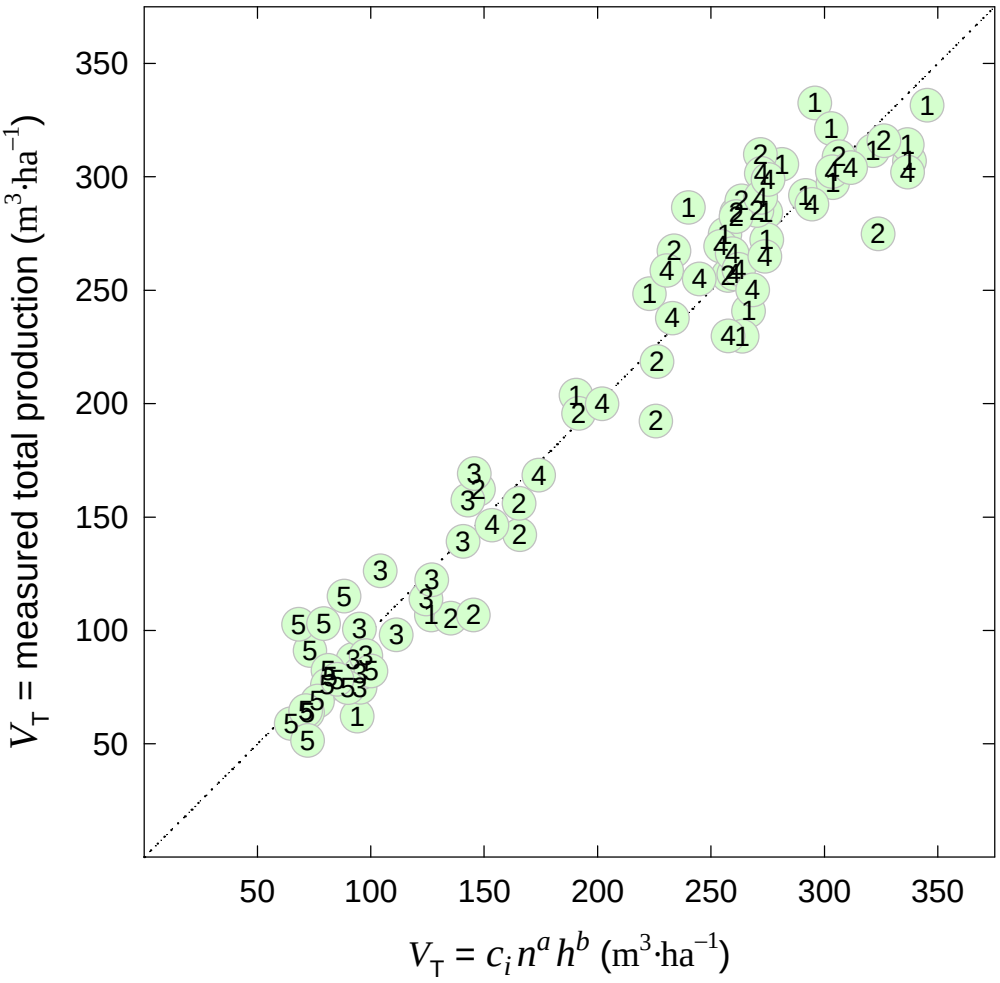

Fig. 2. Stem wood production plotted against values of the equation $V_T = c_i n^a h^b$ where n is the number of living trees·ha^{-1} at 18 years, h their mean height (m), and V_T is the total production at 51–52 years (m^3·ha^{-1}). Parameters estimated by least-squares: a = 0.42, b = 1.42, and c_i = the 'site factor' of each trial:

Field trial	98	113	115	116	116B
Plotted i	1	2	3	4	5
c_i	173.3	168.4	122.7	150.6	43.1

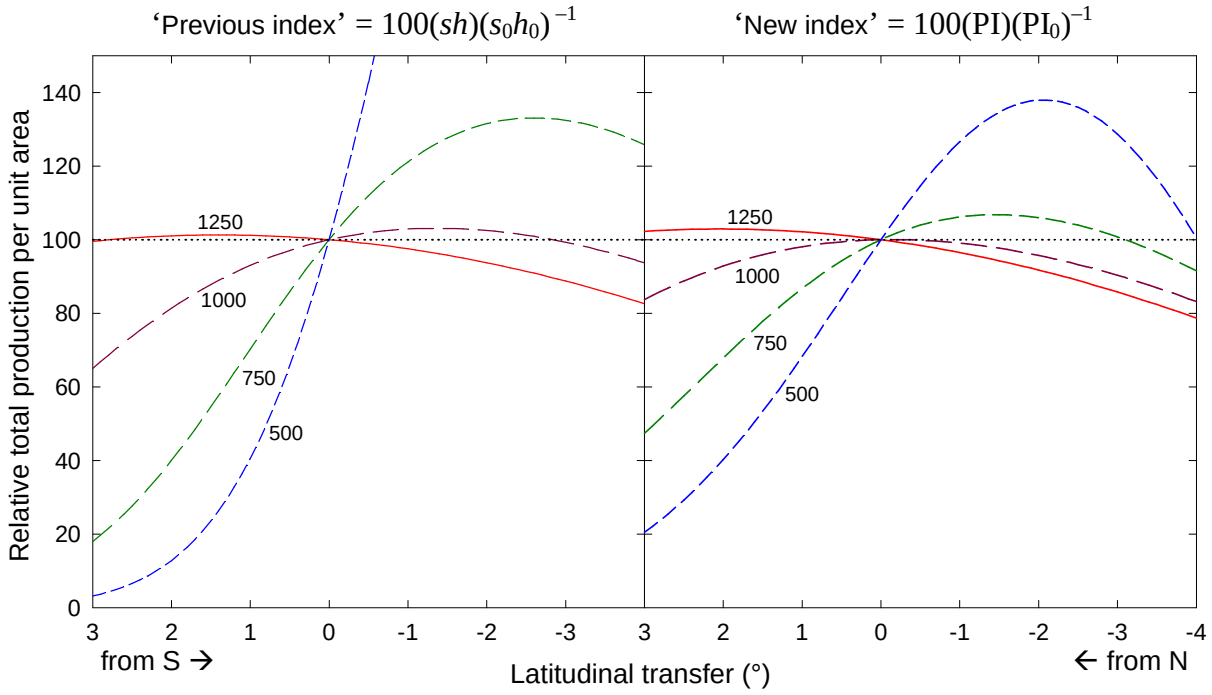

Fig. 3. Relative total production per unit area in varied climatic conditions (temperature sums ranging from 500 to 1250 degree-days) calculated as $100(sh)(s_0h_0)^{-1}$ and $100(\mathrm{PI})(\mathrm{PI}_0)^{-1}$, respectively, where $\mathrm{PI} = s^{0.42}h^{1.42}$. – Applied to Scots pine at sites with temperature sums according to Odin *et al*. (1983). Survival and mean height values for young stands for transferred (s and h) and local (s_0 and h_0) Scots pine were calculated from 'Persson's functions' for growth and survival in north Sweden (Persson & Ståhl 1993, Persson 1994b). The maxima of the curves suggest optimal transfers for maximum production. The 'new index' suggests the need for less southward transfer to harsh sites.

Tree height is an early growth trait that is distinct and easy to record, long before stem diameter and tree volume can be usefully estimated. Predictable mortality varies mainly as a result of climatic conditions. It is cumulative but, for Scots pine in Sweden, the rate gradually decreases during the first 5–20 years, depending on site harshness. Thus these two traits may be observed in relatively young plantations and field trials.

The product of survival (s) and mean tree height (h) at 10–20 years after planting is a frequently utilized measure of relative production potential, although sh is known to overestimate differences in production over longer periods of time (Marklund 1981). The importance of s tends to be over-emphasized, and that of h, which indicates the growth potential, under-emphasized. There have been some attempts to correct for this bias (see, for instance, Marklund

1981), but still no general approach is available. Consequently, forecasts to date only allow selection of the best material up to a relatively young age: 30 years or one third of a rotation (Persson 1994a, Rosvall *et al*. 1998).

Here we used a function concept similar to that of Bjørgung (1959), who derived a similar formula to predict stand volume from the number of trees per unit area and the dominant height. The production index (PI), described herein, could be used for more long-term forecasts – up to at least half a full rotation. One assumption is that our experimental data represent sufficient variation and representative combinations of hardiness and growth ability. However, other factors could invalidate the general applicability of the index. Experience indicates that high mortality increases patchiness in a stand (see, for instance, Diggle 1982). In this case, dense initial spacing reduces the likelihood of patchiness after thinning, giving more trees to sample from. More sparsely planted stands (around 2000–2500 seedlings per hectare is common nowadays) may, therefore, be slightly less productive than the estimates presented here. It is reasonable, however, to assume that relative comparisons between varying seed sources should, essentially, be correct. This hypothesis requires further testing on other field trials with sparser initial spacing (cf., e.g., Eiche 1966).

The validity of including trial 116B Laxå in this study is open to question because of its southerly location. It was included after thorough consideration, mainly in order to provide more data from sites with relatively low growth rates. Also, of all trials, 116B had the most incomplete data set (many missing trees), but its inclusion was justified by the fact that the site-specific factors (c_i) captured so much variation in site conditions. One remaining uncertainty concerns possible imbalances between provenance entries with regard to missing trees. The relatively robust results indicate that this is not of great importance, which is also a conclusion for the other field trials. Trees have in most cases turned into ‘missings’ fairly early in the stand history and thus they generally contribute very little when the total production exceeds 50 $m^3 \cdot ha^{-1}$. The same applies to the estimates of removed volume by thinning after measurement in 1968, when approximate tree volumes were estimated by breast height cylinders. The insignificance of the influence was verified by replacing the total production with standing tree volume in 2002 for estimating the parameters *a* and *b*. The results were surprisingly similar (data not shown).

We hope that the results can be generalized to cover a whole rotation period, which would be likely to place still more emphasis on growth at the expense of early survival ability. However, the cautious tree planter may wish to decrease the risk of failure (mortality) and thus use especially hardy seedlings for harsh sites. It should be noted that the use of the PI derived here is a retrograde step in this respect, since hardiness is more favored by the hitherto commonly used *sh* index (Fig. 3).

For the Scots pine breeder, the PI represents another tool for improving the relative weight placed on the two most important traits for northern Sweden: Survival ability and growth capacity. It remains for breeders to carry out a profitable implementation when selecting advanced generations.

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Genetic Evaluation Using the TREEPLAN® System

T.A.McRae[1], G.W. Dutkowski[2], D.J. Pilbeam[3], M.B. Powell[1] and B. Tier[4]

Southern Tree Breeding Association Inc., Mount Gambier[1], SA, and Bunbury[3], WA, Australia.
[2] Cooperative Research Centre for Sustainable Production Forestry,
University of Tasmania, Hobart, Australia.
[4] Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia.

ABSTRACT

The TREEPLAN® genetic evaluation system is designed for the efficient and accurate prediction of genetic values in trees for breeding and deployment purposes. TREEPLAN® fits a reduced individual tree model BLUP, and can model multiple genetic groups, handle clonal data, fit multi-trait models, accommodate heterogeneous variances, and fit site specific models. TREEPLAN® is fully integrated with a web based data management system that efficiently handles data and pedigree information. The Southern Tree Breeding Association (STBA) is using TREEPLAN® for genetic evaluation in the Australian tree improvement programs for *Pinus radiata*, *Eucalyptus globulus* and *E. nitens*. TREEPLAN® enables data across generations to be combined in a multi-trait analysis to produce single lists of genetic values for each trait for given production environments. TREEPLAN® is easy to use and has the 'industrial strength' to handle large amounts of unbalanced data with complex pedigree structures. The systematic approach adopted by the STBA for genetic evaluation makes full use of all related pedigree and performance data. This has led to cost savings and other operational efficiencies in the programs, while increasing genetic gain. It can be difficult to predict the commercial performance of genotypes in plantations using data from trees measured at an early age in small plot experiments. Despite this, genetic values should be expressed in terms of breeding objective traits, which are those influencing profit and/or other community issues. Economic weights are applied in multi-trait indices to derive profit equations for purposes of ranking genotypes on economic worth. These profit indices are then used by STBA for selection of trees for breeding purposes and by member companies for decision making in commercial deployment programs.

INTRODUCTION

The Southern Tree Breeding Association (STBA) was formed in 1983, and runs national tree improvement programs for *Pinus radiata* and *Eucalyptus globulus* in Australia. These two species comprise about seventy percent of the national plantation estate of 1.6 million hectares, with *P. radiata* primarily used for solid wood products and *E. globulus* for pulp and paper production. The establishment of cooperative breeding programs for *P. radiata* (1983) and *E. globulus* (1994) resulted in the consolidation of genetic resources and amalgamation of existing breeding programs of a number of member companies. As a consequence, large amounts of performance data for both species that had been collected over time on trees from hundreds of research experiments and genetic trials were made available to the Association. The STBA is continuing breeding of advanced generations, with testing and selection activities resulting in the accumulation of more data and traits for genetic analysis. The TREEPLAN® genetic evaluation system (Kerr *et al*. 2001, 2002; McRae *et al.* 2003) was developed for the purpose of analysing this performance data on a program wide basis, as existing software was inadequate for the task.

It is generally accepted that best linear unbiased prediction (BLUP) is the optimal and preferred statistical method for breeding value prediction. Although BLUP is not new technology, its industry wide application in trees had been limited in the past by a lack of computer power and software capability. Genetic analysis was usually done on subsets of data from single generations with limited sites and the fitting of single trait models. As a result, genetic evaluation was done inefficiently, breeding values were often biased, and it was also difficult to compare genetic values for trees included in different analyses. Although the STBA adopted the individual tree additive genetic model BLUP in its tree improvement programs during the 1990s (Jarvis *et al*. 1995), its application was limited to relatively small and uncomplicated data sets until the recent development of the TREEPLAN® system. The STBA with assistance from the livestock industry (Animal Genetics and Breeding Unit) and its other research members developed a working version of TREEPLAN® in 2001, which had the capability to process performance data on a national and/or international scale. The TREEPLAN® system was specifically designed to apply best practice analytical technologies to large scale commercial tree improvement programs like those managed by the STBA.

TREEPLAN® has allowed data with genetic linkage across generations, sites, years and age classes to be combined into a single multi-trait analysis to produce, for all genotypes, a complete list of genetic (breeding) values for each trait and environment combination. TREEPLAN® is relatively easy to use and has the 'industrial strength' to handle large amounts of unbalanced data with complex pedigree structures. TREEPLAN® fits a reduced individual tree model for purposes of efficiency. TREEPLAN® can model multiple genetic groups, handle individual and clonal data, fit multi-trait models with more than 50 traits, accommodate heterogeneous variances (allows for differing heritability), fit site specific statistical and genetic mixed models, and also weights information across environments to account for genotype by environment interaction (GxE), and time to allow for age:age correlations.

A strength of the TREEPLAN® system is that it is fully integrated with a modern web based data management system that efficiently handles large databases with full pedigree information. The data management system acts as a dynamic repository for data and pedigree information and has the capability to handle multiple species. The system not only facilitates efficient storage and retrieval of data for genetic evaluation, but also the delivery of genetic values and other information to STBA member companies and nominated parties through the Internet. As new trials and traits are assessed the data are validated and entered into a database, and preliminary analyses are done on a single site basis using ASREML (Gilmour *et al.* 2001) or similar software to estimate variance and covariance components which are used to allow for differences in productivity and heritability between sites. When TREEPLAN® is run on the full data set, breeding values for all trees (genotypes) in a specified population are updated using all information available on the individual genotype itself, any correlated traits, and information from all relatives in the pedigree. Prediction of genetic values is now a more dynamic and relatively straightforward process, such that TREEPLAN® breeding values are updated regularly as traits are measured, data compiled and validated. TREEPLAN® is now being used to routinely update genetic values in *E. globulus*, *P. radiata* and *E. nitens* on a program wide basis, and is easily adapted for other species.

This systems approach that has been adopted by STBA in TREEPLAN® genetic evaluation makes much more effective use of all pedigree and performance data, but also has led to other operational efficiencies, including cost savings in the programs.

The Genetic and Statistical Models

The statistical approach used in TREEPLAN® is designed for maximal efficiency as it includes all the design effects used in simpler analyses, but can incorporate in a combined analysis all of the data that has been collected for different traits and across all pedigrees. It fits a linear mixed model of the form:

$$\mathbf{y} = \mathbf{Wf} + \mathbf{Xr} + \mathbf{Yu} + \mathbf{Zs} + \mathbf{e}$$

where: **y** is the vector of observations on one or more traits; **f** is the vector of fixed site and design effects, with its incidence matrix **W**; **r** is the vector of random design effects, with its incidence matrix **X**; **u** is the vector of random additive genetic effects (breeding values) with its incidence matrix **Y**; **s** is the vector of random specific combining ability effects (SCA) with its incidence matrix **Z**; and **e** is the vector of residuals.

The estimates of the fixed and random design and genetic effects are obtained by solving the mixed model equations (MME's) (Henderson 1984) using the Gauss-Seidel iteration:

$$\begin{bmatrix} W'\mathbf{R}^{-1}W & W'\mathbf{R}^{-1}\mathbf{X} & W'\mathbf{R}^{-1}Y & W'\mathbf{R}^{-1}Z \\ X'\mathbf{R}^{-1}W & \mathbf{X}'\mathbf{R}^{-1}\mathbf{X}+\left[I\otimes\mathbf{G}_r\right]^{-1} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Y} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ Y'\mathbf{R}^{-1}W & Y'\mathbf{R}^{-1}X & Y'\mathbf{R}^{-1}Y+\left[A\otimes\mathbf{G}_a\right]^{-1} & Y'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}W & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}Y & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z}+\left[I\otimes\mathbf{G}_s\right]^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{f}} \\ \hat{r} \\ \hat{u} \\ \hat{s} \end{bmatrix} = \begin{bmatrix} W'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ Y'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix}$$

where, the new terms represent variance-covariance matrices of the errors (**R**), random design effects ($\mathbf{G_r}$), additive genetic effects ($\mathbf{G_a}$), and specific combining effects ($\mathbf{G_s}$) and the relationships between the additive genetic effects (**A**, the additive (or numerator) relationship matrix) and independent random effects (**I**), and ⊗ is the Kronecker product. The **R** and $\mathbf{G_r}$ matrices are specific to each site to allow site specific design and error (co-)variances.

This model offers substantial advantages over the models usually used in forest genetic trial analysis. Breeding values (and other genetic effects) are estimated for all traits, for all trees in the pedigree – both parents and offspring, in a single analysis. Where a trait has not been measured on a tree then the best prediction is made of its breeding value using information from relatives and from traits correlated at the genetic, design or error levels. If there is no such information, then the estimate is at the population mean, but the variance of the estimates grow as the amount of information, and thus its reliability, increases. The use of correlated traits allows correction for the effect of selection in measurement, as long as the data used for selection is included. The solutions to the mixed model equations give the highest correlation between true and estimated values, provided that the variances and covariances are known. This is a substantial improvement over best linear prediction (BLP), where the fixed effects are assumed to be known. The STBA has previously used BLP for breeding value estimation in *P. radiata* (White *et al.* 1992 ab). The mixed model equations are extremely robust, and can be readily extended to more complex models.

The model uses the numerator relationship matrix (**A)** to track the proportion of genes in common between trees in the pedigree and gives solutions for all of them without any secondary processing of the data in what has been called an individual tree model (ITM). It easily handles half-sib and full-sib pedigrees, and simple rules have been worked out (Henderson 1976) to create the inverse that is used in the MME's. The matrix can be modified for the types of pedigrees that are common in forest genetic trials: fixed provenance or selected parentage (such

as seed orchard) effects (Quaas 1988), partial selfing (Dutkowski and Gilmour 2001), and even pollen mixes (Perez-Enciso and Fernando 1992).

In forestry, parents of first-generation progeny are typically trees from native stands (or plantations) sampled from many different geographical regions that represent different provenances or races. Because provenances are quite genetically distinct it is important to assume that E($\boldsymbol{g}$) ≠ 0, where $\boldsymbol{g}$ is the vector of genetic values. Male parents are usually unknown and female parents are assumed to be unrelated. Seeds from the female parents (founders) are collected from various localities spread across a wide geographical area. Thus, it is reasonable to consider that progeny are from more than one genetically divergent sub-population. TREEPLAN® relates all foundation parents on the basis of their original provenance to genetic groups. In practice, data sets are likely to be far more complex, for example, a male parent (pollen) might be identified as belonging to a particular population, such as a routine or an improved population. Founders introduced from another unrelated breeding program might also constitute a different genetic group. The modified mixed model equations of Quaas (1988) are used to derive solutions to $\boldsymbol{g}$.

Trees can be partially self-fertile, generating pedigrees where two progeny may be selfed sibs (both progeny result from selfing), a selfed sib and an outcrossed sib, full-sibs or half-sibs. In the *E. globulus* breeding program, most progeny tested in the first-generation are derived from open-pollinated seed collected from founder trees in native forest stands. Until many more second-generation progeny (from controlled pollination crosses) are included in the analysis, the accuracy of breeding value prediction is dependent on how well the relationship coefficients between sibs of open-pollinated trees can be defined. Dutkowski and Gilmour (2001) have outlined simple rules to modify the NRM when a selfing rate in native stands is assumed. These rules can be further extended to account for the equilibrium level of inbreeding in the stand and the level of coancestry in trees local to the female parent from which seed was collected. Sparse and isolated stands of trees are expected to have a higher level of inbreeding among the progeny than dense stands. This functionality is currently being implemented in TREEPLAN®.

The software uses an equivalent gametic model for computational efficiency in the prediction of breeding values for trees without offspring (the majority).

$$y_i = m_i + \frac{u_f + u_m}{2} + s_j + f_i + e_i$$

where: m_I is the mean, y_i, s_j and e_i are as defined above, u_f and u_m represent the breeding values for the tree's female and male parents respectively, and f_i represents Mendelian sampling in the formation of the tree's genotype. That is, $0.5u_f + 0.5u_m$ represent 'average' gametes from each parent, and f_i represents the deviation from the average of the gametes received by the progeny. The genotypic and gametic models are equivalent models, in that the solutions to the unknowns will be exactly the same for both models. Their combined use is called a 'reduced' individual tree model (Quaas and Pollak 1980).

Details of key features of the TREEPLAN® system and its routine application of BLUP in forestry have been previously reported by Kerr *et al.* (2001, 2002) and McRae *et al.* (2003), and are only briefly discussed in this paper.

Trait Mapping to Selection Criteria

The mapping of multiple measured traits to a smaller number of meaningful selection criteria (SC) traits is a desirable feature of TREEPLAN®. In theory, each trait measured on each site could be treated as a unique trait, as long as all the variances and correlations are known. In practice, however, such an approach is computationally infeasible, not all variances and correlations are known, and dealing with output would be very confusing to the breeder, because of the many traits involved. The mapping of measured traits to SC traits, results in a reduction (consolidation) in the number of traits for which breeding values are predicted in a multi-trait analysis. This mapping gives TREEPLAN® its flexibility and ease of use as the breeder can easily define the SC of interest in a given analysis. The mapping allows the breeder to consolidate data with different forms and scales of measurement, different ages and sites, as long as it can be realistically assumed that all the measured traits are highly genetically correlated and can be treated as the same trait.

As an example, diameter at breast height (DBH) in *E. globulus* is measured at different ages and the SC traits defined are: DBH ≤4 yrs, DBH 5–8 yrs, DBH 9-12 yrs and DBH =12 years. As well as age differences, geographical location and site type are other possible criteria for proposing new SC traits out of the one generic trait such as growth. For example, it may be necessary to partition the SC trait, DBH ≤4 yrs, further in a multi-site run, according to province, state or soil type, in order to account for GxE. Although GxE due to scale effects is effectively removed by data transformation (standardisation), we must properly account for GxE where it results in a change of ranking of genotypes across environments. In practice, the best method to handle GxE is to consider the same character measured in two different environments as two different but correlated traits (Falconer and Mackay 1994). A trait measured at different locations can be considered biologically the same SC trait when the genetic correlation is high (for example, ≥0.8). Studies with more extensive data sets are currently being done in Australia to estimate across site correlations and better define the target production environments for *P. radiata* and *E. globulus*. Previous studies designed to quantify the magnitude and nature of GxE for these species have been based on too limited data sets.

Only traits displaying significant genetic variance in preliminary single-site analyses are included in genetic evaluation. The definitions of selection criteria, development of rules for the trait mapping, and estimation of correlations between criteria are based on periodic analyses of data in the database and meta-analyses of other estimates.

Heterogeneous Variances

Breeding programs collect data from trials spread across a diverse range of site types and age classes. Some traits are or have been assessed using different protocols and scales. For example, growth may have been measured as tree height, stem diameter or tree volume; and stem form assessed using several scales with different levels of precision. The variance of performance traits such as growth usually increases with size, growth rate and age of trees. A linear transformation of the data such that the phenotypic variance is unity and a simple model with standard genetic design and error variances is an approach often used in plant and animal breeding. This approach is probably sub-optimal as recent work (Dutkowski *pers. comm.*) has shown that this assumption is unrealistic in many instances. A disadvantage of this approach for tree breeding is that a constant heritability would need to be assumed across all sites, despite some sites being more homogeneous. Tree breeders usually have the benefit of large designed trials that usually provide good estimates of genetic, error and design feature (co-) variances for

each site. TREEPLAN® takes advantage of the availability of these estimates to transform the data for each trait to unit additive variance on a site by site basis and uses a model with site specific error and design feature variances to produce the genetic estimates. The recent analysis has also shown that this approach is very close to the ideal multivariate approach.

Clonal Data

Individual trees can be replicated using various forms of vegetative propagation. Clonal tests are common in *P. radiata* and are also used in some eucalypt breeding programs. TREEPLAN® currently treats clones as the same individual and matches unique clone identities to a single genotype. Clonal replication is important for accurately predicting clonal deployment values, but also for improving the precision of an individuals breeding value. An enhanced version of TREEPLAN® is currently being developed that will be capable of predicting genetic values, including additive and non-additive genetic effects, for individual clones, recognising the potential for somaclonal variation and propagation effects.

Running the TREEPLAN® system

An efficient data management system is critical for accessing data and pedigree information to produce breeding values quickly. The TREEPLAN® analytical system is fully integrated with a data management system (STBA-DMS^{TM}) which operates via a web based interface. TREEPLAN® could be run independently of the STBA-DMS^{TM}, but its interactive nature makes the process of genetic evaluation far more straightforward and efficient. It also facilitates data entry and analysis from various locations. The STBA-DMS^{TM} is mainly designed for storage and retrieval of tree data for the purposes of genetic evaluation. It is flexible and accommodates different species of trees. User access is restricted and data is password protected to the level of traits within trials. This allows us to easily complete multiple TREEPLAN® runs for the membership, firstly using only generic data, but then also including confidential data for traits belonging to a restricted group of members. This provides the flexibility needed in large cooperative tree improvement programs to satisfy individual client needs by producing customised genetic values.

TREEPLAN® extracts genetic parameters, data and run specifications from the STBA-DMS^{TM}, and making changes to specifications for a new TREEPLAN® run is straightforward. That is, it is a simple process to include (exclude) new trials and/or more traits in a multi-trait BLUP analysis. As new trials are assessed, the data is validated and entered. Multi-variate analyses are first done on a trial by trial basis using ASREML and the variances and correlations for all significant design and random genetic components estimated are stored in the STBA-DMS^{TM}. TREEPLAN® is then run with the complete database updating all genetic values for all genotypes, including individuals, founders and families.

Genetic Values for Breeding Objective Traits and Economic Indices

TREEPLAN® predicts breeding and genetic values for Selection Criteria which are the traits that are measured in trials and genetics experiments. These traits are usually used as surrogates for the Breeding Objective traits, which are those that directly affect profitability. For instance, currently the STBA uses three breeding objective traits for *E. globulus*: Harvest Volume, Basic Density, and Kraft Pulp Yield. Genetic values for the breeding objective traits can be readily estimated from the selection criteria using the method of Schneeberger *et al*. (1992), as long as

the correlations between the selection criteria and the breeding objective traits are known, or can be estimated, and the variance of these traits is similarly known. Breeding objective traits for each genotype are then combined into a single economic index with different economic weights applied to each trait, reflecting its commercial importance. Economic breeding objective functions have been developed for kraft pulp markets in *E. globulus*, and are being developed for alternative products in *E. globulus*, and for various products and markets in *P. radiata*.

Genetic Evaluation in *E. globulus* and *P. radiata*

TREEPLAN® is being used routinely by the STBA to predict genetic (breeding) values for trees included in its databases for *E. globulus* and *P. radiata*. Table 1 lists details of example data sets used in some recent runs of TREEPLAN®.

Table 1 — Example data sets used in recent runs of TREEPLAN® for *P. radiata* and *E. globulus*.

	Species	
	Pinus radiata	*Eucalyptus globulus*
Generations	3	2
Trials included in Analysis	78	90
Number of Selection Criteria Traits Analysed	21	11
Genetic (founder) Groups fitted	11	25
Families	3,110	1,610
Genotypes included in Analysis	134,767	152,170

Pinus radiata. Breeding values for 21 selection criteria for 132,700 genotypes were updated recently (Powell *et al.* 2004). This included data from 78 first-, second and third-generation trials spread across southern Australia. At this stage, growth selection criteria reflect six different production regions defined in the National Plantation Inventory for Australia (Wood *et al.* 2001) by four age classes (0-5 yrs, 6-12 yrs, 13-24 yrs and >24 years). Branch angle, branch quality, branch size, and stem straightness comprise the form traits. Basic density (0-12 yrs and =13 years) and spiral grain (0-6 yrs and ≥6 years) constitute wood quality traits. Data for disease and pest resistance/tolerance traits (*Phytophthora cinnamomi*, *Dothistroma septospora*, pine pitch canker (*Fusarium circinatum*), Cyclaneusma spring needle cast and Essigella pine aphid) are currently being incorporated. Some hundreds of historical first- and second-generation trials will be included as resources permit, with links to first-generation trials of all major lineages already included.

Current breeding objective traits are, harvest volume, whole tree basic density, branching and stem straightness (Powell *et al.* 2004). The current breeding objective function and coefficients in the selection indices for *P. radiata* have not been derived formally using economic data. However, the increased emphasis on density and other wood quality traits reflects the perceived economic importance of these traits as profit drivers in industry. The undesirable characteristics of juvenile wood that affect quality of timber, including grade recovery, strength, distortion,

surface checks, and finishing properties for structural timber are also increasingly more of an issue at harvest as rotation length reduces.

The STBA and CSIRO-FFP in partnership with the Forest and Wood Products Research and Development Corporation (FWPRDC) are currently undertaking research to clearly define economic breeding objectives for *P. radiata* which are more commercially relevant to STBA members and industry, including the impacts of juvenile wood properties. A study by Wu *et al.* (2004) has included estimation of genetic parameters among key breeding objective (harvest age) and SC traits measured at younger ages. Preliminary results by Ivkovich *et al.* (2004) using bio-economic models suggest mean annual increment, sweep, branch size and modulus of elasticity (MOE) will be important breeding objective traits for *P. radiata*. These traits and associated economic weights are likely to be adopted in selection indices by STBA in its breeding program and by industry in deployment systems in the near future.

Eucalyptus globulus. Breeding values for 152,170 genotypes in the national *E. globulus* database were recently updated in a multi-trait analysis with 11 SC traits using TREEPLAN® (Pilbeam *et al.* 2004). This included genetic values for native provenances (sub-races), native stand (founder) trees, first- and second-generation progeny. Data was from 90 trials, including 16 second-generation progeny trials, spread across Tasmania, Victoria, South Australia and Western Australia (Pilbeam *et al*. 2004), and includes all trials for which measurements are available. Breeding values for growth are predicted in four production regions by three age classes (0-4 yrs, 5-8 yrs and 9-12 years). Basic density, by two age classes, pilodyn penetration and NIRA predicted pulp yield comprise wood quality traits. Data for pest and disease resistances (defoliation), kraft pulp yield, NIRA cellulose content, collapse, shrinkage and tree form traits will be incorporated with time. Trees in the CSIRO collections (Gardner and Crawford 1987, 1988) are used to establish a baseline for monitoring genetic improvement over time.

The primary economic breeding objective for the national *E. globulus* tree improvement program is to maximise the net present value per hectare ($NPV) from forests grown for kraft pulp production. The breeding objective traits are harvest volume (VOL), whole tree basic density (DEN) and kraft pulp yield (KPY). Table 2 gives summary statistics for trees included in a recent TREEPLAN® run for *E. globulus*. The economic weights currently used by STBA were derived after the method of Borralho *et al.* (1993), and adapted by Greaves *et al.* (1997) and Dutkowski *et al.* (2000). Although the primary objective of most STBA member companies is to produce wood chips for kraft pulp markets, there is an increasing interest in sawlog regimes and other alternative products (Volker 2002). Research studies (Greaves *et al.* 2004; Whittock *et al.* 2004) are investigating alternative breeding objective functions for other products and markets including carbon revenues.

Table 2 — TREEPLAN® breeding values for volume (VOL), wood density (DEN) and pulp yield (KPY) for *E. globulus*. Averages for baseline, founder parents, and first- and second-generation trees are given for comparative purposes with selected groups of genotypes. Gains as a percentage of base productivity are given in brackets (%).

	VOL		**DEN**		**KPY**		**$Index**
*Base Productivity**	*313 m^3/ha*		*542 kg/m^3*		*55.7 %*		
CSIRO collection *(616 trees)*	0		0		0		0
Base Generation *(all native stand founder trees)*	0.42	(0%)	0.88	(0%)	0.01	(0%)	$27.69
First Generation	3.10	(1%)	0.47	(0%)	-0.02	(0%)	$45.06
Second Generation	51.41	(16%)	3.11	(1%)	-0.90	(-2%)	$466.93
Top 20 trees $Index	34.49	(11%)	72.88	(13%)	-0.72	(-1%)	$1,964.95
Top 20 trees VOL	132.69	(42%)	-19.10	(-4%)	-1.42	(-3%)	$814.55
Top 20 trees DEN	-8.09	(-3%)	87.47	(16%)	-0.74	(-1%)	$1,770.27
Best Provenance $Index	-0.61	(0%)	20.96	(4%)	-0.02	(0%)	$486.30
Worst Provenance $Index	-125.65	(-40%)	23.20	(4%)	1.18	(2%)	-$697.11

Future Enhancements

In partnership with AGBU and FWPRDC, STBA is developing an enhanced version of TREEPLAN® with additional features including: (1) Better modelling of intra-site environmental variation using spatial and competition models, (2) Incorporation of information at the DNA level (markers and candidate genes), (3) Modelling of dominance and epistatic effects to allow for the full exploitation of these non-additive genetic effects in family and clonal deployment populations, and (4) Development of a clearer understanding of GxE to better target different production environments.

CONCLUSIONS

The TREEPLAN® genetic evaluation system has facilitated the routine application of individual tree model BLUP in the Australian tree improvement programs for *P. radiata*, *E. globulus* and *E. nitens*. The system was developed for processing performance data from efficient rolling front breeding programs with overlapping generations, and has resulted in significant cost savings in genetic evaluation. TREEPLAN® has allowed data with genetic linkage across generations, sites, years and age classes to be combined into a single multi-trait analysis to produce for all

genotypes a complete list of genetic (breeding) values for each trait and environment combination. TREEPLAN® is easy to use and has the 'industrial strength' to handle large amounts of unbalanced data with complex pedigree structures. TREEPLAN® is fully integrated with a web based data management system that efficiently handles data and pedigree information, and delivery of genetic information to customers. TREEPLAN® generates accurate breeding and genetic values for measured traits using objective performance data collected in genetics trials. For commercial relevance, these values are converted into breeding objective traits in selection indices that are used for making breeding and deployment decisions.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support and contribution of data and information of STBA member companies. Appreciation is extended to representatives of STBA research members (CSIRO-FFP, CRC-SPF, Forest Science Centre, Forest Research and the Animal Genetics and Breeding Unit) for their valued inputs.

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Table of Contents - Posters

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Poster Presentations
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Overview Of European Larch Breeding And Improvement In The Czech Republic, Current State And Prospects

Josef **Frydl**
Forestry and Game Management Research Institute (FGMRI)
Jílovište – Strnady, 156 04 Praha 5 – Zbraslav, Czech Republic

European larch (*Larix decidua* Mill.), it is a very important forest tree species in the Czech Republic where Sudeten larch is the autochthonous population. This regional population has performed well both in experimental plantations and forest practices in the majority of European countries and also outside Europe, e.g. some regions of the USA and Canada. Sudeten larch is valued for its fast growth, considerable biomass production and especially as for the ecovalence, adaptability to various environment conditions. This is why this variety of European larch has been recommended to be cultivated in many European countries. Because of the importance of this regional population is of international character, Sudeten larch is known all over the world. There is considerable information, within the framework of provenance research particularly, about Sudeten larch which has been a subject of interest of both science and forest practice during past decades, both in the Czech Republic and elsewhere. Sudeten larch is widely recognised as an important basic material for both genetic research and practical-oriented breeding.

In the Czech Republic, the genetic variability of European larch has been studied there has been studied, in the frame of increasing the stability and productivity of forest ecosystems in relationship to geographic changeability, adaptation abilities to site and civilisation load. Besides the classical methods (provenance trials, hybridisation projects), new methods of molecular biology (e.g. isoenzyme and DNA analyses) are used for the changeability study, which enables to acquire information about the genome of this forest tree species much faster. In the Czech Republic, breeding programs elaborated for European larch are focused above all on preservation of genetic variability of populations (declaration of gene bases, establishment of seed orchards and clone archives). Breeding processes, which can be used for these purposes, including biodiversity preservation, are studied in details, as well as new biotechnological methods for micropropagation of well tested varieties from European larch breeding programs are searched. The target of breeding, as the practical application of knowledge from forest genetics in forestry practice, is to form and recommend the suitable populations and verified improved assortments of European larch for practical use in the Czech Republic forest management.

Stratified Sublining

D. Lindgren[1], S. Ruotsalainen[2,3] and M. Haapanen[2,4]
[1]Swedish University of Agricultural Sciences, 901 83 UMEÅ, Sweden, [2]The Finnish Forest Research Institute, [3] 58450 Punkaharju, Finland, [4] 01301 Vantaa, Finland

Stratified sublining arranges the breeding material in unrelated sublines ranked for breeding value. As a direct result of the stratification, the distribution of breeding values of the best unrelated clones, which will be used in seed orchards, is amplified. The strategy can be seen as "elite-main" or "PAM" driven to its extreme.

Stratified sublines can be constructed as follows (Ruotsalainen and Lindgren 2000):

1. Rank tested founders (F_0 breeding population) for breeding value;
2. Mate adjacent founders (Positive Assortative Single-pair Mating which is equivalent of creating F_1 stratified sublines derived from 2 founders);
3. Test individuals in offspring for breeding value;
4. Select the two best offspring in each F_1 family;
5. Rank the offspring pairs for their mean breeding value (F_1 breeding population);
6. Mate the adjacent offspring pairs (the best with the best and the second best with the second best);
7. Now F_2 stratified lines have been formed, each subline comprising 4 individuals. Each individual in the breeding stock will thus have 4 founders with a similar rank as grandparents. F_2-individuals in different sublines are not related whereas individuals within sublines are either full sibs or double first cousins.

Stratified sublining is powerful in supporting clonal seed orchards, as well as many other ways to transfer genetic gain from the breeding population to the production population, at least two breeding generations ahead. In the first generation, stratified sublining is identical to positive assortative mating (PAM), which has turned out to be effective in combining diversity with genetic gain. Two generations ahead, compared with assortment to sublines at random, which has been considered for *Pinus taeda* (McKeand and Bridgwater 1998), the superiority of stratified sublining, mostly due to the effect of PAM, is above 15%. Stratified sublining is also highly capable of supporting family forestry, as crosses of the best parents will be tested, and as unrelated sublines with superior breeding value will be created. This will constitute a superb starting material for developing clones for clonal forestry.

Starting with the maximum number of sublines (half the number of founders) makes it possible to completely avoid inbreeding for at least three generations of breeding. As the development of coancestry remains strictly confined to sublines, the number of unrelated selections available to form a seed orchard is equal to the number of sublines in the breeding population. This number is halved if all sublines are pairwise merged to obtain unrelated trees to regeneration matings. Since inbreeding within sublines must be kept rather low to avoid harmful effects, the potential of drawing unrelated selections to seed orchards may be significantly reduced due to the merging after three or more (depending on the size of the founder population) generations of breeding. At this point, different management strategies can be used. Feasible options involve, for example, enriching sublines with fresh material, or allowing the accumulation of inbreeding within

sublines, or abandoning the sublining completely. The long term prospects are likely to be better or at least as good as if stratified sublining had never been applied. In other words, it is not likely to appear a penalty in the far future for applying stratified sublining in the first generations.

Stratified sublining will be implemented in the recently developed Finnish breeding strategy. Therefore, the concept as a component in the Finnish breeding strategy and options to strengthen the implementation were considered. In the following a summary is given of the planned way of implementation of stratified sublines in Finnish breeding programs for Scots pine and Norway spruce.

To further boost the effect of stratification and to obtain additional genetic gains from future seed orchards, the Finnish breeding strategy involves the idea of distributing breeding, testing and selection efforts unequally, making the effort positively dependent on genetic value of the material being improved. This principle is implemented throughout the breeding cycle. In the first generation turnover, the founders forming the first-generation breeding population (160 individuals) are single-pair mated with regard to breeding value (in the way described above). Those of the founders that are ranked to the highest quarter are, however, double-pair mated to to allow more options for recombination of their gene mass and reduce the risk that their genemass is degraded by an unfortunate choice of partner, as well as a way to increase the number of offspring. Furthermore, the target sizes of F_1 families are larger (160 seedlings) for the best quarter of the parents than for the average parents (120 seedlings) or for the lowest quarter of parents (80 seedlings). The F_1 families (the recruitment population) are grown in forward selection trials that last from 5 to 10 years depending on species. At this age, the best individuals within each full-sib family are phenotypically selected for further testing. Roughly three times as many selections (candidates) are drawn from within the larger families (representing offspring of the best parents) than from the smaller ones. In the two stage selection system applied, the candidates are then clone or progeny tested for about 12 to 15 years to more accurately determine their true breeding values. The genetic testing is essential for the new round of stratification to be successfully carried out at the end of the second breeding cycle.

The number of candidates selected from each full-sib family to the new breeding population varies in relation to the mean breeding value; three individuals are selected from the best full-sib families (determined as the mean breeding value of the top 3 candidates), two individuals from the average families, and one (possibly none) individual from the lowest ranking families (Ruotsalainen and Lindgren 2001). As the result, the second generation breeding population, comprising 100 individuals, will have an unbalanced structure where the size of the stratified subline is six, four or two trees for the highest ranking, the average and the lowest ranking candidates, respectively. This method results in a overrepresentation of the gene mass of the best founders whereas a high number of low ranking founders will still be represented, but with relatively little genetic contributions.

One of the many options for the management of stratified sublines could be to use gene mass of the best founders to strengthen the lowest ranking lines. The relatedness between sublines would probably not be too big a problem as the low ranking lines are anyway unlikely to support seed

orchards in the near future. Breeding material available has often characteristics which justify or require modifications in the construction of sublines.

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Estimated Genetic Paramaters And Gain From Two Series Of *Pinus Brutia* Ten Open Pollinated Progeny Trials In Mediterranean Low Elevation Breeding Zone

Öztürk, H.[1] Isik, K[2]. ;Siklar S[1].; Alan, M[1,] Ezen, T[1]., Korkmaz, B[1]., Gülbaba, A. G[3]., Sabuncu, R[4].;Tulukçu, M.[1]; Derilgen, S.I.[1]

[1] Scientist, Forest Tree Seeds and Tree Breeding Research Institute P.O.B. 11 Gazi/ANKARA/ 06560 TURKEY
[2] Professor, Akdeniz University, Faculty of Arts and Sciences, Antalya/TURKEY
[3] Scientist, Eastern Mediterranean Research Institute, Karabucak/Tarsus/ TURKEY
[4] Scientist, Western Mediterranean Research Institute, Antalya/ TURKEY

In this study, two series of open pollinated progeny trials, which were established for low elevation Turkish red pine breeding zone (0-400m), were investigated. Open pollinated seeds collected from 168 clones in six seed orchards were used to establish first series of progeny trials in Fethiye, Antalya and Ceyhan in 1998. The second series of progeny trials originating from natural stands in subsequent year. Tree height was observed at age 4 in both series, and breeding values of the families for height were estimated by using BLUP method. B type genetic correlation were used to investigate genotype-environment interaction the among sites.

Individual heritability was estimated 0.15±0.02 for tree height in the first series of progeny trials, and, 0.22±0.04 for second series. Family mean heritability for height in the first and the second series were 0.48±0.06 and 0.64±0.05 respectively. B type genetic correlations between sites ranged from 0.58 to 0.63 in the first series and 0.51to 0.88 in the second series.

Genetic gain for height (relative to controls) at age 4, which obtained from phenotypic seed orchards was 8.1%. After rouging, by leaving the best 20 clones in each seed orchard, genetic gain reached 13% for height. Genetic gain from the first generation genotypic seed orchards consisting the best 30 clones was estimated 24.9% in the first series and 14.6% in the second series.

Evaluation Of Genetic Parameters For Branch Quality In Maritime Pine (*Pinus Pinaster* Ait.)

O. License[1], **P. Alazard**[2] and A. Raffin[3]

[1]Graduate Student, [2]Research Engineer and Tree Breeder, AFOCEL, [3]Research Engineer and Tree Breeder, INRA
AFOCEL, Domaine de Sivaillan Les Lamberts, 33480 Moulis en Médoc, France
INRA, UMR Biogeco, 33612 Cestas Cedex, France

Large genetic gains have been realized through seed orchards from breeding of maritime pine in Aquitaine (south western France). Gain in growth and stem straightness should reach 30% for present varieties compared to non improved material. The breeding programme is now focusing on new selection objectives such as wood quality, especially with branch quality traits. The aim of this study was to estimate the genetic parameters (heritability, genetic coefficient of variation, genetic correlations) of different selection criteria for branch quality, in order to evaluate their interest for the breeding programme.

In addition to usual growth and form measurements (tree height, circumference at 1.30 m, stem straightness) and disease notations (*Dioryctria sylvestrella* and *Melampsora pinitorca* presence or absence), 4 branch quality traits were assessed using a score system (polycyclism, presence of branch defects, branch thickness and branch angle), on a 8 year old progeny trial planted in 3 contrasting sites with 35 complete blocks of single tree plots on each site. The trial comprised 95 half sib families together with seed orchard seedlots and non improved seedlots controls.

Despite low to moderate heritabilities, significant gains on branch quality traits are likely to be achieved, due to high coefficients of variation. These results should allow to select elite trees as parents for the next generation of seed orchards (SO4), using a compromise index between growth, stem straightness and branch quality. The use of a score system combining branch defects, thickness and insertion angle proved to be useful in data analysis and for the integration of branch quality as a quantitative trait in the breeding index.

Early Performance and Genetic Parameters for Six European Chestnut Populations Tested in Greece

P.G. Alizoti[1] and F.A. Aravanopoulos[1]

[1]Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece (E-mail: alizotp@for.auth.gr)

European Chestnut (*Castanea sativa* Mill.) is a multipurpose tree species of great economic importance to Europe, with an extended distribution that harbors populations under different management practices, as natural, coppice, naturalized populations and orchards. Gene flow among domesticated and wild populations, due to transfer of germplasm across Europe for economic purposes, raised issues concerning the extend and nature of genetic variation among and within populations, adaptation of populations to specific environments and their potential to adapt in future environmental changes. Six natural European chestnut populations, representing two contrasting environments of the species' distribution in Greece, Italy and Spain, were assessed for survival, growth and adaptive traits, at the age of four, in two provenance-progeny trials established in Greece. Each population was represented by twenty six open pollinated families. The main objectives of the study were to: (1) determine whether the six European populations differ for survival, growth, and adaptive traits, (2) characterize the genetic architecture among and within the populations, and (3) determine whether the existing genetic variation is amble enough to secure future adaptation to environmental changes and make decisions concerning genetic conservation.

Remarkable differences were recorded among chestnut populations for all traits studied. The significant interaction among populations and sites, indicated different population plasticity to divergent sites and environmental conditions. Significantly better survival was recorded for the populations originating from South Spain, France and Greece. Southern populations initiated growth earlier, while there northern populations exhibited better height growth. Populations originating from Greece and Spain were characterized by more basal shoots, while the greater branch number was recorded for the northern Greek population.

Family differences within populations were large for all the traits studied. Height growth and bud flushing were under moderate genetic control, which varied greatly among individual populations. Among branching traits the strongest genetic control was estimated for the number of primary branches. Coefficient of additive genetic variance ranged among 9-15% for growth traits, 5-13% for bud flushing and 18-30% for branching traits, indicating the potential for artificial selection.

This early evaluation showed that there is amble genetic variation among and within European chestnut populations for growth and adaptation traits, and indicated the potential for selection and breeding, as well as the evolutionary potential of natural populations to adjust to future changes of the environment. Long-term evaluation is crucial, as it will provide evidence on early selection effectiveness and the confidence to tackle the issue of the species' conservation strategy.

Repeatability for Oleoresin Yield Determinations in Southern Pines

J. H. Roberds[1] and B. L. Strom[2]

Flow of constitutive oleoresin is believed to be a major component of tree defense against attack by the southern pine beetle (*Dendroctonus frontalis* Zimmermann). Pines that exude large quantities of oleoresin are considered to be most capable of preventing or obstructing colonization by this destructive insect herbivore (Hodges et al. 1979; Cook and Hain 1987; Strom et al. 2002). We evaluate a tree's capacity to resist attack by assessing resin yield over a fixed time interval from one or more small wounds made on a tree's bole at or near breast height. These wounds are administered so as to sample resin from a zone proximal to the bark, the region containing tissues directly affected by *D. frontalis* during an attack. For resin yield measured in this way, it is desirable to determine the relative contribution of variation among multiple measurements taken from individual trees compared to phenotypic variation existing in populations.

Repeatability (r) is a population parameter that provides information pertinent to this issue. It is by definition, a measure of the correlation between multiple measurements taken on the same individual and represents the fraction of the total variation in populations attributable to variation among individuals. In quantitative genetics, estimates of repeatability are routinely used to obtain upper bounds for broad sense heritability, and to determine the appropriate number of measurements needed per individual to provide accurate estimates of individual trait means and breeding values (Falconer and MacKay 1996; Mrode 1996).

To acquire information about the distribution of variation for oleoresin yield within stands of three southern pine species, we estimated repeatability for measurements taken in the vicinity of breast height for this trait. Resin samples were collected from eight populations located in the central Gulf region of southern United States. Although our primary interest is focused on values for loblolly pine (*Pinus taeda* L.), we also obtained estimates for two populations of longleaf (*Pinus palustris* Mill.) and for a single population of slash (*Pinus elliottii* Engelm. var. *elliottii*) pine. Estimates from these populations primarily occur in the interval r = 0.5 to 0.7,

[1] Research Geneticist, Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service, 23332 Highway 67, Saucier, MS 39574-9344; [2] Research Entomologist, Bark Beetles and Invasive Insects, Southern Research Station, USDA Forest Service, Alexandria Forestry Center, 2500 Shreveport Highway, Pineville, LA 71360.

indicating moderately high repeatability. These results suggest that for oleoresin yield in populations growing on average and better sites, greater variation is likely to be found among trees as opposed to within trees.

For traits in which it is possible to take multiple measurements on each tree, variation among tree means can be partitioned into true variance among trees plus an error variance that depends on variation among measurements within trees and the number of measurements per tree. Error variance of this type decreases with increasing repeatability and also with increasing numbers of measurements per individual. If repeatability values are close to one, a single measurement per tree is sufficient to produce low error variance, but with low repeatabilities, additional measurements are required to reduce this error variance to appropriate levels. For the range of repeatabilities we observed for breast-height resin yield, two or three measurements per tree suffice to provide satisfactory reduction of error variance for most estimation purposes.

Tree resin defense is almost always evaluated from samples collected at or near breast height. This practice is dictated by logistical convenience; however initial attacks by the southern pine beetle most frequently occur at bole heights of three to five meters (Coster et al. 1977). Such attack behavior makes it desirable to obtain a method for assessing resin yield in this upper bole region from measurements taken near breast height. Because of this need, we developed equations for predicting tree resin yields at a bole height of 4.5 meters from yield assessments made at a height of 1.5 meters. An allometric approach combined with regression methods was employed to produce linear prediction equations based on logarithms for the two resin yield variables. Data collected from resin samples taken from two loblolly pine populations located in central Louisiana were used to estimate equation parameters. Equations for both populations provided reasonable fits to the sample data (R^2 = 0.68, 0.76), and in each population, regression coefficients were found to be significantly greater than zero and significantly less than one (b = 0.592, 0.845). Corresponding intercept estimates for the two populations were also in the interval, zero to one (a = 0.299, 0.111), with only the value for the first population being significantly greater than zero. Under a hypothesis of equivalent tree resin yields at the two heights, estimates for the regression coefficients would not be expected to differ from a value of b = 1.0, and intercept terms would not be expected to differ from a value of zero. Our results indicate that resin yields at the two heights, although correlated (r = 0.72, 0.84), are not equivalent across the entire range of resin yields observed at a height of 1.5 meters, especially in the portion of the range consisting of high yield values. As a consequence, high resin yielding trees appear to be somewhat more resistant when evaluated at a height of 1.5 meters than they actually are, since their resin yields tend to be lower at a height of 4.5 meters.

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New Swedish Seed Orchard Program

Ola Rosvall[1] and **Per H. Ståhl**[2]

[1]Senior Researcher, [2]Research Director
Skogforsk, The Forestry Research Institute of Sweden, Umeå[1] and Uppsala[2], Sweden

Swedish forestry is now gradually switching their seed source of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L Karst.) from the first to the second round of seed orchards, and at the same time in cooperation establishes a third set of orchards. Tree breeding enhances genetic gain in wood production by about 10% every 20 years, which fits well with the planting of new seed orchards at the same intervals. Swedish orchards reach full seed production after 15 years and have a life span of approximately 40 years.

Generally, at present, 25% increase in long-term wood production per unit area can be reached with new seed orchards. By 2010–2020 a gain of 35% can be achieved. Norway spruce will proceed more rapidly than Scot pine since clonal testing is applied in the spruce breeding program. About 40% pollen contamination in production seed orchards and up to 20% natural regeneration in planted stands causes realized gain to vary between 16 and 28%.

There are 14 seed zones for each of Scots pine and Norway spruce. An ordinary seed orchard is made up from 25 clones, which are deployed in higher frequency the greater the breeding value. The genetic diversity corresponding to at least status number = 15. Scots pine orchards are planted at 3 m spacing within rows at a distance of 7 meters. The tree rows are regularly pruned into hedges where the first cut is carried out when they have reached 3 m height. Spruce orchards are preferably planted at 2.5 by 7 m spacing in order to enhance early seed crops. They are later thinned to 5 m within-row spacing. The tree tops are cut off after mast years, whereupon new leading shoots will regenerate.

The third round of orchards is estimated to cost $12.5 millions including establishment and management until the first seed crop. This corresponds to $0.0025–0.005 per seedling or $0.08 per additional cubic meter of wood produced. The internal rate of return is estimated to 7%.

There is a tradition in Sweden to run seed orchards in cooperation. In the 3:d round of orchards the forest owners' associations in Sweden are new partners among the large forest companies, the national forest enterprise, and independent forest nurseries. Each collaborator owns his share of an orchard, which is managed by an entrepreneur, generally a forest nursery. To facilitate efficient administration cooperation is carried out in three regional groups. Skogforsk initiated the national program, produced the basis for decision making, and is coordinating the program under supervision of a board of representatives from the three cooperative groups.

Effect Of Spacing And Pruning Height On Early Production In A Scots Pine Clonal Seed Orchard

Curt Almqvist

Senior researcher, The Forestry Research Institute of Sweden (Skogforsk), Uppsala, Sweden

Drögsnäs experimental seed orchard was established 1990 in central Sweden (Lat 59°37' N, Long 12°57' E, alt. 80 m.a.s.l.). The objectives of the orchard were to evaluate the time to production and the production capacity for different combinations of spacing and pruning heights. 16 different combinations of spacing and pruning height were tested. Spacing ranged from 178 stems/ha (7.5×7.5 meter) up to 4000 stems/ha (1.0×2.5 m), and the pruning heights ranged from 2 to 7 meters.
The objective of the pruning regimes was to initiate the pruning when the grafts reached a height 0.2-0.5 meter below the intended height of the plot. Through annual pruning the crown was formed and the grafts reached the aimed height in five years. After that the annual pruning continued with the aim to reduce the height development to a maximum of 0.1 meter per year.

Pollen production started at the same age and increased at the same rate in all treatment combinations. In 1999 pollen was produced on 23 % of the grafts. In 2001 the percentage of grafts producing pollen had increased to 74 %. The following years, 2002 and 2003, the percentage increased further to on average 80% and 89% respectively.

Cone production has been measured annually 1996–2002. The highest total cone production for the period 1996–2002 (7 years) 89 hl cones/ha was obtained on plots with 4000 stems/ha, and the lowest total production 11 hl cones/ha was obtained on plots with 178 stems/ha. The cone production corresponds to a total seed production in seven years of 62 Kg seed in the plots with 4000 stems/ha. The highest average production occurred in 2001, with the highest production, 33.9 hl cones/ha on plots with 4000 stems/ha and 2-meter pruning height. The lowest production this year, 6.1 hl cones/ha, was obtained on plots with 178 stems/ha aiming at 7 meter. As for pollen production grafts on plots with low spacing and not yet being pruned shows the highest per graft cone production.

A Clonal Seed Orchard of Wild Cherry (Prunus avium L.) Selection of Clones and Spatial Design

B.A. De Cuyper[1]

Forest policy in Belgium strongly promotes the use of indigenous hardwoods for re- and afforestation and for stand conversion. For wild cherry (*Prunus avium* L.) this option is even more motivated by the acknowledgement of its high silvicultural, ecological and economical importance. Wild cherry is a tolerant tree species allowing the establishment of mixed forest stands and is a relatively fast growing hardwood producing very high quality timber. Furthermore, the species is often mentioned as a potential alternative to poplar for the afforestation of abandoned and set-aside farmland.

This line of policy generates a strong demand for high quality forest reproductive material, which cannot be met by the currently available basic material. There is only a limited potential for the selection of seed stands due to the occurrence of wild cherry as individual trees or small clusters scattered throughout mixed forest stands. Seed orchards have been created in the past, yet are relatively unproductive, mainly due to their restricted area.

The selection and breeding programme attempts to remedy the discrepancy between supply and demand by creation of a new generation of clonal seed orchards characterised by (i) a high yield and (ii) a high genetic quality and diversity of the offspring.

MATERIAL AND METHODS

The establishment of a clonal seed orchard of wild cherry involves the achievement of a twofold objective: (i) the selection of the clones and (ii) the spatial design of the orchard.

Clones will be selected in a basic collection of 168 genotypes, i.e. phenotypically superior plus trees selected within 27 populations. Vegetative replica of these plus trees were planted in seven multi-clonal plantations.
Firstly, the genetic diversity of the basic collection was assessed using 14 microsatellite markers. Estimation of the genetic distance D between individuals was based on the proportion of shared alleles: $D = 1 - P$ with $P = \Sigma_u S/2u$ where the number of shared alleles S is summed over all loci u.
The half-sib progeny resulting from seed harvest in the multi-clonal plantations was evaluated on vigour, morphological and phenological traits and on disease resistance. For each trait, the narrow sense heritability h^2_A as well as the general combining ability GCA was determined, allowing the construction of a selection index: $I = \Sigma_i (h^2_A.GCA)_i$ with i = 1 to n and n = number of traits.

[1] Senior Scientist. Institute for Forestry and Game Management, Gaverstraat 4, B-9500 Geraardsbergen, Belgium

Once clones have been selected, there spatial arrangement within the seed orchard has to be designed. Wild cherry is entomophilous, with bumble bees (*Bombus* spp.) acting as main pollen vectors. Bumble bees display a particular foraging behaviour, which leads one to suspect a small-scaled patch-like pollination pattern within wild cherry populations.
As a test-case, one of the above-mentioned multi-clonal plantations was chosen, consisting of 65 accessions and surrounded by ten 'natural' wild cherry populations situated at various distances. In order to unravel the pollen flow (i.e. assessment of internal mating patterns as well as pollen input from outside the plantation) a parenthood analysis was carried out using 14 microsatellite markers. The objective was to trace the father tree of 30 half-sibs randomly chosen within the offspring of 16 selected accessions (i.e. mother trees)

Once the internal mating pattern has been essessed, the spatial arrangement of clones in the future seed orchard will be based on the knowledge of

i. Their flowering phenology based on the observation of the proportion of receptive flowers each 48 hours during 38 days.
ii. Their self-incompatibility genotype using consensus and allele-specific PCR.

RESULTS AND DISCUSSION

Scoring of 14 microsatellite markers revealed 8 identical genotypes within the basic collection. As wild cherry often regenerates through root suckering, plus trees selected within the same population can indeed be identical.
Based on the value of the selection index, 63 genotypes were chosen as constituents of the future seed orchard. Their genetic distance varied between 0.41 and 0.87.

Parenthood analysis showed that 68 % of the pollen donors were located within the multi-clonal plantation. Regarding the internal pollen flow, about 75 % of the pollen came from fathers located within a radius of 10 m from the mother tree, i.e. twice the planting distance (Figure 1).

Tracing of external pollen donors revealed that 82 % of the pollen input came from fathers situated within a wild cherry population located at a distance of 490 m from the plantation studied.

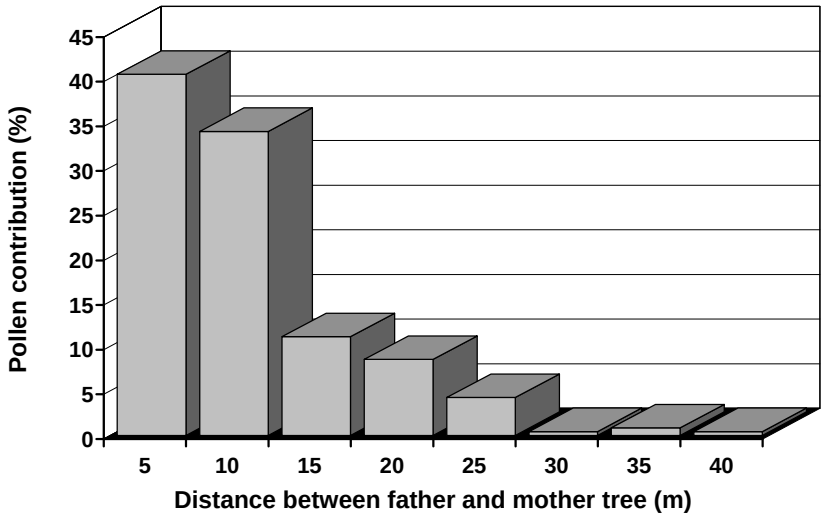

Figure 1. Relation between pollen contribution of father trees and their distance from the mother tree in a multi-clonal plantation of wild cherry.

In conclusion, a small-scaled patch-like pollination pattern is observed within wild cherry populations. The standard for minimum spatial isolation of a seed orchard can be set at 500 m.

The self-incompatibility genotypes of the 63 selected accessions were determined and all (semi)compatible combinations were identified. In addition, six 'new' *S*-alleles, previously unknown in sweet cherry cultivars, were detected and numbered S_{17} to S_{22}.

Observation of the flowering phenology resulted in a cross-tabulation of the overlap in flowering period for all possible combinations of selected accessions. Only combinations with an overlap exceeding 25 % were considered to be phenotypically cross-compatible.

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USDA Forest Service, Forest Health Protection, Resistance Screening Center Services

Carol H. Young

Resistance Screening Center Manager, USDA Forest Service
Resistance Screening Center, 1579 Brevard Road, Asheville, NC, USA

The Resistance Screening Center (RSC) is operated by the Forest Health Protection unit of the USDA Forest Service, Southern Region, State and Private Forestry. The Center is located at the Bent Creek Experimental Forest near Asheville, NC, USA. The Center evaluates seedlings for resistance to disease, primarily fusiform rust (caused by *Cronartium quercuum* F. sp. *fusiforme*) and pitch canker (caused by *Fusarium circinatum*) as a service to tree improvement specialists, seed orchard managers, scientists, government agencies, research institutions, universities, and private industry. Testing enables clients to obtain information on the relative resistance of their materials in much less time than is possible in field progeny tests. The RSC has the flexibility to modify current screening procedures to accommodate specialized requests. This allows researchers to use the RSC as an additional experimental tool. In a research assistance capacity, the RSC has played an important role in newly developed understanding of genetic interactions in the pine-fusiform rust pathosystem and will continue to do so in the foreseeable future. By using information from the Resistance Screening Center tests, trees producing resistant progeny can be identified or questions may be answered concerning such things as the nature of pathogen variation or the effectiveness of fungicides. The RSC remains open to service screening work or research endeavors in an effort to improve forest health.

White Pine Blister Rust Resistance In *Pinus Monticola* And *P. Lambertiana* Seedling Families Following Artificial Inoculation With Two Sources Of Blister Rust

R.A. Sniezko[1] and A.J. Kegley[2]

[1]Center Geneticist and [2]Biological Scientist, USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, Oregon, USA 97424

Western white pine (*Pinus monticola*) and sugar pine (*P. lambertiana*) are highly susceptible to the non-native invasive pathogen, *Cronartium ribicola*, the cause of white pine blister rust. In Oregon and Washington the USDA Forest Service has utilized artificial inoculation of seedlings to evaluate western white pine (WWP) and sugar pine (SP) families for genetic resistance to *C. ribicola*. Several types of resistance have been documented, including a hypersensitive response in the needles (HR). In 1997 two different sources of blister rust were used to inoculate a common set of WWP and SP families. One source of rust was virulent (*vcr2*) to HR in WWP, and the other source (*AVCr2*) was not. Both species were inoculated concurrently with a single source of rust; inoculations were approximately two weeks apart. Seedling families included in this trial were half-sib progeny of field selections, families that showed HR or bark reaction responses in previous tests, orchard seedlots, and several full-sib checklots from another Forest Service resistance program (Region 1). First symptoms of rust appeared unusually early in these trials. For WWP, dramatic differences among the two inoculum types were present in percentage seedlings with stem symptoms (SS) for seedlots known to have HR (100% vs. 49% for the *vcr2* and *AVCr2* inoculums, respectively); mortality was 100% for these families when inoculated with the *vcr2* source. SP families with HR showed similar levels of SS and survival regardless of inoculum source. Two SP families purported to be homozygous dominant (*Cr1Cr1*) for HR showed a low frequency of SS and mortality under both inoculum sources. WWP bark reaction families showed high levels of SS with both inoculum types (97% and 91%) and showed little or no difference in level of bark reaction (29% and 31%) or survival (15% and 20%). Several of the WWP open-pollinated bark reaction families showed the highest survival of any non-HR seedlots over both inoculum sources, slightly exceeding the Region 1 full-sib families. In contrast, the SP bark reaction families had generally lower levels of bark reaction (7.9% and 0.7%) and survival (4.3% and 3.7%). The level and range of bark reaction, SS, and survival for the previously untested WWP and SP half-sib families was generally low.

The European Research Programme FRAXIGEN: Ash for the Future, Defining Ash Populations for Conservation, Regeneration and Ecological Adaptation – Works in Greece

K. A. Spanos[1], R. M. Papi[1,2], D. P. Mylonas[1], D. Gaitanis[1] and D. A. Kyriakidis[2]

Abstract: Microsatellite DNA markers (nSSRs, cpSSRs) have been used to study genetic diversity and phylogeography of *F. ornus* and *F. angustifolia* native populations in Greece. Genetic diversity was high within populations whereas differentiation was low among populations. Artificial pollinations have been carried to study reproductive biology of the two species and also inbreeding effects. Pollination treatments applied in *F. ornus* demonstrated that selfing can result in lower number of seeds and lower seed germination in comparison to cross either with male or hermaphrodite. Pollen from male trees proved to be more effective compare to pollen from hermaphrodites in relation to the number of seeds produced and seed germination. Possible inbreeding impacts of selfing should be studied in a long term progeny testing. In field testing, selected seed sources were found not to be significantly different ($P>0.05$) for survival, whereas the significance between planted sites was high ($P<0.01$). In relation to height growth, significant differences were found between tested seed sources ($P<0.1$) and also between planted sites ($P<0.01$). Results demonstrated low interactions ($P>0.05$) between seed sources and planted sites for the recorded parameters (survival, height growth). Long term testing (at least 10 years) of field trials is advisable for evaluating genetic adaptability of the tested seed sources in different environments.

Keywords: Genetic diversity, population, microsatellites, pollination, inbreeding, seed source, adaptation, *Fraxinus.*

INTRODUCTION

In this work, *Fraxinus ornus* and *F. angustifolia* indigenous populations have been identified and selected to study genetic variation, reproductive biology and ecological adaptation under the framework of the European research project FRAXIGEN (EVK2-CT-2001-00180). Nuclear and chloroplast DNA markers (nSSRs ?a?cpSSRs) developed for *Fraxinus excelsior* (Brachet et al. 1999, Heuertz et al. 2001, Morand-Prieur et al. 2002, Papi et al. 2003) and new ones were used to estimate genetic variation of both species and to investigate gene flow within and among populations. The same molecular markers were used to study reproductive system and to estimate the extent and rate of relationship between individuals. The three species have different types of mating system, which affect their biology and the level of genetic diversity. They have also two different reproductive systems (autogamy and allogamy) and two types of pollination (wind-pollination and insect pollination) (Binggeli and Power 1991, Dommee et al. 1999, Spanos

[1] N.AG.RE.F. – Forest Research Institute, 57006 – Vassilika, Thessaloniki, Greece

[2] Aristotelian Univ. of Thessaloniki, Department of Chemistry, Laboratory of Biochemistry, 54006 Thessaloniki, Greece

et al. 2004, Verdu et al. 2004). To study biology and inbreeding effects, controlled pollinations carried out in two *F. ornus* and one *F. angustifolia* populations.

It is generally acceptable, that local genotypes have the capacity to adapt to local conditions and should be preferred where it is appropriate. Timber categorization and certification adopts this policy for conservation of genetic diversity and use of local provenances (Ennos et al. 1998, Spanos et al. 2004). Study of adaptation of natural ash populations to their environment, and how selection based on economic criteria has affected their adaptive capacity is also one of the FRAXIGEN goals. By the use of a network of reciprocal transplants experiments (RTEs), established under natural conditions, the rate and the extent of local adaptability (reproductive vigor, seed and seedling survival, competition with other species, long term adaptation) will be estimated. Production of a guide-book for seed collection, utilisation and conservation of ash natural resources is also intended.

MATERIALS AND METHODS

Ten (10) indigenous populations of *Fraxinus ornus* and nine (9) of *Fraxinus angustifolia* have been selected (Table 1 & 2) to study genetic variation, reproductive biology and ecological adaptation of the two species. Seed and leaf material was collected (in autumn/late spring) in the years 2001, 2002 and 2003. Microsatellite primers designed for *Fraxinus excelsior* and new ones developed for *F. ornus* (BIONOSTRA, Spanish Company) have been tested for SSR variation in *F. ornus* and *F. angustifolia* populations. Primers used are listed as follows (Table 3): FEMSATL4, FEMSATL11, FEMSATL16, FEMSATL19, M2-30, FR16, FR39 and FR41 Amplifications of the nuclear SSR loci have been completed for all populations and allele scoring is in progress. Conserved cpDNA primers (ccmp2, ccmp3, ccmp4, ccmp6, ccmp7 and ccmp10) (Table 3) have also been used to identify chloroplast DNA regions in both species for phylogeographic analysis. Leaf and seed material was collected from the tested populations and DNA was extracted (using DNeasy Plant Mini Kit, Qiagen) in the years 2003/2003/2004. PCR amplifications were performed (MJ Research Peltier Thermal Cycler) and PCR products were multiplexed for gel separation. LI-COR IR^2 DNA sequencer was used for SSR loci separation (6% w/v polyacrylamide gel electrophoresis). To study reproductive biology and inbreeding effects, controlled pollinations have been carried out in two *F. ornus F. angustifolia* populations (Table 4). Pollinations were carried out in spring (*F. angustifolia* -February, *F. ornus* - April/May) 2002 and 2003 using specially designed pollination bags. Seed was harvested in autumn (November) 2002 and 2003 (separately for each tree and each treatment). Parameters recorded were as follows: mean number of seeds/infrutescence, mean seed weight (mean of 10 seeds) and cumulative germination (% sown seeds). For the field trials, seed of each population was subjected to warm (2 weeks, 20-25° C) and cold stratification (10 weeks, 0-4° C) to enhance germination. After stratification, seed was sown (March, 2002) in the soil from which each population originate. Germination occurred in April/May, 2002 -and seedlings were left to grow at the FRI nursery till planted in the field sites (February/March, 2003). Field trials (reciprocal provenance trials) were established using 1-year-old seedlings to test vigor and local adaptation. Parameters recorded were: survival (% planted seedlings) and height increment.

Table 1. Natural populations of *Fraxinus ornus* identified and used for seed and leaf collection

Code number	**Population**	**Latitude (°)**	**Longitude(°)**	Altitude (m) (a.s.l.)
9FOR	Melia	41.00.573 N	26.04.451 E	348
10FOR	Stavroupolis	41.14.384 N	24.39.905 E	332
11FOR	Nevrokopi	41.16.604 N	23.44.341 E	817
12FOR	Nigrita	40.51.764 N	23.24.013 E	542
13FOR	Poligiros	40.26.500 N	23.19.100 E	577
14FOR	Kastania	40.26.370 N	22.24.412 E	300
15FOR	Stomio	39.52.599 N	22.39.751 E	380
16FOR	Lamia	38.57.165 N	22.24.131 E	520
17FOR	Lidoriki	38.39.473 N	22.13.107 E	920
22FOR	Kalavrita	37.56.291 N	22.04.093 E	870

Table 2. Natural populations of *Fraxinus angustifolia* identified and used for seed and leaf collection

Code number	Population	**Latitude (°)**	**Longitude(°)**	Altitude (m) (a.s.l.)
8FAN	Melia	40.58.300 N	26.07.400 E	155-170
9FAN	Komotini	40.59.506 N	25.23.300 E	10-15
10FAN	Nestos	40.53.704 N	24.46.470 E	18-20
11FAN	Doirani	41.14.701 N	22.46.357 E	50
12FAN	Ierissos	40.27.550 N	23.49.000 E	10-20
13FAN	Omolio	39.53.575 N	22.37.186 E	16-20
14FAN	Evia	38.49.144 N	23.25.25 E	120
15FAN	Louros	39.09.190 N	20.45.778 E	50
16FAN	Kalavrita	37.56.291 N	22.04.093 E	870

Table 3. Microsatellite DNA markers used to study genetic diversity and phylogeography of the two *Fraxinus* species.

Genetic diversity (nSSRs)		Phylogeography (cpSSRs)	
Fraxinus ornus	*Fraxinus angustifolia*	*Fraxinus ornus*	*Fraxinus angustifolia*
FEMSATL4	FEMSATL4	ccmp2	ccmp2
FEMSATL16	FEMSATL11	ccmp3	ccmp3
M2-30	FEMSATL16	ccmp4	ccmp4
FR16	FEMSATL19	ccmp6	ccmp6
FR39	M2-30	ccmp7	ccmp7
FR41		ccmp10	ccmp10

Table 4. Pollination treatments applied to *Fraxinus ornus* and *F. angustifolia* populations.

Population	Treatment			
Fraxinus ornus				
21FOR	S [1]	HM x HM [2]	HM x M [3]	OP [4]
23FOR	S	HM x HM	HM x M	OP
Fraxinus angustifolia				
11FAN	S	HM x HM	HM x M	OP

[1] S - self pollination, [2] HM x HM - cross with hermaphrodite, [3] HM x M cross with male, [4] OP - open pollination.

RESULTS AND DISCUSSION

Results on genetic diversity (all tested populations) and population differentiation of *F. ornus* and *F. angustifolia* are shown in Table 5. Genetic diversity levels were found high within populations whereas differentiation level was low among populations.

Table 5. Preliminary analysis of genetic diversity and differentiation of *F. ornus* and *F. angustifolia* populations tested in Greece.

	Diversity		**Differentiation**	
	Ho	**Hs**	**G_{ST}**	**F_{ST}**
F. ornus	0.820	0.948	0.018	0.022
F. angustifolia	0.709	0.849	0.080	0.090

Results of pollination treatments are presented in Table 6 and 7. Results revealed significant differences ($P<0.01$) between the treatments for the number of seeds/infrutescence. Selfing in all cases (tested trees) produced the lowest rates (Table 6). Open pollination and cross with male pollen (treatments) were more effective (overall mean 208,2±22,0 and 147,8±10,6, respectively) in comparison to selfing and cross with hermaphrodite pollen (40,6±9,0 and 87,9±7,1 respectively). Significant differences ($P<0.05$) were also found between the different trees for the mentioned parameter (Table 6).

Table 6. Mean number (± s.e.) of seeds/infrutescence for the different pollination treatments in *Fraxinus ornus* (Chortiatis, Thessaloniki).

	Pollination Treatment				
Tree	S	HM x M	HM x HM	OP	Mean * [1]
1	-	91.3±28.0	97.5±9.8	129.0± 24.9	*102.2±10.7*
3	111.2 ±0.2[1]	277.2±81.2	36.7 ±10.1	253 ±30.5	*174.5±31.1*
4	2.5 ±1.5	131.7±20.6	10.75±3.7	273.8 ±11.9	*129.6±30.9*
6	-	329.4±31.5	88.7±64.0	469.8 ±112.9	*265.7±48.1*
7	76.9 ±27.0	134±40.4	74.7±20.8	423 ±133.9	*142.8±33.5*
8	7.5 ±0.9	118.3±8.3	109.4±11.6	103±26.9	*102.3±8.1*
9	5.0±2.0	150±22.0	125.6±20.4	167.2±43.0	*132.1±16.6*
10	-	212.3±14.8	102.5±13.5	86.6±21.0	*127.5±21.5*
11	-	62.2±6.9	76.2±31.7	66.3±18.3	*66.2±8.3*
12	8.8 ±2.5	228.2±54.4	22.4±11.5	148.4±55.9	*118.4±28.9*
13	114.1 ±44.6	196±33.5	-	334.6±63.8	*206.6±30.8*
14	11.4±2.4	83.4±11.6	95 ±15.3	115±24.8	*70.7±8.0*
Mean**[1]	*40.6±9.0*	*147.8±10.6*	*87.9±7.1*	*208.2±22.0*	

[1] * significant difference (P<0.05), ** significant difference (P<0.01) (ANOVA -test), - no data.

Results on cumulative germination of seed produced by the different pollination treatments are shown in Table 7. Significant differences were found between treatments (P<0.05 for all trees) and also between trees (P<0.05 for all treatments) (ANOVA - test) for germination. The seed produced from selfing gave the lowest germination percentages in all trees. The treatment HM x HM (cross with hermaphrodite) resulted in lower germination in some trees (e.g. tree No. 3 & 12) compared to HM x M (cross with male).

Table 7. Cumulative germination (% sown seeds) of *Fraxinus ornus* seed produced by different pollinations.

Tree		Date					
		17/4/2004	23/4/2004	30/4/2004	7/5/2004	14/5/2004	21/5/2004*[1]
	S	0.67	12.00	24.00	34.33	34.33	34.33
3	HMxHM	1.68	13.45	42.86	62.18	63.03	63.03
	HMxM	0.33	20.67	46.00	58.00	58.00	58.00
	OP	5.33	57.67	80.33	84.00	84.00	84.00
8	S	0.00	0.00	10.00	10.00	10.00	10.00
	HMxHM	7.67	55.33	75.33	79.67	80.33	80.33
	HMxM	3.67	21.33	62.00	78.67	78.67	78.67
	OP	6.30	41.70	61.70	75.00	75.00	85.00
9	S	0.00	20.00	30.00	30.00	30.00	30.00
	HMxHM	3.67	26.33	46.67	59.33	59.33	59.33
	HMxM	6.33	33.67	56.33	74.00	74.00	74.00
	OP	6.70	39.30	68.00	79.30	82.00	82.00
12	S	3.23	11.29	19.35	20.97	20.97	20.97
	HMxHM	10.98	39.02	43.90	45.12	45.12	45.12
	HMxM	12.33	56.67	75.00	78.33	78.33	78.33
	OP	14.30	55.00	70.00	72.30	72.30	72.30
13	S	1.00	18.67	47.67	60.67	62.00	62.00
	HMxHM	22.87	69.77	76.74	78.68	78.68	78.68
	HMxM	14.33	51.67	80.00	82.33	82.33	82.33
	OP	23.70	62.00	82.00	84.70	84.70	84.70
14	S	0.00	1.33	15.33	31.33	31.33	31.33
	HMxHM	0.00	3.00	19.00	51.33	52.00	52.00
	HMxM	5.67	24.00	45.00	57.00	57.00	57.00
	OP	14.30	52.70	77.70	91.00	91.00	91.00

[1] * significant difference ($P<0.05$, ANOVA -test, between treatments and between trees).

Results on survival and growth of the tested seed sources in the field trials are presented in Tables 8 and 9. Significant differences ($P<0.01$) for survival were found between the planted sites but not between the tested seed sources ($P>0.05$, ANOVA -test). Survival was higher in Nevrokopi (94.9 %) and Poligiros (82,7%) in comparison to the other sites, and this is most

possibly due to environmental differences (soil/humidity). Interaction was found not significant (P>0.05).

Table 8. Survival (% of planted seedlings) of *Fraxinus ornus* seed sources planted in the tested sites[+].

SITE	Provenance Code 10FOR	11FOR	12FOR	13FOR	14FOR	15FOR	Mean **
Stavroupolis (10)	89.5	63.3	79.6	71.1	68.0	90.0	*76.9* [bc]
Nevrokopi (11)	96.9	97.0	95.3	87.1	96.4	96.8	*94.9* [a]
Nigrita (12)	62.5	41.3	60.2	62.4	58.9	52.7	*56.3* [d]
Poligiros (13)	82.6	79.3	89.0	85.9	76.5	82.6	*82.7* [b]
Kastania (14)	53.7	81.2	73.7	68.7	72.5	79.4	*71.5* [c]
Stomio (15)	35.9	26.7	50.0	35.8	52.9	59.1	*43.4* [e]
Mean [ns]	*70.2*	*64.8*	*74.6*	*68.5*	*70.9*	*76.8*	*71.00*

[+] ns - not significant difference (P>0.05), ** significant difference (P<0.01, ANOVA -test), means with the same superscript letter do not differ significantly (P>0.05, Duncan test).

In Table 9 height increment results of the tested provenances in the planted sites are presented. Significant differences at 10% level (P<0.1) were found between the tested seed sources. Seed source 14FOR and 15FOR gave the best results. Significant differences (P<0.01) were also recorded for the different planted sites. Site Nevrokopi gave the highest height increment (overall mean 81.2 mm). The interaction provenance x site was not significant (P>0.05) for height growth.

Table 5. Height increment (year 2003) of *Fraxinus ornus* provenances in the tested sites (mean ± standard error, mm) [+].

SITE	Provenance Code 10FOR	11FOR	12FOR	13FOR	14FOR	15FOR	Mean
Stavr(10)	39.5±4.8	22.2±2.0	31.6±3.0	28.7±3.4	34.9±5.9	25.2±3.3	31.1±1.7 [a]
Nevr(11)	83.3±8.8	74.8±10.6	73.2±8.1	99.8±10.8	65.7±9.5	83.1±11.1	81.2±4.1 [d]
Nigr(12)	55.1±6.6	42.6±8.9	54.5±7.2	51.5±7.8	56.0±11.0	43.3 ±6.0	51.6±3.3 [b]
Polig(13)	68.3±8.5	49.4±4.6	67.5±7.7	50.6±5.2	86.4±8.7	88.8±7.9	67.4±3.1 [c]
Kast(14)	57.6±9.8	68.5±9.1	66.1±7.6	58.5±10.0	77.1±12.3	71.8 ±8.7	67.2±3.5 [c]
Stom(15)	41.8±4.0	39.5±13.9	60.1±6.7	53.3± 9.5	72.8±11.8	54.6±10.7	53.8±3.5 [b]
Mean [1]	63.5±7.7 [fg]	57.1±7.3 [g]	62.5±7.4 [fg]	59.5±7.4 [fg]	72.2±7.9 [ef]	69.8±7.4 [fg]	63.6±7.5

[+] means with the same superscript letter do not differ significantly (P>0.1 within row or P>0.05 within column, T- test).

Differences between different seed sources/provenances as well also between planted sites for survival and growth of other broadleaves have reported in the past (Clausen 1984, Worrell 1992, Cundall et al. 1998, Worrell et al. 2000, Cundall et al. 2003). In our case study of early

provenance trials, we found highly significant differences between the planted sites and this is obviously due to different ecological conditions. There were not found significant differences between seed sources for survival but differences in height growth were significant (P<0.1). The interaction between seed sources and planted sites was low for both parameters.

CONCLUSIONS

- Nuclear and chloroplast microsatellite DNA markers ((nSSRs, cpSSRs) have been used to study genetic diversity and phylogeography of *F. ornus* and *F. angustifolia* native populations in Greece. Results demonstrated high genetic diversity inside the populations and low differentiation among populations.
- Artificial pollinations have been applied to study *F. ornus* and *F. angustifolia* reproductive biology and inbreeding affects. Pollination treatments applied in a *F. ornus* population demonstrated that selfing can result in lower number of seeds and lower germination in comparison to cross either with male or hermaphrodite. Pollen from male trees proved to be more effective compare to pollen from hermaphrodite individuals in relation the number of seeds produced and seed germination. Possible inbreeding effects of selfing should be studied in long term progeny testing.
- Reciprocal provenance trials demonstrated no significant differences between seed sources for survival, but significant between the planted sites. Significant differences (P<0.1) for height growth were found between tested seed sources and also between planted sites (P<0.01). Low interaction seed source x planted site were found for both parameters. Long term testing (at least 10 years) of provenance trials is advisable for adaptability expression of tested seed sources in the different environments.

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Genetic Diversity And Structure In Two Oak Species Populations In Israel; *Quercus Ithaburensis* Desc. *And Q. Bossieri* Reut.

L. Korol, G. Shklar and G. Schiller
vckorol@agri.gov.il
Department of Agronomy and Natural Resources, Agricultural Research Organization,
The Volcani Center, P.O.Box 6, Bet Dagan, 50250 Israel.

Two deciduous oak species grow in Israel. The *Quercus aegilops* L. *ssp. ithaburensis* [Desc.] (The Tabor oak), a thermophilous species growing at altitudes of between 50 and 1000 m a.s.l., on the eastern shores of the Mediterranean Sea. The area of distribution from the north to the south encompasses the Golan Heights, the upper Jordan valley, the Lower Galilee, the Mt. Carmel range, Samaria and the Coastal plains down to the vicinity of Tel Aviv. The second species, *Quercus boissieri* Reut., also grows on the Golan Heights, the Upper Galilee, the Carmel range, Samaria and Judea. The occurrence of these two species in Israel represents the most southern fringe of their global distribution in the Middle East. They are among the many Mediterranean oaks species of which we lack genetic knowledge that is needed as the basis for genetic conservation and forest management.

We hypothesized that as the result of strong fragmentation of the forest area since antiquity and the existence of the North-South and West-East geo-climatic cline, differences in the genetic diversity and structure among *Q. ithaburensis* and *Q. boissieri natural* populations in Israel are inevitable. The objective of the study was to acquire knowledge on within- and between - populations genetic diversity by means of RAPD molecular markers.

The analysis of 17 populations of *Q. ithaburensis* revealed that the total genetic differentiation (H_T) was 0.414, the average gene diversity within the populations (H_S) was 0.362 and the average differentiation among the populations G_{ST} was 0.126. The UPGMA analysis based on genetic distances revealed three main clusters of the populations that are coherent with the geographic regions of the country: 1- the Golan-Heights and Upper Galilee group, 2- The Lower Galilee group, 3- The Mt. Carmel, Samaria and Coastal plain group. The analysis of 14 relict populations of *Q. boissieri* revealed that the total genetic differentiation (H_T) was 0.398, H_S was 0.335, and G_{ST} was 0.162. Each population is unique in its genetic composition, the within-population high genetic diversity overshadows the differentiation among the populations. No relations could be established between the genetic and the geographic parameters.

Whereas the results of the genetic analysis in *Q. ithaburensis* support our hypothesis on the genetic differentiation of populations according to the geo-climatic conditions of a particular site, the results of the genetic analysis in *Q. boissieri* do not support it. Contrary to *Q. ithaburensis*, which is a thermophilous species and has probably spread through the area since the end of the last glaciations because of warming, *Q. boissieri* populations in the Lower Galilee, the Carmel range and in the Samaria and the Judean range might be relicts from the periods in which the spread of this species was based on the adaptation to colder and wetter continental climates. This pattern resembles the occurrence and distribution patterns of glacial relict plants such as *Pistacia atlantica*, *Pistacia khinjuk* at high elevations on Mt. Hermon in the north of Israel and on Mt. Santa Katrina about 600 km to the south in the Sinai peninsula.

Vitrification, A Feasible Cryopreservation Method For Conversation Of Silver Birch

L.A. Ryynänen[1] and **T.S. Aronen[1]**

[1]Senior Researcher, Dr.
Finnish Forest Research Institute, Punkaharju Research Station
Finlandiantie 18, FIN-58450 Punkaharju, Finland

Forest genetic resources, as a part of biodiversity and nature conservation, are nowadays highly appreciated in all the Nordic countries. In forest tree breeding their value has been understood for a long time, and different *ex situ* gene conservation strategies are applied in connection to breeding programmes. Cryopreservation – storage of tree germplasm in liquid nitrogen at ultra-low temperature – provides a modern tool for conservation of forest genetic resources.

Cryopreservation is an alternative or a duplicate storage for the traditional *ex situ* clone collections. In silver birch (*Betula pendula* Roth), in which the initiation of micropropagation from bud explants originating in mature trees is possible, cryopreservation can be applied to conserve specific genotypes. Endangered elite trees, and trees expressing rare, valuable or interesting characteristics can thus be saved in the minimum of space and maintenance, and without the risk of contamination, somaclonal variation or genotypic changes due to external factors.

Slow cooling cryopreservation protocols have been applied both for silver birch *in vivo* buds and *in vitro* meristems since the middle of 1990's. In the method the samples are frozen slowly at a defined cooling rate to a terminal temperature of 38°C before immersion in liquid nitrogen. Slow cooling allows cryodehydration of the cells; only extracellular ice is formed, when water is removed from the cells in which the solutions are concentrated to the point where they can be cryostored without intracellular freezing. However, expensive equipment must be utilized to carry out slow freezing.

In vitrification, i.e. fast cooling cryopreservation method no equipments are needed, but samples loaded with highly concentrated cryoprotectants are passed rapidly through the temperatures where ice crystal growth occurs, by immersing them directly in liquid nitrogen resulting no intracellular or extracellular ice crystal formation. In this study, a new vitrification protocol for cryopreservation of axillary *in vitro* buds of silver birch is presented. The protocol starts with four week's cold hardening of donor shoots bearing buds under SD conditions. After that excised axillary buds are precultivated in medium containing high sucrose concentration followed by preatreatment in medium containing glycerol and sucrose. Finally the buds are treated with PVS2-cryoprotectant mixture for even as long as 120 min after which they are directly immersed in liquid nitrogen. An average recovery of vitrified axillary silver birch buds is over 80%.

For both applications of cryopreservation, slow cooling protocol and vitrification, genetic fidelity of the cryopreserved material is of uppermost importance. No genetic or phenotypic changes have been detected after regeneration of plants using slow-cooling cryopreservation protocol for silver birch. Testing of genetic fidelity of vitrified material has still to be finished

before material can be considered as reliable for any research, breeding or silvicultural activities.

Application of breeding methods for the preservation and reproduction of gene sources of silver fir (*Abies alba* Mill.) and other species of genus *Abies* well adapted to conditions found in the Czech Republic.

J. Frydl[1], J. Kobliha[2]

Abstract: The main objective of this research project, is to progress the development of tree breeding techniques applied to the gene sources of silver fir (*Abies alba* Mill.) found in the Czech Republic; to enhance the species preservation, reproduction, by increasing its representation, volume production and general resistance, including partial substitution by new varieties. Methodical evaluation of research plots will contribute to other findings relating to the genetic variability of Silver fir, in particular, the vitality and production potential of silver fir partial populations. New findings should also be used within the framework of both seed crop management and seed zoning purposes.

This project also includes the interspecific and intraspecific hybridisation within the *Abies* genus. Hybrid progeny tests have been already established. In which hybrid progenies are compared with *Abies alba* Mill. and various exotic fir species. The best individuals within these hybrid progenies have been selected and subsequently propagated by cuttings. Clonal tests of the hybrid material have been also established.

Keywords: Czech Republic, breeding methods, provenance research, silver fir (*Abies alba* Mill.), gene resources, preservation and reproduction, research plots, seed zoning, interspecific and intraspecific hybridisation, clonal tests, vegetative and generative propagation.

INTRODUCTION

Silver fir (*Abies alba* Mill.) is considered to be the most productive domestic species, for the conditions of Central and Western Europe. In addition to high volume production, silver fir possesses other positive characteristics, allowing it to tolerate and often thrive on heavy-textured and settled soil, in particular on those pseudogley soil conditions found at mid and higher altitudes, where there is no viable alternative tree species. Besides volume production, silver fir contributes to the creation of desirable humus forms, by virtue of its needle fall, and promulgates good stability, especially when found as a component within a mixed stand.

A noticeable Europe-wide decline in silver fir over recent decades gave much cause for concern, especially among countries where silver fir is an important component of forest structure. In the Czech Republic however, this phenomenon is of long-term character, having been documented

[1]Research Scientist, Forestry and Game Management Research Institute (FGMRI/VULHM), Jiloviste – Strnady, Czech Republic
[2]Professor, Czech University of Agriculture in Prague (CAU/CZU), Czech Republic

by a series of information publications (Svoboda 1943, Bednar, Hosek, Raynoch, 1963, etc.). The reason for this decline is complex. The clear–cutting system of forest management connected with mass utilization of Norway spruce and Scots pine, used to be supposed as being the main factor. This type of forest management, especially when employed over long-term periods, is also known to be followed by increased threats to forest stands. These include climatic factors (frost, drought), changes to soil chemistry, insect and fungal diseases, and damage from game to which silver fir natural seeding is particularly susceptible. Colonisation of felled sites by other naturally regenerated species and the use of other species as restocking material are among the other factors leading to the decline of silver fir.

During last two decades, it has been observed, at a limited and localised level, a deceleration in the decline of silver fir, and in some areas an indication of improvement of previously "declining" stands. Nevertheless, the overall continuation of silver fir decline still exists, including losses of valuable gene sources, and a real danger of silver fir extinction in certain areas of the Czech Republic (e.g. Ore Mts., Jizerske hory Mts., etc.).

PRESERVATION AND REPRODUCTION OF SILVER FIR GENE SOURCES IN THE CZECH REPUBLIC

Historically, the preservation and reproduction of the silver fir gene source within the Czech Republic was managed in a variety of ways:

Natural forest reserves were established, which included the preservation and reproduction of the gene source as an objective of management. Silver fir is, as an admixed species, represented in 70 such natural forest reserves within the Czech Republic (Marsakova – Nemejcova, Mihalik et all, 1977).

Silver fir is also represented as admixture in 56 gene bases having been declared in various areas of the Czech Republic (Rambousek, 2002, 2003). Also, there is a relatively extensive area of forest stands with silver fir certificated for seed crop (reduced total area for silver fir is calculated to several thousands hectares from total area of these certificated stands of category A (221,02 ha).

Gene sources of silver fir are also preserved and reproduced both in seed orchards (1,60 ha) and seed stands (13,31 ha) plantations having been established by seed from stands certified for seed crop.

Although silver fir does not rank among the main economically important tree species considering its current low representation within species composition of Czech Republic forest stands, the potential of this species, nevertheless, presents itself as a subject of both forest research and practice interest. That fact is reflected in the numerous publications, which have previously been published in the Czech Republic. Mostly, these publications relate to the problems of silver fir decline. In particular, there are completed protocols in place, aimed at the preservation of silver fir as a species itself. Also to the preservation of individual regional silver fir gene sources, including breeding treatments having been established with the aim of, both

reaching the targets mentioned above, and to the effective use of silver fir in Czech Republic forest management.

It is therefore necessary to consider all treatments oriented to silver fir preservation and increasing this species representation in the Czech Republic forest stands as one of the most important, both within forest research and forest management disciplines.

BRIEF SURVEY OF PREVIOUS RESEARCH RESULTS

Within the research activities undertaken during the last decades, there have been a considerable number of findings, having important implications for both forest practice management and other forest research.

As examples, it is possible to mention the following, both of high importance for their utilization in the Czech Republic forest management:

- It has been found that silver fir provenances from the Slovak Republic (Carpathian areas) are comparable in economic value, volume production, wood quality and health characteristics to that of silver fir provenances from the Czech Republic (Sudeten Hercynian areas). On the basis of these results, it is possible, in the case of a lack of domestic reproductive material, to envisage reproductive material of Slovak origin being used within the Czech Republic.

- On the basis of provenance research, the positive prospects of grand fir (*Abies grandis* /Dougl./ Lindl.) and partly of noble fir (*A. procera* Rehd.), have also been recognised for possible use within the Czech Republic forest management. On the basis of these results, it was possible to formulate proposals of suitable geographic areas for eventual import of these species' reproductive material to the Czech Republic. Partial results having been obtained within the scope of interspecific hybridisation would indicate higher tolerances of some hybrid combinations to changing ecological conditions.

New common research project of Forestry and Game Management Research Institute (FGMRI/VULHM) Jiloviste - Strnady and Czech University of Agriculture in Prague (CAU/CZU) philosophy and its essential aims

In the Czech Republic, there has been prepared, proposed and approved, a new common research project of Forestry and Game Management Research Institute Jiloviste – Strnady (FGMRI/VULHM) and Czech University of Agriculture in Prague (CAU/CZU), for the period of 2004 – 2007.

This new research project is designed to explore the application of breeding methods for the preservation and reproduction of gene sources of silver fir (*Abies alba* Mill.) and other species of genus *Abies* well adapted to the Czech Republic conditions.

Essential aims:

- To undertake and evaluate silver fir breeding and improvement treatments, for the preservation and reproduction of this species' gene sources, having the aim of contributing to the enhancement, both for the preservation and expansion of silver fir representation in the Czech Republic forest stands, and the improvement in health condition and vitality for this species. From the methodical evaluation of selected research plots already established by FGMRI/VULHM Jiloviste – Strnady in previous periods, other conclusions regarding silver fir variability, and especially of its individual population vitality and volume production potential will be derived.

- These results should also be used, among others, for seed crop rectification in certified forest stands and also seed zoning purposes.

- Research activities will also be aimed at the understanding of another interspecific hybridization, including evaluation of clonal progeny of genus *Abies*, etc.

As the new project assignments are to be carried by two co–operative scientific work places (FGMRI/VULHM Jiloviste – Strnady and CAU/CZU in Prague), the planned activities are divided into two main parts:

- FGMRI/VULHM Jiloviste – Strnady will evaluate health condition and volume production, including evaluation of other qualitative and quantitative characteristics, in the series of 19 provenance plots established by FGMRI/VULHM in the period of 1973 - 1976 in (for) silver fir both optimal and non–optimal (inappropriate – extreme) ecological conditions.

- Evaluation of the oldest provenance plot with silver fir established in 1961 will present an important complementation of research activities planned to be completed by FGMRI/VULHM Jiloviste – Strnady.

- 10 research plots with Czech and Polish silver fir provenances established by FGMRI/VULHM during 1990 – 2001 in the Czech Republic will also be evaluated by research team of FGMRI/VULHM Jiloviste – Strnady. The results of this evaluation will contribute to other considerations and conclusions connected with silver fir reproductive material zoning specifications, which will include consideration in relation to possibilities and real needs of Polish silver fir reproductive material importation.

- FGMRI/VULHM Jiloviste – Strnady will evaluate the health condition and volume production, which will include an appraisal of other qualitative and quantitative characteristics, in a series of provenance plots established in the period 1980 – 1986 with various exotic, and introduced species of genus *Abies* (e.g. *Abies balsamea, A. grandis, A. concolor, A. procera, A. cephalonica, A. pinsapo, A. fraserii, A. lasiocarpa, A. nordmanniana, A. cilicica,* etc.). Obtained results will contribute to another consideration concerning the prospects of exotic and introduced species of genus *Abies* in the Czech

Republic forest management.

- CAU/CZU in Prague will aim research activities to obtain new findings about flowering and fructification of hybrid clonal progeny of genus *Abies* in hybrid seed orchards.

- CAU/CZU in Prague will also evaluate progeny from those hybridization projects already completed in previous periods, with the aim of selecting the most appropriate variants for further breeding programs.

- CAU/CZU in Prague will carry out research into the different growth characteristics of plant material having been propagated by generative and vegetative means.

CONCLUSION

This research project is consistent with, and relevant to the needs of the Czech Republic forest management. The resolution of the problems associated with our long–term efforts to preserve and increase the silver fir gene resource, and the species representation in Czech forest stands, will considerably extend the usefulness and augment the number of findings obtained so far. This project will also emphasise the possibilities of their practical use in the Czech Republic forest industry.

The results from this research will add considerably to other findings concerning the genetic variability of silver fir, especially in relation to the vitality and production potential of silver fir partial populations. New findings from provenance research of both domestic and exotic species of genus *Abies* will contribute to the specification required for reproductive material resource zoning, transfer and importation. These research activities, focusing on both interspecific and intraspecific hybridisation, include the testing of progeny from vegetative and generative propagation. This is consistent with demands to develop new methods for use in the areas of preservation and conservation, while increasing the valuable genetic resources of silver fir, including the practical use of other well-adapted species of genus *Abies*.

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ENCLOSURES

Photo 01. Silver fir (*Abies alba* Mill.) provenance plot No. 211 established in 1961. FGMRI/VULHM Jiloviste – Strnady, locality Bane. (Photo Josef Frydl 2004)

Photo 02. Silver fir (*Abies alba* Mill.) provenance plot No. 211 established in 1961. FGMRI/VULHM Jiloviste – Strnady, locality Bane. (Photo Josef Frydl 2004)

Photo 03. Silver fir (*Abies alba* Mill.) provenance plot No. 211 established in 1961. FGMRI/VULHM Jiloviste – Strnady, locality Bane. (Photo Josef Frydl 2004)

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Photo 07. Silver fir (*Abies alba* Mill.) provenance plot No. 67 established in 1975. Part of plot with some exotic species of genus *Abies*. Forest Enterprise Pelhrimov.
(Photo Josef Frydl 2000)

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Map Construction Based on Orthologous Makers, Wood Property Traits and Candidate Genes in *Pinus sylvestris*.

S. Pettersson, R. García-Gil and A. Fries [1]

The use of DNA markers for marker-assisted selection (MAS) has received considerable attention among plant and animal breeders in the past 10-15 years. Although MAS is thought to make an immense contribution in tree breeding, the application is still rare. Before the technique can be efficiently used, a dense comparative map composed of accurate QTL (Quantitative Trait Locus) positions and QTL-candidate genes co-locations must be constructed.

Pinus sylvestris L. (Scots pine) has the widest distribution of all pine species that extends from the south of Spain (38^{o} N) to the north of Scandinavia (68^{o} N), and from western Scotland (6^{o} W) to eastern Siberia (135^{o} E). More than half of Sweden is covered by forest and *Pinus sylvestris* (Scots pine) stands account for 39%. Scots pine is one of the most relevant species since it is the most important source of wood. The Swedish forest industry accounts for 13% of the total export activities in Sweden (Ekberg, 2003). Improvement of the selection on economical favourable traits, such as frost hardiness, tree height, stem diameter, fibre and wood quality in this species would make a considerable cost reduction.

The Scots pine cross that will be used was made in 1988 at the Forestry Research Institute of Sweden in Sävar (Skogforsk). This cross was chosen for three important reasons (1) the progeny size is exceptionally large (1000 individuals) compared with the small progeny sizes used in all previously-published QTL analysis in pine; (2) QTL analysis was already performed in an small set of this progeny (100 individuals) across four years, which will allow for further QTL stability evaluation (Lerceteau et al., 2001; Yin et al., 2003) and (3) the cross is currently used in a conventional breeding program, this means that the effectiveness of our method can be tested directly on a real breeding program.

In the present project we intend to increase power of QTL position estimation by increasing the mapping pedigree size (up to 500 individuals). The same controlled cross was formerly used to construct an AFLP framework map based on 100 individuals (Lerceteau et al., 2001; Yin et al., 2003). In order to align our map with other pine genetic maps available in the literature we will use orthologous markers such as SSR and ESTPs. QTLs for wood properties, height and frost hardiness will be located together with a set of candidate genes. The same methodology used for QTL location will be applied to located ELPs (Expressed Level Polymorphsisms) of our candidate genes. ELP location is a tool for the study of interrelation of gene action (Jansen and Nap 2001).
This will be the first comparative map that integrates, QTLs, candidate genes and ELPs in *Pinus sylvestris*.

[1] Graduate student, Assistant professor and Assistant professor respectively.
Department of Forest Genetics and Plant Physiology, SLU, Umeå, Sweden.

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The Design And Application Of Universal DNA Microarrays For The Management And Improvement Of Tropical Timber Species In SE Asia.

C.H. Cannon[1], M.J. Asif[2], and C.S. Kua[3]

[1]Assistant Professor, [2]Postdoctoral Research Associate, [3]Research Advisor,
Department of Biology, Texas Tech University, Lubbock, TX USA

The application of genomic evidence in the management and improvement of SE Asian tropical timber species is a particularly challenging problem. Timber harvests from any one producer normally include numerous species from several different plant families. Hundreds of high-quality marketable timber species exist in Asian forests but virtually nothing is known about genomic structure, content, or variation in most of these species. Standard genetic marker systems, like SSR and AFLP analyses, require a labor and cost-intensive period of exploration for informative markers, which are often restricted to a single species or a group of closely related species. The statistical analyses required by these marker systems are often complicated and relatively inconclusive. These limitations greatly reduce the number and variety of timber species to which genomic techniques can be applied. To solve this problem, we propose a technique, using DNA microarray technology, which can detect genomic differences between any two or more sets of samples.

First, a large set of anonymous 25 base pair DNA probe sequences are produced. Candidate target sequences are chosen based upon a set of selection criteria, to optimize their usefulness in downstream applications. The selection criteria can differ according to the objectives of the genomic study. In our case, we chose to focus on DNA sequences that could perform optimally as PCR primers and would be rapidly evolving (non-coding sequence). These anonymous DNA sequences are then placed upon a microarray slide and hybridized with at least two different samples. These samples can consist of individual organisms or be composite mixtures, representing populations, geographic locations, or evolutionary lineages (the important 'unit' in the particular genomic study). The presence of unique DNA sequences in one sample can then be used to develop future PCR-based markers. This approach requires only a single successful hybridization and all subsequent verification and development can be performed using basic techniques and skills. Also, the results would consist of simple presence/absence tests on a standard agarose gel.

We are currently performing a case study on an endangered group of timber species collectively known as 'RAMIN' (*Gonystylus bancanus*). Our current objective is to detect geographically

structured genomic variation so that the physical origin of a particular shipment of logs can be determined. This information would provide objective and conclusive enforcement of current trade policy and prevent wide scale illegal logging and smuggling. Once trade has been normalized, a wide range of applications for this type of genomic information can be imagined, from improvement of disease and pest tolerance to the conservation of overall genetic diversity in timber stocks. We are currently performing the necessary experiments to demonstrate that this technique will provide the necessary genomic information. Results of these experiments will be forthcoming.

DNA Fingerprinting Of Tropical Timbers

C.H. Cannon[1], **M.J. Asif[1]**, R. Wickneswari[2], K.S. Lee[2], W.B. Maynard[3], and C.T. Tan[4]

[1]Texas Tech University, Dept. of Biology, Box 43131, Lubbock, TX 79409-3131, USA;
[2]Universiti Kebangsaan Malaysia, Plant Genetics and Biotechnology, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, 43600 Bangi, Selangor;
[3]Tropical Forest Trust, 16 Station Field Industrial Estate, Kidlington, Oxon, OX5 1JD, UK;
[4]Perak Integrated Timber Complex, No. 8 Lebuh Lasam, 30350 Ipoh, Perak, Malaysia.

South East Asia is one of the most important timber producing regions in the world. Illegal harvest and smuggling is currently a major cause of unsustainable damage to forest areas. To better enforce trade policies, both timber producers and buyers need a marker system which clearly demonstrates the geographic origin of timber. Physical methods are commonly used but these are not permanent and can be forged. Evidence based upon DNA, which cannot be affected by environmental conditions or human action, would be the most powerful. Many questions still remain though concerning the best way to develop genetic markers, create adequate georeferenced databases, and implement the marker system in the field.

To explore various options, we performed a DNA fingerprinting study of Meranti Bukit (*Shorea platyclados*) using standard SSR loci. A large number of samples were collected from four locations in peninsular Malaysia. SSR analysis is currently the marker system of choice for most population level studies because they combine several features of the ultimate marker system; high polymorphism per locus, co-dominance, abundance, and evenly dispersed across the genome. We explored two different detection systems in an effort to compare the results and create an efficient high-throughput test.

Initially, SSR fragments were size fractionated using a PAGE gel system. Given the problems associated with accurate allele sizing and the labor-intensive nature of this system, we also chose to utilize a capillary electrophoresis system. Here we standardized a method of fragment analysis for microsatellite markers using unlabelled primers using small amount of PCR product, a Spectromedux System® automated DNA sequencer fragments were detected using ethidium bromide. Alleles varying by 1 bp were clearly separated and alleles differing in more than 1 bp showed distinct peaks. Allele sizing was readily achieved using the software and large amount of data could be analyzed in a short time. The six microsatellite loci used were highly polymorphic in *S. platyclados* populations. A large allelic variation was observed within and between different populations. However, only a few unique alleles were observed to be present in different individuals of a population.

Ultimately, we were not satisfied with the results from this study. Accurate and reliable sizing of SSR fragments is a challenge no matter what the detection system. Additional DNA sequencing of selected fragments also revealed a surprising amount of 'noise' and potential for PCR-induced error. Also, the final conclusion relies upon a complicated statistical analysis which only provides a rough likelihood of geographic origin. We think more efficient and reliable systems must be developed.

Possibilities of Breeding Teak (*Tectona grandis*) in Costa Rica assisted by AFLP markers

EMANUEL ARAYA[1], OLMAN MURILLO[1], GABRIEL AGUILAR[2], OSCAR ROCHA[2]

[1]Escuela de Ingeniería Forestal, Instituto Tecnológico de Costa Rica; [2]Escuela de Biología, Universidad de Costa Rica

Abstract

Teak (*Tectona grandis*) is a tropical tree species naturally distributed in Southeast Asia, where it is also widely planted. It is planted as exotic species in Africa, South and Central America. Tree improvement activities have been initiated in several countries including Costa Rica, which has become the largest teak developer in Latin America.
Tectona grandis sprouts were collected in a 4-year-old progeny test from the best two and the worst two families for volume as well as for stem quality traits. A DNA extraction protocol described before was modified in order to yield high quality DNA. AFLP reactions were performed as described previously. Seven selective primer combinations (E-ACG + M-CCG, E-ACT +M-CCG, E-AGC + M-CCG, E-ACG + M-CTC, E-ACT + M-CTC, E-AGC + M-CTC, E-AGG + M-CCA) resulted in 330 scoreable markers. A large number of markers were obtained when families where analyzed separately from its performer. Genetic distances based on AFLP markers frequencies for volume and quality traits were used to construct a dendrogram among families. The best quality performer families grouped widely separated from the worst performers, sharing 61.63% of their genetic elements. In volume, worst performer families grouped sharing 45.85% of their genetic elements. Best performer families did not group clearly, but shared 70.74% of their genetic elements.

Key words: AFLP, breeding, gene marker, progeny test, teak, *Tectona grandis*

Introduction

Teak (*Tectona grandis*) is a tropical tree species widely distributed in Southeast Asia, growing in countries such as India, Burma, Thailand and Indonesia (Kaosa-ard, 1981; Schubert, 1974). Teak is used for many purposes, but the most important end use is timber, preferably for fine furniture and other high valuable products (Kjær and Foster, 1996).
The species is distributed over an area with large variation in edaphic and climatic condition. Today it is widely planted in South East Asia and as exotic species in Africa, South and Central America (Ball *et a*l. 1999). These new areas with teak plantings in Africa and Central America have offered teak further variation in conditions of growth. Continuing selection of individuals adapted to local climates and soils may have formed ‘landraces’ in these new areas, each with its own distinctive characteristics (Kjær and Foster, 1996). Teak has been used for decades in plantation establishment either as an indigenous or an exotic species. The species is easily established in plantations. This makes teak one of the most promising species for plantations in the tropics, although the soil requirement is rather specific (Keogh, 1996). Tree improvement activities have been initiated in several countries including Costa Rica (Murillo and Meza, 1992).

It has become the largest teak developer in Latin America. Teak is interesting from a tree improvement point of view because it is used on a large scale, the timber is of high value and it is prevailingly regenerated artificially, which allows introduction of improved genetic material (Kjær and Foster, 1996).

During the last 20 years, advances have emerged in the technologies available for assessing genetic diversity at the molecular level. Genetic markers (isozymes, RFLPs, AFLPs, RAPDs, and more recently microsatellites) have been developed and applied for genome mapping, population genetics and marker assisted breeding (Young *et al*., 2000; Grattapaglia *et al*., 2000). These technologies are becoming in an important tool for forest tree breeding and conservation genetic studies. The genetic diversity of teak from India, Thailand and Indonesia has been determinated by isozyme variation of provenances (Kertadikara and Prat, 1995). Kjær and Siegismund (1996) evaluated allozyme diversity and genetic distance for two Tanzanian and two Nicaraguan landraces of teak. A previous work in the Java Island of Indonesia was developed with *Tectona grandis*, trying to achieve a highly reliable clone management of teak plus trees by using RAPD (Watanabe *et al*., 2004).

Early selection and testing of superior teak trees in breeding activities is important because of long term testing. Genetic markers may play a significant role in future teak breeding programs. Therefore, this study aims to explore the possibilities of AFLPs as an important teak breeding tool in Costa Rica.

Materials and Methods

Plant Material

Leaves were collected in a 4-year-old progeny test from the best two and worst two families for volume as well as for quality traits (Table 1). The progeny test belongs to an active teak breeding program the dry Pacific of Costa Rica. The test is conformed by 30 families established in a 6-half-sib progenies by 6 blocks in a complete randomized design. SAS Proc. mixed model was used and ranking of families obtained.

Table 1. Families selected in this study for quality and volume traits

	Families selected	
Variable	Best performer	Worst performer
Quality	27, 14	19, 1
Volume	2, 20	1, 35

DNA extraction

A protocol described by Muhammad *et al.* (1994) has been modified in order to yield high quality DNA. Tissue was ground with liquid nitrogen before extraction. Approximately 50-100 mg of tissue were put in a 1.5 mL tube. The tissue were ground with 500 µL of the buffer extraction (20 mM sodium EDTA and 100 mM Tris-HCl, adjust pH to 8.0 with HCl, 1.4 M NaCl, 2.0% (w/v) CTAB, 2% (w/v) PVP and 0.2 % of beta-mercaptoethanol) and additional 250 µL were added later. The tubes were incubated at 60°C for 20 minutes. After incubation 700 µL of chloroform:octanol (24:1) were added and tubes mixed gently by inverting 20 times. The mixture was centrifuged at 6000 rpm for 15 minutes at 4°C and 400 µL of the aqueous phase was

transferred into a new 1.5 mL tube. A second chloroform:octanol extraction was performed when the aqueous phase was brown-colored or turbid. In order to precipitate DNA, 0.5 volume of 5M NaCl and two volumes of cold 95% ethanol were added and refrigerate for 1 hour at -20°. Alternatively, DNA can be precipitated at 4-6°C overnight. The solution was centrifuged at 10 000 rpm for 10 minutes at 4°C. The supernatant was pour off and the pellet kept at the bottom of the tube. The pellet was washed with 70% ethanol and the ethanol removed without drying the DNA. The DNA was dried at 37°C for 1 hour and then dissolved in 100 µL TE (Tris-HCl 10mM and EDTA 1mM). For RNA treatment 1 µL RNase was added to each tube and incubated at 37°C for 1 hour. After extraction, DNA was cleaned using a DNeasy Plant Mini Kit from QiaGen Inc. DNA was kept at -20°C until its use. Extracted DNA quality was determinated running samples in an agarose gel (0,8%). In addition, extracted DNA was subjected to two restriction enzyme digestions (EcoR I and Hind III) for 1 hour and also subjected to agarose gel (1%) electrophoresis with undigested DNA as control.

AFLP reactions

AFLP reactions were performed as described by Vos *et al*. (1995) with available products and kits (Applied Biosystems AFLP™ Plant Mapping). All AFLP reactions were performed at Keim Genetics Laboratory at Northern Arizona University, Flagstaff, Arizona. Pre-amplification step was performed with primers complementary to the EcoR I and MseI adaptors. The PCR reactions were performed in a MJ Research (Gradient Cycler) thermocycler using the following PCR conditions: 72ºC for 2 minutes, 94ºC for 20 seconds (Step 2), 56ºC for 30 seconds, 72ºC for 2 minutes, Go to Step2 for 19 more times, 60ºC for 30 minutes and 4ºC forever.
Initially, nine primer combinations were evaluated by combining EcoR I selective primers with MseI selective primers: ACG, ACT, AGC combined with CCG and CTC; AGG, ACA, ACC combined with CCA. PCR reactions were performed in a MJ Research (Gradient Cycler) thermocycler with the steps as follows: 94ºC for 2 min, 94ºC for 20 sec, 66ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 65ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 64ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 63ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 62ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 61ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 60ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 59ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 58ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec, 57ºC for 30 sec, 72ºC for 2 min, 94ºC for 20 sec (Step 32), 56ºC for 30 sec, 72ºC for 2 min, Go to step 32 for 20 more times, 60ºC for 30 min and 4ºC forever. After amplification, the samples were denatured adding 1 µL of formamide and heating for 5 minutes at 95 ºC; then, tubes were immediately placed on ice. Products of selective amplifications were analyzed in an ABI PRISM ® 3100 Genetic Analyzer.

Data analysis

Data obtained from the ABI PRISM ® 3100 Genetic Analyzer were initially screened with the ABI PRISM® GeneScan® Analysis Software (Version 3.7) and then analyzed with the ABI PRISM® Genotyper® 3.7 NT Software. Each marker was coded as present (1) or absent (0) for each individual, thus creating a binary data matrix. Information from primer combinations was analyzed by using PopGen software (Version 1.32). This software was also used to construct a dendrogram in order to group families.

Results and Discussion

DNA extraction

The CTAB based method for DNA extraction evaluated yields high quality DNA. Degradation of DNA was not observed when samples were run in a 0.8% agarose gel. Differential solubility in the presence of CTAB allows the separation of polysaccharides from nucleic acids (Rogers and Bendich, 1988). CTAB binds strongly to DNA, removes proteins and prevents DNA degradation (Valadez and Kahl, 2000). In addition, the methodology evaluated was low time-consuming. DNA from sixty samples can be extracted in a working-day when tissue was previously ground with liquid nitrogen. However, we have observed the same amount of samples can be handled with tree species having softer leaves than *Tectona grandis*.
Results of DNA restrictions with EcoR I and Hind III showed complete digestion. DNA was extracted with a modified protocol from Muhammad *et al.* (1994), as described before. These authors report a complete digestion with three endonucleases (EcoR I, Eco RV and Hind III) working with *Vitis* and *Ampelopsis* species. We also have observed that the extraction protocol evaluated yields high quality DNA working with different tree species. A complete DNA restriction has been observed when we use EcoR I and Hind III.

AFLP reactions

In order to evaluate the suitability of *Tectona grandis* DNA for its use in AFLP analysis, reactions were performed as described before. Initially, nine primer combinations were tested on sixteen samples from different families. Two primer combinations were not used in further analyses because these resulted in non-scoreable markers in most of the samples. The following seven primer combinations were finally retained for analyses: ACG, ACT, AGC combined with CCG and CTC; AGG combined with CCA. The number of markers scored for samples are shown in Table 2.

Table 2. Number of markers scored for seven primer combination evaluated in *Tectona grandis*

Primer combination	Number of markers scored
E-ACG + M-CCG	51
E-ACT +M-CCG	44
E-AGC + M-CCG	64
E-ACG + M-CTC	47
E-ACT + M-CTC	49
E-AGC + M-CTC	25
E-AGG + M-CCA	50
Total scoreable markers	330

Family information obtained from AFLP reactions

The application of the seven primer combinations on seven families resulted in 330 scoreable markers (Table 2), of which 311 were polymorphic. Further analyses were developed separately, in order to obtain information for families categorized as best progeny test performer and worst

progeny test performer. Quality and volume traits were considered for analyses. The number of polymorphic markers and the percentage of polymorphism loci per family are shown in Table 3.

Table 3. Number of polymorphic loci and percentage of polymorphic loci for quality and volume traits in two teak family categories. Best and worst progeny test performer data were analyzed separately for each family category and for each variable studied.

	Family Category			
	Best performer		Worst performer	
Trait	Number of polymorphic markers	% of polymorphic loci	Number of polymorphic markers	% of polymorphic loci
Quality	187	56.66	166	50.45
Volume	168	50.91	154	46.66

The AFLP reactions produce many bands from a single assay. From the complex genomes of trees, the basic AFLP assay produces too many bands to analyze (Young *et al*., 2000). As shown in Table 2, more than 330 bands were generated by using seven primer combinations. Stenefon *et al*. tested seven primer combination on *Araucaria angustifolia* and obtained 625 markers. In a study in *Quercus petraea* eight primer combinations were evaluated obtaining 374 scoreable markers (Coart *et al*., 2002). The fact AFLP technique produces many bands, analysis has been found to be more informative than RAPD and RFLP analysis (Savelkoul *et al*., 1999). A critical characteristic of genetic markers is its reproducibility. Since relatively small amounts of DNA are digested and detection of AFLP fragments does not depend on hybridization, partial digestion and faint patterns, which are sources of irreproducibility with restriction fragment length polymorphism (RFLP) genotyping, can easily be avoided. Furthermore, the possibility of using stringent PCR annealing temperatures renders the AFLP analysis method more reproducible and robust than RAPD analysis (Savelkoul *et al*., 1999).

AFLP technique as a potential tool for teak breeding

Genetic distances based on AFLP markers frequencies for volume and quality traits were used to construct a dendrogram (UPGMA) among families. The best quality performer families (27 and 14) were grouped widely separated from the worst performers, sharing 61.63% of their genetic elements. The worst performer families did not grouped but they shared 89,53% of their genetic elements. In volume trait, worst performer families grouped sharing 45.85% of their genetic elements. Best performer families did not group clearly, but they shared 70.74% of their genetic elements.

In this study we have obtained a large number of markers when testing seven primer combinations. A previous work based on RAPD was developed with *Tectona grandis*, trying to achieve a highly reliable clone management of teak plus trees. It is reported only 26 fragments when 24 primers were evaluated (Watanabe *et al*., 2004). This suggests that information from AFLP can be used for clone managing in teak trees more accurately than RAPD markers.

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Ten-Year Response of Diverse Families of Loblolly Pine to Fertilization

S.E. McKeand, J.E. Grissom, H.L. Allen, and B.P. Bullock[1]

Genetic gains from tree improvement programs have been large in loblolly pine, since geographic and within-provenance variation for growth and adaptive traits is very large. General trends in productivity variation are that families from southern and eastern coastal sources grow faster than families from northern, western, and interior sources. Contrasting the response to nutrient stress of two very different provenances of loblolly pine such as from the "Lost Pines" region of Texas and the Atlantic Coastal Plain may give us insight into the adaptive significance of different ecophysiological traits. Previous work indicates that the Drought-Hardy Texas (DHT) sources are generally more stable across environments, while productivity of eastern sources depends more on the environment. Eastern sources were very responsive to environmental enhancement, since productivity was high on the better sites, but very low on the droughty sites.

MATERIALS AND METHODS

The study site is located in Scotland County, NC adjacent to the U.S. Forest Service / N.C. State University SETRES (SouthEast Tree Research and Education Site). The soil is very infertile, somewhat excessively drained. Open-pollinated families from the Atlantic Coastal Plain of the Carolinas and from the "Lost-Pines" or drought-hardy area of Texas were included in the study.

A split-split-plot design was used with the two nutrient treatments as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Each family plot consists of 64 interior measured trees planted at 1.5 m by 2 m spacing in 100-tree plots. The study was replicated across 10 blocks. Fertilizer was applied annually to maintain a balanced supply of all nutrients in the fertilized plots. All trees were measured annually for height and starting in year 4 for breast height diameter.

RESULTS AND DISCUSSION

Fertilizer Response: Growth responses to fertilization were very large and significant each year (Fig 1 & 2). Through age ten years, height was 68% greater in the fertilized plots and stem volume per hectare was 167% greater, compared to the non-fertilized plots. Nutrient amendments dramatically increased uniformity within the 64-tree family plots. The average within-plot CV for 10-year height was 18.6% for the control plots and 9.1% for the fertilized plots. The average within-plot standard deviations for height were also significantly different ($P \leq 0.05$) and were 1.15 m. for the control plots and 0.97 m. for the taller fertilized plots.

Provenance and Family Variation: For the first 8 growing seasons, the five families from the Atlantic Coastal Plain grew faster than the five Drought-Hardy Texas families in both fertilizer

[1] Professor, Tree Improvement Analyst, Professor, and Assistant Professor, respectively Department of Forestry, NC State University, Campus Box 8002, Raleigh, NC 27695-8002.

regimes (Fig 1 & 2). We anticipated that under the harsher environmental conditions in the control plots that the DHT families would perform relatively better. However, the ACP families were superior in both environments, and the provenance by treatment interactions for height in all ten years were not close to being significant (all P values > 0.20). Beginning at age ten years, rank changes at the provenance level were observed for volume increment and basal area (data not shown). In the fertilized treatment, the DHT trees had higher volumes and basal areas than the ACP trees, opposite of the trends in the nonfertilized plots and during the earlier growing seasons in both environments. Survival differences between the provenances are thought to account for the rank changes in volume observed at age ten (data not shown). The stand development of the ACP trees is more advanced than that of the DHT trees, and density-dependent mortality appears to be more prevalent in the fertilized ACP plots.

Families within provenances also differed for growth traits. The family means at age ten for the ACP families varied from 64 m^3/ha to 81 m^3/ha in the control plots and from 161m^3/ha to 202 m^3/ha in the fertilized plots. The DHT families also differed in the control plots (60m^3/ha to 72 m^3/ha) and in the fertilized plots (176 m^3/ha to 201 m^3/ha). Unlike at the provenance level, there was no treatment by family interaction for growth traits at any age.

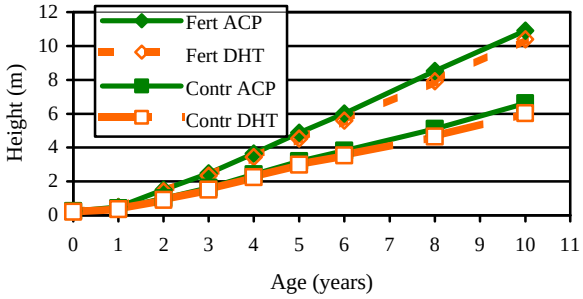

Figure 1. Height growth for the ACP and DHT provenances in fertilized and control plots over the ten years of the trial.

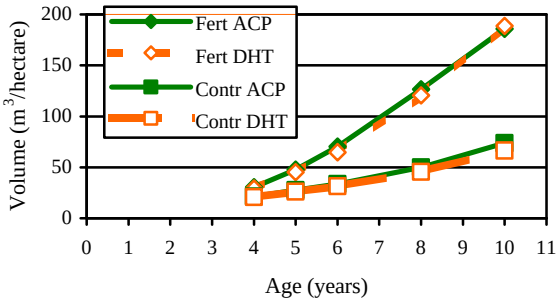

Figure 2. Volume growth for the ACP and DHT provenances in fertilized and control plots ages four to ten years.

CONCLUDING REMARKS

As stand development progresses, especially in the fertilized plots, both growth and survival of the Atlantic Coastal Plain provenance and the Drought-Hardy Texas trees will be monitored closely. The long-term performance of these diverse provenances in response to environmental extremes will allow us to evaluate genetic differences in mortality functions, growth rates, and genotype by environment interactions.

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Water Activity As A More Reliable Method Than Moisture Content Applied To Pollen And Seeds Moisture Management .

Patrick *BALDET* [1], Michel VERGER [2].

[1] Cemagref, Forest Ecosystems Research Unit, Domaine des Barres, F- 45290 Nogent sur Vernisson. [2] INRA, UE 995, Avenue de la Pomme de Pin, Ardon, BP 20619, F- 45166 Olivet cedex

Pollen and Seed moisture management, as a key factor influencing storability, is a continual challenge for gene conservation. Moisture content (MC) procedure is commonly used to assess the degree of humidity of forest pollen and seeds to be stored. The range of optimal moisture content for storage of seeds and pollen has been defined for numerous species both by know-how and conducting tests.

However, this method is not always reliable because it does not take into account the chemical properties of water remaining in dried materials; this occurs because moisture content in organic matters is the consequence of two main factors : The Equilibrium Relative Humidity (HRE) of air surrounding them and the proportion and ability of hygrophilous constituents, like starch or proteins, to absorb and retain water. Consequently, a given moisture content may result from different combinations of HRE and proportion of hygrophilous constituents. Forest seeds and pollen characteristics and composition are weakly predictable, this is mainly due to a high level of genetic diversity which is sought after for forest reproductive material and non or partly controlled growing conditions of trees.

In contrast, the concept of activity of water (Aw) quantifies the vapour pressure generated by the moisture present in hygroscopic products like organic substances. It is known as the chemical potential of water. Aw has been widely used for decades by food industry because it is very well-known that reactivity and depreciating ability of hydrolytic reactions, enzymes activity, lipid oxidation or biotic agents growth, are dependent on Aw rather than on MC. Consequently, Aw is more consistent to define stability moisture for organic materials preservation. In addition, Aw assessment is a quick and non destructive test, it requires only an hygrometer fitted with a special attachment to enclose samples.

The relationship between Aw and MC can be pictured by the sorption/desorption isotherm. Different models are available to analyse sorption isotherms in order to define water activity values of greater stability. The relation between Aw and MC is rather stable for homogeneous organic materials like sugars or flours, this allows to use equally MC or Aw for moisture management. On the contrary, the experimental construction of sorption isotherms of different pollen and seed lots, demonstrated a large variability for MC and Aw relationship among different seed or pollen lots for a given species. These results confirmed the interest of water activity assessment versus moisture content evaluation.

Aw values of greater stability for pollen and seeds storage are given according to sorption isotherms analyse and bibliographic references. Aw assessment is now profitably used as a routine by the two main French forest seed dealers and state seed orchard managers for both seed and pollen moisture management.

Genetic and Cultural Effects on Stem Taper for Loblolly Pine

J.R. Sherrill, T.J. Mullin, B.P. Bullock, S.E. McKeand, and R.C. Purnell[1]

Total inside-bark volume is the most important selection criterion in tree breeding programs. Tree breeding programs estimate total inside-bark volume based on diameter at breast height (DBH) and total height. These variables used to estimate volume do not account for stem taper or bark thickness. Large taper or bark thickness differences among families could cause erroneous selections or inaccuracies in gain estimates. Families with large height and DBH measurements, but high taper or bark thickness, could be selected in place of more cylindrical or thinner barked families that have higher wood volumes.

Foster (1988) found that stem taper in loblolly pine is generally controlled by additive gene action. Additive gene action will benefit breeders desiring less taper if there is adequate variation in the natural population to make improvements. A subsequent study with rooted cuttings found a family difference in taper but not between propagule types for ages 7 and 10 (Stelzer, et al. 1998).

Pederick (1970) did not detect any significant half-sib family differences in inside-bark taper and felt that inside-bark diameter and height could adequately predict wood volume. This loblolly pine study found that the estimated inside-bark volume, calculated from DBH and total height, could be over or underestimated by 3.3% due to bark thickness. Bark thickness was found positively correlated with diameter inside-bark growth ($r = 0.66$), meaning that selection for faster diameter growth may result in thicker bark. Pederick (1970) reported a relatively high individual-tree narrow-sense heritability for bark thickness of 0.60.

MATERIALS AND METHODS

A genotype by cultural treatment study at Bainbridge, GA was measured in the 13th growing season. This study was a two by two factorial of weed control and fertilizer treatments in a split-plot design. The control had no herbicide applications until age five and no fertilizer treatments. The herbicide treatment consisted of early woody and herbaceous competition control and all treatments were aerially released at age five. The fertilization treatment was ground applied five times up until age 9.

There were 25 open-pollinated first- and second-generation families arranged as individual trees within each main treatment plot, each replicated 5 times. Some known relationships existed between mothers. Approximately 40 individuals from each of 25 families were sampled, 10 from each treatment. Two trees from each family were sampled in most replication/treatment plots.

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1 Graduate student, Research professor, Assistant professor, and Professor, respectively Department of Forestry, NC State University, Campus Box 8002, Raleigh, NC 27695-8002. Manager, Genetics and Tree Improvement, International Paper Co., 1201 W. Lathrop Ave., Savannah, GA 31402

Sectional data were collected along the stem every 1.2 meters to a 7.6-centimeter top. Inside-bark diameters were used in Smalian's log volume equation (Avery and Burkhart 2002) to find the inside-bark volume of each stem section. In addition to diameters, total height and height to live crown were measured to calculate crown ratio as a percentage of the total height.

RESULTS AND DISCUSSION

Using the methods of Amateis and Burkhart (1987) for fixed models, variance components in mixed models were tested. Type III ANOVA was used to detect differences in height-DBH relationships, stem form, and taper among families. The volume model used was:

$$Y_{ijk} = \mu + X + R_i + T_j + RT_{ij} + F_k + XF_k + TF_{jk} + e_{ijk} \quad [1]$$

where Y_{ijk} is whole-tree inside-bark volume in meter^3, X is DBH^2*total height (D^2H) in mm^2*m (continuous fixed effect), R_i is the random block effect, T_j is the fixed treatment effect, F_k is the random family effect, and e_{ijk} is the random within plot effect. A square-root transformation was applied to both the dependent and independent numeric variables to reduce heteroscedasticity. This volume model was used to test the significance of intercept and slope terms for family effects. Family was significant in this model for intercept but not slope. Therefore, one D^2H equation may not be appropriate for all families because different families will require different intercepts in regression models.

The height-DBH model is the same as equation 1, except Y_{ijk} is a total height measurement in meters and X is the DBH measurement in mm. Height and DBH were square-root transformed to improve homoscedasticity. The intercept and slope for family was significant in this model. This indicates that height-DBH relationships can account for some of the volume differences between families. However, height-DBH relationships may be accounted for by using D^2H as a predictor of total volume.

A form model was fit similar to equation 1 but Y_{ijk} is a whole-tree inside-bark volume measurement in meters^3 and X is the combined variable A*H. A is the surface area of the stump and H is the total height in meters^3. Both total volume and AH were square-root transformed to induce constant variance. Family was significant in this model for intercept but not slope. Therefore, family differences influenced stem shape.

Family variation in taper was explored with a taper model similar to equation 1 but with Y_{ijk} as a diameter squared (d^2) in mm and X as the height in meters where d^2 was measured. The variable d^2 was log transformed for homoscedasticity. Slope and intercept for family were significant in this model, which indicates families differed in taper.

Gilmour (2003) was used to calculated individual and family heritability for total inside-bark volume, D^2H, and six other traits (Table 1) with corresponding standard errors. The following linear model was used to calculate variance components:

$$Y_{ijk} = \mu + R_i + T_j + RT_{ij} + F_k + RF_{ik} + TF_{jk} + e_{ijk} \quad [2]$$

where Y_{ijk} is an observation of family k in treatment j of replication i. In addition, the genetic correlation between total inside-bark volume and D^2H was calculated as 0.99 (SE 0.0072).

Table 1. Individual tree heritability (h^2_i) and family heritability (h^2_f) for eight traits with corresponding standard errors.

			Standard Error
Total Measured Inside Bark Volume	h^2_i	0.24	0.0965
	h^2_f	0.55	0.1101
DBH^2*Total Height (D^2H)	h^2_i	0.27	0.1024
	h^2_f	0.58	0.1033
Total Measured Outside Bark Volume	h^2_i	0.26	0.1003
	h^2_f	0.57	0.106
Outside Bark Diameter at Breast Height	h^2_i	0.27	0.1035
	h^2_f	0.58	0.1032
Inside Bark Diameter at Breast Height	h^2_i	0.24	0.0942
	h^2_f	0.55	0.1087
Bark Thickness at Breast Height	h^2_i	0.31	0.119
	h^2_f	0.63	0.1018
Total Height	h^2_i	0.18	0.0771
	h^2_f	0.48	0.1127
Crown Ratio	h^2_i	0.07	0.0635
	h^2_f	0.24	0.1786

The genetic parameters listed in Table 1 indicate that most traits measured have a high heritability except for crown ratio. Likewise, Type B genetic correlations for all the traits except crown ratio were high, which indicates genotype by cultural treatment interaction was low for most traits.

CONCLUSION

The results of the TYPE III ANOVA model analysis indicate that D^2H does not account for all of the volume differences among families. A strong positive genetic correlation between D^2H and total inside-bark volume indicates that selection for D^2H is adequate for indirect selection of inside-bark volume. However, predicted gains may be over or under estimated by using one D^2H equation for multiple families.

Genotype by cultural treatment interactions were low for most traits except for crown ratio. This implies that family ranks will not change among tests with different silvicultural treatments. Also, this demonstrates that there is no need to synchronize silvicultural treatments with family block plantings. These results are preliminary and further analysis and interpretations continue.

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Differences In Basic Wood Density Between Radiata Pine Cuttings And Seedlings.

C. Gantz[1], R. González[2], R. Ellis[3].

[1]Research Forester, Wood Quality Program, Forestal Mininco S.A., Los Ángeles, Chile.
[2]R&D Manager, CMPC Celulosa, Nacimiento, Chile.
[3]Research Engineer, CMPC Celulosa, Nacimiento, Chile.

Forestal Mininco S.A. is establishing 80% of its annual plantations using rooted cuttings coming from the best genetic material developed in the tree breeding program. Huge genetic gains in volume growth and stem straightness will be obtained from this material. Nevertheless, the there are not reports regarding the wood quality of trees from vegetative and sexual origins.

In this study wood basic density of radiata pine seedlings and cuttings from the same control pollinated families were compared, in a 13 years old test established in one site.

Five control pollinated families and 8 trees per family for each of the two origins (cutting or seedling) were sampled using 12mm increment cores taken at breast height.

Highly significant differences in basic wood density between families were observed ($p<0.0001$), ranging between 336 kg/m^3 and 375 kg/m^3, but no statistically significant differences in basic density were obtained between cuttings and seedlings (352 vs 356 kg/m^3).

These results suggest that under the conditions of this experiment, no detrimental effects in juvenile wood density would be expected due to the establishment of plantations with rooted cuttings.

Differences In Juvenile Basic Wood Density Among Radiata Pine Natural Provenances Planted In Chile.

C. Gantz[1], R. González[2], R. Ellis[2].

[1]Research Forester, Wood Quality Program, Forestal Mininco S.A., Los Ángeles, Chile.
[2]R&D Manager, CMPC Celulosa, Nacimiento, Chile.
[3]Research Engineer, CMPC Celulosa, Nacimiento, Chile.

In 1993 two radiata pine provenance/progeny tests were established by Forestal Mininco S.A. in central south Chile. The provenances included in this study were: Monterrey, Año Nuevo, Cambria and Guadalupe. Local clonal seed orchard material was also included as a control. The tests were established in two different soil types: sandy soils and red clays. At 11 years of age, the tests were measured and wood samples were obtained for basic wood density determinations. Between 43 and 60 trees/provenence/site were sampled, using 12 mm increment cores.

Highly significant differences in juvenile wood basic density among provenances were obtained ($p<0.0001$). Each provenance formed a discrete group according to their wood density means: Guadalupe provenance had the highest wood density (375.72 kg/m^3), followed by Monterrey (363.04 kg/m^3), Año Nuevo (356.42 kg/m^3), and Cambria (347.94 kg/m^3) provenances. The clonal seed orchard control had a mean basic density between Monterrey and Año Nuevo (358.49 kg/m^3)

Significant differences ($p<0.0001$) were also obtained between site means, but genotype by environment interaction were absent for juvenile wood basic density.

These results agree with those obtained in provenance/progeny tests established in New Zealand and stresses the importance that the Guadalupe provenance could have for a breeding program leading to the improvement of radiata pine juvenile wood density.

Genetic Performance Of Wood Properties Of *Betula platyphylla*

Yang Chuanping Wang Qiuyu Jia Hongbai

Forestry Faculty, Northeast Forestry University, 150040, Harbin, P. R. China

This study is based on materials from five natural stands of *Betula platyphyll* in northeast part of China. The wood characteristics including tracheid length, tracheid width, percentage of cell wall, tracheid wall thickness, wood tissue percentage, tracheid microfiber angles and basic density of the wood are measured by means of wood micro-observation, in order to analyze genetic variation and radial growing trends in above characteristics among and within population. The result indicates that great variation in tracheid length and width among the populations can been seen, but not in the percentage of cell wall, tracheid wall thickness and wood tissue percentage where to show obvious changes among individuals within population. Besides, the differences are also remarkable for both of wood microfiber angles and basic density among and within populations. The tracheid length, tracheid width and wood density increase along the tree age in which thracheid length rises faster than the other two characteristics, whereas the tracheid microfiber angle presents a opposite change. All of these changes reach the maximum point at a certain age, and then keep them in a stable state. Significant correlation among some wood characteristics exists within an individual or in a population, and sometimes among the populations. These results will be a basis for tree improvement of this species in wood quality.

Geographic Variation and Genetic Performance of Growth Characteristics and Wood Properties of *Picea Koraiensis* Nakai.

Wang Qiuyu Yang Chuanping
Forestry Faculty, Northeast Forestry University, Harbin 150040, P. R. China

The materials from three representative provenance trials with 8 provenances of *Picea Koraiensis* Nakai of 19 years old in northeast part of China are measured. The growth characteristics of the provenances are analyzed in order to know the genetic variation among the provenances for this species. The significant variation in height and breast height diameter growth among the provenances is shown, with different geographic adaptability following the increase of tree age. The growth characteristics in Maoershan and Liangshui Trials in 10 years old have some positive correlation with longitude. And a strong relationship between height growth and latitude as well as altitude can been seen when the trial is in 15 and 19 years old.

In addition, wood characteristics of the provenance, such as tracheid length, tracheid diameter, tracheid wall thickness, annual ring as well as wood density are observed. Great difference in above wood properties among the provenances is in existence. Also, some correlations are displayed between growth characteristics and wood properties, for example, the height and breast height diameter growth of the provenance show positive relations with tracheid diameter and annual ring, and negative correlation with tracheid wall thickness and wood density. Finally, growth performance of the provenance in above characteristics is investigated, which could provide more useful information for comprehensive selection of this species for pulpwood.

Somatic Embryogenesis Of Fraser Fir (*Abies Fraseri*)

Gerald S. Pullman, Katie Olsen, **Ulrika Egertsdotter**

Forest Biology, IPST, Georgia Institute of Technology, Atlanta, GA

In conifers, the possibility of using cuttings for clonal propagation is limited due to the ageing effect resulting in insufficient rooting and poor shape of the trees. For some fir species that effect is already excluding the use of cuttings from an age of 5 yrs. Somatic embryogenesis is a promising alternative method for clonal propagation of conifers. The techniques has however not yet been optimized for many species. The potential for using somatic embryogenesis (SE) for clonal propagation of Christmas tree species has been investigated. The method for propagation of *Picea abies*, (Norway spruce) the most common Christmas tree in Europe, by SE is already well established. Protocols for propagation of subalpine fir (*Abies lasiocarpa*) have also been successfully developed. In the present study, we have been exploring the use of SE for another Christmas tree species, Fraser fir (*Abies fraseri*), which is important to the US Christmas tree market.

Different growth media were tested for initiation. Immature whole megagametophytes or with embryos exposed were used as explants for initiating SE. The seeds were collected at different time points to determine the most responsive developmental stage of the explants. Several different seeds sources were included in the study to investigate the importance of the genetic background.

Somatic embryogenesis cultures were initiated from several seed sources. Different culture media were required for different developmental steps. Key components of the culture media were identified and further investigated. Embryogenic cultures from several genotypes have been established and progressed through maturation and germination. A small number of plants have been transferred to soil.

Cloned Genotypes Are More Efficient Than Seedlings for Indirect Selection

Fikret Isik, Bailian Li, Barry Goldfarb, John Frampton and Steve McKeand

Department of Forestry, North Carolina State University, Campus Box 8002,
Raleigh, NC 27695-8002

ABSTRACT

Cloned families of loblolly pine (*Pinus taeda* L.) have proved to be more effective than families with seedling progeny in sampling environmental noise. In this study, co-heritability of wood density and the Resistograph drilling resistance were estimated from clones and seedlings of the same full-sib families. Using co-heritabilities, efficiency of clones versus seedlings for indirect selection of wood density were compared.

Seedlings and clones of the same 9 full-sib families were tested at 2 sites using a RCB field design. Wood density (kg/m3) and the drilling resistance (Amp %) of each tree were recorded when the trees were 12 years old. Full-sib family co-heritabilities from clones (0.63) and seedlings (0.60) were close. However, within-family co-heritability based on clones (0.60) was much higher than the within-family co-heritability based on seedlings (0.25). Breeding values of wood density and drilling resistance based on clones (r=0.82) had a stronger correlation than breeding values from seedlings (r=0.75).

Using clones for indirect full-sib family selection gave 7% greater correlated response than using seedling progeny of the same families. However, indirect selection for within-family selection of density from seedlings and clones was decidedly different. Correlated response from cloned material was 44% more efficient than using seedlings. The efficiency of clones for indirect selection of density originated from higher within-family co-heritabilities and stronger genetic correlations between breeding values of two traits. Cloned genetic tests of loblolly pine, along with other advantages, may also allow more efficient selection of target traits that are difficult, costly or time consuming to measure.

Breeding of Wild Cherry in the Czech Republic

Jaroslav Kobliha, Vladimír Janecek, Martin Hajnala

Prof. Dr. Jaroslav Kobliha, Ing. Vladimír Janecek, Ing. Martin Hajnala
Czech University of Agriculture in Prague, Faculty of Forestry
Kamýcká 957, 165 21 Praha 6 – Suchdol, Czech Republic

During years 1997- 1999 research of forest stands with wild cherry composition was realized in several regions with preliminary selection, measurement and phenotypic valuation of wild cherry trees. In 1999 63 trees were certified in forest stands of Forest Districts of Forests of the Czech Republic (FD FCR) Krivoklát and Nižbor and 30 trees in forest stands of Military Forests (MF) Velichov. In February 1999 grafts from 63 plus trees in FD FCR Krivoklát and Nižbor (central Bohemia) were collected and used for grafting. This material was used for establishment of seed orchard in FD FCR Lužná on prepared plot in former forest nursery in spring 2002. 343 grafted trees of 61 clones were planted in spacing 6 x 6 m (1,13 ha). During January 2000 grafts were collected from 30 plus trees in MF Velichov (western Bohemia). This material was also used for establishment of seed orchard in prepared plot in MF Velichov in spring 2002. 240 grafted trees of 28 clones were planted in spacing 7 x 6 m (1,25 ha)

In spring 1999 research clone archive was established in School Forest Enterprise (SFE) in Kostelec n. C. l. (central Bohemia). This material was obtained from plus trees in South-East of Moravia. 118 grafted trees of 55 clones were planted in spacing 3 x 3 m. In spring 1999 seed orchard of the second generation from genetically verified trees by progeny test from Germany was established in Forests of the Town Prostejov (0,86 ha) (central Moravia). 228 grafted trees of 30 clones were planted in spacing 6 x 6 m. Seed orchard of the same clones was already established in SFE in Kostelec n. C. l. in spring 1998. 155 grafted trees of 30 clones were planted in spacing 6 x 6 m. In spring 2000 reserve clone archive of 26 same clones was established in SFE in Kostelec n. C. l. 141 grafted trees were planted there in spacing 3 x 3 m. From spring 2001 flowering of grafted trees and from summer 2001 damage by aphids is regularly controled.

In autumn 2000 progeny test of individual selected trees in SFE in Kostelec n. C. l. was established in this enterprise. In October 1999 seedlings of individual progenies were measured in nursery. Significant differences between progenies were found. Progeny test was established by plantation of 335 plants of 14 half-sib progenies in spacing 1,5 x 1,5 m. From spring and autumn 2001 plant height is regularly measured. Significant differences in these parameters were found. From summer 2001 damage of plants by aphids is regularly controled. In spring 2000 clonal test of plants from cultures in vitro was established in SFE in Kostelec n. C. l. 140 plants of 28 clones (by 5 plants per clone) were planted in spacing 3 x 3 m. Clones n. 1 – 10 were obtained from selected trees in FD FCR Krivoklát and others from plus trees in FD FCR Nové Hrady and Prachatice (southern Bohemia). From spring and autumn 2001 height is regularly measured. Significant differences between clones in these parameters were found. From summer 2001 damage by aphids is regularly controled. Measurement in several clonal tests in southern Bohemia has been started from summer 2003.

Participant List

Albert Abbott
Clemson University
Dept of Genetics & Biochemistry
Clemson, SC 29634
USA
Phone: 864-656-3060
Fax: 864-656-6879
aalbert@clemson.edu

Sally Aitken
University of British Columbia
3041-2424 Main Mall
Vancouver, BC V6T1Z4
Canada
Phone: 604-822-6020
Fax: 604-822-9102
sally.aitken@ubc.ca

Pierre Alazard
AFOCEL
Domaine de Sivaillan
Moulis en Medoc, 33 480
France
Phone: 33-5-57-88-82-33
Fax: 33-5-57-88-82-34
pierre.alazard@afocel.fr

Evi Alizoti
Aristotle University of Thessaloniki
Dept. of Forestry and Natural Env.
Thessaloniki, NM 54124
Greece
Phone: 011-302-310-992769
Fax:
alizotp@for.auth.gr

Henry Amerson
NC State University
2500 Partners II Bldg.
Raleigh, NC 27695
USA
Phone: 919-513-0012
Fax: 919-515-7801
amerson@unity.ncsu.edu

Bengt A. Andersson
Skogforsk
Box 3, SE-918 21
Savar, SE-918 21
Sweden
Phone: 46-90-150950
Fax: 46-90-150960
bengt.andersson@skogforsk.se

Emanuel Araya
Instituto Tecnologico de Costa Rica
Escuela de Ingenieria Forestal
Cartago, 159-7050
Costa Rica
Phone: 506-550-2511
Fax: 506-550-4182
evalverde@itcr.ac.cr

Tuija S. Aronen
Finnish Forest Research Institute
Finlandiantie 18
Punkaharju, FIN-58450
Finland
Phone: 358-10-211-4233
Fax: 358-10-211-4201
tuija.aronen@metla.fi

George Askew
Clemson University
PO Box 596
Georgetown, SC 29442
USA
Phone: 843-546-1013 x230
Fax: 843-546-6296
gaskew@clemson.edu

Steve Attree
CellFor
PO Box 133
Brentwood Bay, BC V8M2A3
Canada
Phone: 250-544-0787 x223
Fax: 250-544-0769
sattree@cellfor.com

Ryan Atwood
MeadWestvaco
103 Bay Colony Ct
Summerville, SC 29483
USA
Phone: 8436-556-8391
Fax:
rae4@meadwestvaco.com

Yorleny Badilla
Instituto Tecnologico de Costa Rica
Escuela de Ingenieria Forestal
Cartago, 159-7050
Costa Rica
Phone: 506-550-2511
Fax: 506-591-4182
ybadilla@hotmail.com

Claudio Balocchi
BioForest SA
Casilla 70-C
Concepcion,
Chile
Phone: 56-41-390438
Fax: 56-41-390439
cbalocch@arauco.cl

Brian Baltunis
Forest Biology Research Cooperative
10015 NE Waldo Rd
Gainesville, FL 32609
USA
Phone: 352-846-0894
Fax: 352-846-1277
baltunis@ufl.edu

Wayne Barfield
MeadWestvaco
.
USA
Phone:
Fax:

Michael R Becwar
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5162
Fax: 843-851-4595
mrbecwa@arborgen.com

Andy Benowicz
CellFor
PO Box 133
Brentwood Bay, BC V8M1R3
Canada
Phone: 250-544-0787x249
Fax: 250-544-0769
abenowicz@cellfor.com

Peter Birks
Buffalo Creek Silvics
120 Tecumseh Ct
Wendell, NC 27591
USA
Phone: 919-606-6363
Fax: 919-553-3129
birks_forestry@mindspring.com

Tom Blush
MeadWestvaco
PO BOX 1950
Summerville, SC 29484
USA
Phone: 843-851-4768
Fax: 843-875-7185
thomas.blush@meadwestvaco.com

Anne-Christine Bonfils
Natural Resources Canada
580 Booth St., 12D2
Ottawa, ON KIA0E4
Canada
Phone: 613-947-9039
Fax: 613-947-9035
abonfils@nrcan.gc.ca

Floyd E Bridgwater
USDA Forest Service
3041 Cornwallis Rd
Research Triangle Park, NC 27709
USA
Phone: 919-549-4019
Fax: 919-549-4047
fbridgwater@fs.fed.us

Kathleen M Brosemer
Forest Genetics Ontario
510 Queen Street East, Suite 24
Sault Ste. Marie, ON P6A 2A9
Canada
Phone: 705-942-4907
Fax: 705-942-9140
kbrosemer@fgo.ca

Amy Brunner
Oregon State University
Dept of Forest Science
Corvallis, OR 97331
USA
Phone: 541-737-8488
Fax:
amy.brunner@oregonstate.edu

Richard L. Bryant
International Paper Company
719 Southlands Rd.
Bainbridge, GA 39819
USA
Phone: 229-246-3642
Fax: 229-243-0766
richard.bryant@ipaper.com

Mary Burdon
Private Bag 3020
Rotorua,
New Zealand
Phone: 64-7-343-5742
Fax: 64-7-343-0952
rowland.burdon@forestresearch.co.nz

Rowland Burdon
New Zealand Forest Research Institute
Private Bag 3020
Rotorua,
New Zealand
Phone: 64-7-343-5742
Fax: 64-7-343-0952
rowland.burdon@forestresearch.co.nz

Tom Byram
Texas Forest Service
Bldg 1042 Agronomy Rd TAMU 2585
College Station, TX 77843-2585
USA
Phone: 979-845-2523
Fax: 979-845-3272
psieling@tfs.tamu.edu

David Canavera
MeadWestvaco
PO BOX 1950
Summerville, SC 29484
USA
Phone: 843-851-4774
Fax: 843-875-7185
david.canavera@meadwestvaco.com

Christina M Cannistra
ArborGen
180 Westvaco Rd
Summerville, SC 29483
USA
Phone: 843-851-4598
Fax: 843-851-4595
cmcanni@arborgen.com

William C Carlson
Weyerhaeuser
WTC 1B10, PO Box 9777
Federal Way, WA 98063-9777
USA
Phone: 253-924-6880
Fax: 253-924-6736
bill.carlson@weyerhaeuser.com

Mike Carson
Carson Associates Ltd
34 Parawai Rd
Rotorua, 3202
New Zealand
Phone: 64 7 357 4244
Fax: 64 7 357 4744
mike.carson@forest-genetics.com

Sue Carson
Carson Associates Ltd
34 Parawai Rd
Rotorua, 3202
New Zealand
Phone: 07 357 4244
Fax: 07 357 4744
sue.carson@forest-genetics.com

Shujun Chang
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5163
Fax: 843-851-4595
sxchang@arborgen.com

Xiaoyang Chen
Beijing Forestry University
Qinghua E Rd 35, Haidian District
Beijing, 100083
PR China
Phone: 86 10 62338005
Fax:
xychen@bjfu.edu.cn

Vincent Chiang
NC State University
Campus Box 7247
Raleigh, NC 27695
USA
Phone: 919-513-0098
Fax: 919-515-7801
vincent_chiang@ncsu.edu

Kamal Chowdhury
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5060
Fax: 843-851-4595
mkchowd@arborgen.com

Alexander Clark
USFS
320 Green St
Athens, GA 30602
USA
Phone: 706-559-4323
Fax: 706-559-4317
aclark@fs.fed.us

John J. Clark
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5063
Fax: 843-832-6484
jjclark@arborgen.com

Marie B Connett
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5087
Fax: 843-832-2164
mbconne@arborgen.com

Mary Cook
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4583
Fax: 843-851-4595
mjcook@arborgen.com

Paul Cotterill
Private Bag 10
Clayton South, Victoria 3169
Australia
Phone: 61-419-818-314
Fax:
paul.cotterill@csiro.au

Russell Cox
Tennessee Division of Forestry
PO Box 2666
Knoxville, TN 37901
USA
Phone: 865-594-6432
Fax: 865-594-5430
russell.cox@state.tn.us

Barbara Crane
USDA Forest Service
1720 Peachtree Rd NW
Atlanta, GA 30309
USA
Phone: 404-347-4039
Fax: 404-347-4154
barbaracrane@fs.fed.us

Patrick Cumbie
Weyerhaeuser Company
PO Box 238
Oglethorpe, GA 31068-0238
USA
Phone: 478-214-2446
Fax: 912-526-8615
patrick.cumbie@weyerhaeuser.com

Michael W Cunningham
International Paper Company
PO Box 1391
Savannah, GA 31402
USA
Phone: 912-238-7615
Fax: 912-238-7160
mike.cunningham@ipaper.com

Dick Daniels
University of Georgia Wood Quality
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-7298
Fax: 706-542-8356
ddaniels@forestry.uga.edu

Mark S Davis
Florida Division of Forestry
11650 Munson Hwy.
Milton, FL 32570
USA
Phone: 850-957-6160
Fax: 850-957-6143
davism@doacs.state.fl.us

Bart De Cuyper
Institute for Forestry & Game Mgt
Gaverstraat 4
Geraardsbergen, Flanders 9500
Belgium
Phone: 32 54 43 61 77
Fax: 32 54 43 61 60
bart.decuyper@lin.vlaanderen.be

Christian De Veer
BioForest SA
Casilla 70-C
Concepcion,
Chile
Phone: 56-41-390438
Fax: 56-41-390439
cdeveer@arauco.cl

Christine Dean
Weyerhaeuser Company
Technology Ctr, WTC 1A5 PO Box 9777
Federal Way, WA 98063-9777
USA
Phone: 253-924-6892
Fax: 253-924-6736
christine.dean@weyerhaeuser.com

Jeff DeBell
Washington DNR
PO Box 47017
Olympia, WA 98504
USA
Phone: 360-407-7578
Fax:
jeff.debell@wadnr.gov

Jed DeHaven
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4143
Fax: 843-851-4595
jedehav@arborgen.com

Mark Dieters
University of Queensland
School Land and Food Sciences
St Lucia, QLD 4072
Australia
Phone: 61-7-33657410
Fax: 61-7-33651177
m.dieters@uq.edu.au

Stephen P DiFazio
Oak Ridge National Laboratory
1 Bethel Valley Rd
Oak Ridge, TX 37830
USA
Phone: 865-574-7844
Fax: 865-576-1393
difazios@ornl.gov

Phil Dougherty
MeadWestvaco
PO BOX 180
Summerville, SC 29484
USA
Phone: 843-851-4750
Fax: 843-875-7185
pmd6@meadwestvaco.com

Nicklso Dudley
Hawaii Ag Research Center
99-193 Aiea Hts. Dr.
Aiea, HI 96701
USA
Phone: 808-486-5334
Fax:
ndudley@harc-hspa.com

Bonnie P Dumas
MeadWestvaco Corp
PO Box 1950
Summerville, SC 29484
USA
Phone: 843-851-4725
Fax: 843-875-7185
bonnie.dumas@meadwestvaco.com

Philip H Dunham
MeadWestvaco Corp
PO Box 1950
Summerville, SC 29484
USA
Phone: 843-851-4726
Fax: 843-875-7185
phd4@meadwestvaco.com

Greg Dutkowski
CRC for Sustainable Production Forestry
Private Bag 12
Hobart, Tasmania 7008
Australia
Phone: 0408238495
Fax: 61-3-6226-7942
g.dutkowski@utas.edu.au

Bill S. Dvorak
CAMCORE, NC State University
1110 Grinnells Lab, Box 7626
Raleigh, NC 27695
USA
Phone: 919-515-6426
Fax: 919-515-6430
bill_dvorak@ncsu.edu

Ulrika Egertsdotter
Georgia Institute of Technology
500 10th St NW
Atlanta, GA 30332
USA
Phone: 404-894-0363
Fax: 404-894-4778
ulrika.egertsdotter@ipst.gatech.edu

Yousry A El-Kassaby
University of British Columbia
3616-2424 Main Mall
Vancouver, BC V6T 1Z4
Canada
Phone: 604-822-1821
Fax: 604-822-9102
y.el-kassaby@ubc.ca

Tore Ericsson
Skogforsk
Box 3, SE-918 21
Savar, SE-918 21
Sweden
Phone: 46-90-150950
Fax: 46-90-150960
tore.ericsson@skogforsk.se

Johan Eriksson
Swedish Univ. of Ag. Sciences
Skogforsk, Box 3
Sava, Umea SE-91821
Sweden
Phone: 46-90-15-09-54
Fax: 46-90-15-09-60
johan.eriksson@skogforsk.se

Sam Foster
USDA Forest Service
1601 N. Kent St., 4th Floor
Arlington, VA 22209
USA
Phone: 703-605-5277
Fax: 703-605-0279
gfoster@fs.fed.us

Kirk R Foutz
ArborGen
PO Box 840001
Summerville, SC 29484-8401
USA
Phone: 843-851-6484
Fax: 843-851-4595
krfoutz@arborgen.com

John Frampton
NC State University
Campus Box 8002
Raleigh, NC 27695
USA
Phone: 919-515-7580
Fax: 919-515-8149
john_frampton@ncsu.edu

Josef Frydl
Forestry & Game Mgt Research Institute
Jiloviste-Strnady
Praha 5 - Zbraslav, 156 04
Czech Republic
Phone: 00 420 257 892 271
Fax: 00 420 257 921 444
frydl@vulhm.cz

Carlos Gantz
Forestal Mininco SA
Avda Alemania 751
Los Angeles,
Chile
Phone: 56-43-405351
Fax: 56-43-312701
cgantz@formin.cmpc.cl

Dave M Gerwig
MeadWestvaco
PO Box 739
Ravenel, SC 29470
USA
Phone: 843-556-8391
Fax: 843-556-7584
dmg8@meadwestvaco.com

Nancy Gillette
Pacific Southwest Experiment Station
PO Box 245
Berkeley, CA 94701
USA
Phone: 510-559-6474
Fax:
ngillette@fs.fed.us

Barry Goldfarb
NC State University
CAMPUS BOX 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-4471
Fax: 919-515-3169
barry_goldfarb@ncsu.edu

Susumu Goto
University of Tokyo
Yamabe, Furano
Hokkaido, 079-1561
Japan
Phone: 81-167-42-2111x22
Fax: 81-167-42-2689
gotos@uf.a.u-tokyo.ac.jp

Daniel Grans
NC State University
2300 Avent Ferry Rd, Apt. G-7
Raleigh, NC 27606
USA
Phone: 919-836-1621
Fax:
dagrans@unity.ncsu.edu

Michael Greenwood
University of Maine
5755 Nutting Hall
Orono, ME 04469-5755
USA
Phone: 207-581-2838
Fax: 207-581-4257
greenwd@umenfa.maine.edu

Jim Grissom
NC State University
Campus Box 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-5453
Fax: 919-515-3169
jim_grissom@ncsu.edu

Matti Haapanen
Finnish Forestry Research Institute
Box 18
Vantaa, FIN-02770
Finland
Phone: 358-10-2112521
Fax:
matti.haapanen@metla.fi

Richard B. Hall
Iowa State University
124 Science II
Ames, IA 50011-3221
USA
Phone: 515-294-1453
Fax: 515-294-2995
rbhall@iastate.edu

Kyung-Hwan Han
Michigan State University
126 Natural Resources
East Lansing, MI 48824-1222
USA
Phone: 517-353-4751
Fax: 517-432-1143
ander174@msu.edu

John Hendrickson
Temple-Inland
PO Box 659
Leesburg, AL 35983
USA
Phone: 256-526-5190
Fax: 256-526-4383
johnHendrickson@templeinland.com

Michael Henson
Primary Industries Trading
.
Australia
Phone:
Fax:

Takashi Hibino
OJI Paper
Nobono 24-9
Kameyama, Mie 519-0212
Japan
Phone: 81-595-85-0122
Fax: 81-595-85-1528
takashi-hibino@ojipaper.co.jp

Maud Hinchee
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5078
Fax: 843-832-2164
mahinch@arborgen.com

Gary Hodge
NC State University
1110 Grinnells Lab, Box 7626
Raleigh, NC 27695
USA
Phone: 919-515-6427
Fax: 919-515-6430
grh@ncsu.edu

Heather Holley
ArborGen
Box 840001
Summerville, SC 29466
USA
Phone: 843-851-5062
Fax:
hmholle@arborgen.com

Stephen Horner
Scotia Pacific Co
PO Box 712
Scotia, CA 95565
USA
Phone: 707-764-4128
Fax: 707-764-4155
shorner@scopac.com

Benjamin Hornsby
University of Georgia Wood Quality
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-0917
Fax: 706-542-8356
bsh5594@forestry.uga.edu

Glenn Howe
Oregon State University
321 Richardson Hall
Corvallis, OR 97331
USA
Phone: 541-737-9001
Fax: 541-737-1393
glenn.howe@oregonstate.edu

Scott C Howell
University of Georgia Wood Quality
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-0917
Fax: 706-542-8356
showell@forestry.uga.edu

Dudley Huber
CFGRP
PO Box 110410
Gainesville, FL 32611-0410
USA
Phone: 352-846-0898
Fax: 352-846-1277
dahuber@ufl.edu

Fikret Isik
NC State University
Box 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-1234
Fax: 919-515-3169
fisik@ncsu.edu

Larry Jackson
Applied Industry Analysis Ltd.
PO Box 5611 Wellesley St
Auckland, Auckland 1036
New Zealand
Phone: 64-9-524-5122
Fax:
lrj@industryanalysis.co.nz

Gunnar Jansson
Skogforsk
Uppsala Science Park
Uppsala, SE-751 83
Sweden
Phone: 46-18-18-15-56
Fax: 46-18-18-86-00
gunnar.jansson@skogforsk.se

Keith J.S. Jayawickrama
Oregon State University
321 Richardson Hall
Corvallis, OR 97330
USA
Phone: 541-737-8432
Fax: 541-737-1393
keith.jayawickrama@oregonstate.edu

Randy Johnson
USDA Forest Service
3200 SW Jefferson Way
Corvallis, OR 97331-4401
USA
Phone: 541-750-7290
Fax:
randyjohnson@fs.fed.us

David Jones
University of Georgia Wood Quality
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-2686
Fax: 706-542-8356
pdj7477@forestry.uga.edu

Lewis Jordan
University of Georgia Wood Quality
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-9724
Fax: 706-542-8356
lcj6444@forestry.uga.edu

Dominic P Kain
Australian National University
84 Dryandra St O'Connor
Canberra, ACT 2602
Australia
Phone: 61-2-6248-7863
Fax:
dominic.kain@anu.edu.au

Robert P Karrfalt
USDA Forest Service
1007 N 725 W
W Lafayette, IN 47906
USA
Phone: 765-583-3502
Fax: 765-583-3512
rkarrfalt@fs.fed.us

Angelia J Kegley
USDA Forest Service
34963 Shoreview Dr
Cottage Grove, OR 97424
USA
Phone: 541-767-5703
Fax: 541-767-5709
akegley@fs.fed.us

Bob Kellison
Institute of Forest Biotechnology
PO Box 13399
RTP, NC 27799
USA
Phone: 919-549-8896
Fax: 919-549-9710
bob_kellison@ncbiotech.org

Larita Kellison
Raleigh, NC
USA
Phone:
Fax:

John N King
British Columbia Forest Service
PO Box 9519, Stn Prov Govt
Victoria, BC V8W 9C2
Canada
Phone: 250-387-6476
Fax: 250-387-0046
john.king@gems7.gov.bc.ca

Bohun Kinloch
Institution of Forest Genetics
PO Box 245
Berkeley, CA 94701
USA
Phone: 510-704-7745
Fax:
bkinloch@fs.fed.us

Matias Kirst
Cornell University
,
USA
Phone:
Fax:
mk366@cornell.edu

Jenny Knoth
International Paper
PO Box 2668
Savannah, GA 31402
USA
Phone: 912-238-6119
Fax:
jenny.knoth@ipaper.com

Jaroslav Kobliha
Czech Univ of Agriculture in Prague
Kamycka 1176, Praha 6 - Suchdol
Prague, Czechia 165 21
Czech Republic
Phone: 00420-224382878
Fax:
kobliha@fle.czu.cz

Robert Kodrzycki
ArborGen
PO Box 840001
Summerville, SC 29494
USA
Phone: 843-851-4133
Fax: 843-851-4595
rjkodrz@arborgen.com

Dorina Korol
Tzalaf 3
Yavne, Israel 81200
Israel
Phone: 972 8 9426428
Fax:
vckorol@bezeqint.net

Leonid Korol
Agr.Res.Org. Volcani Centre Israel
POB 6, Bet Dagan, Israel
Beit Dagan, Israel 502520
Israel
Phone: 972 3 968 36 78
Fax:
vckorol@agri.gov.il

Thomas L Kubisiak
USDA Forest Service
23332 Hwy 67
Saucier, MS 39574
USA
Phone: 228-832-2747x213
Fax: 228-852-8130
tkubisiak@fs.fed.us

Susumu Kurinobu
Forest Tree Breeding Center
3809-1, Ishi, Juo
Ibaraki, 319-1301
Japan
Phone: 81-293-32-7045
Fax: 81-293-32-7306
kurinobu@affrc.go.jp

Clem Lambeth
Weyerhaeuser Company
PO Box 1060
Hot Springs, AR 71901
USA
Phone: 501-624-8510
Fax: 501-624-8505
clem.lambeth@weyerhaeuser.com

Gregory N Leach
International Paper Company
4025 Hwy 178
Jay, FL 32565
USA
Phone: 850-675-0929
Fax: 850-675-0938
greg.leach@ipaper.com

Steve J. Lee
Forestry Commission
Northern Research Station
Roslin, Midlothian EH25 9SY
Scotland, UK
Phone: 44-131-445-6926
Fax: 44-131-445-5124
steve.lee@forestry.gsi.gov.uk

Ann G. Leffel
13275 Laurel Rd
Brogue, PA 17309
USA
Phone: 717-927-9557
Fax:
baleffel@blazenet.net

Robert C. Leffel
ANS-USDA (Retired) & PA Chapter TACF
13275 Laurel Rd
Brogue, PA 17309
USA
Phone: 717-927-9557
Fax:
baleffel@blazenet.net

Mark R Lesser
Lakehead University
955 Oliver Rd
Thunder Bay, On P7B 5E1
Canada
Phone: 807-346-7794
Fax: 807-343-8116
mrlesser@lakeheadu.ca

Bailian Li
NC State University
Box 8002
Raleigh, NC 27695
USA
Phone: 919-515-6845
Fax: 919-515-3169
bailian_li@ncsu.edu

Bill J Libby
Zobel Forestry Assoc
28 Valencia Rd
Orinda, CA 94563
USA
Phone: 925-376-5896
Fax: 925-376-7928
wlibby2@aol.com

Iris Libby
28 Valencia Rd
Orinda, CA 94563
USA
Phone: 925-376-5896
Fax: 925-376-7928
classyick@yahoo.com

Dag Lindgren
Swedish Univ. of Ag. Sciences
SE 901 83 Umea Sweden
, 901 83
Sweden
Phone:
Fax:
dag.lindgren@genfys.slu.se

Judy A Loo
Canadian Forest Service
PO Box 4000
Fredericton, NB E3B5P7
Canada
Phone: 506-452-3398
Fax: 506-452-3525
jloo@nrcan.gc.ca

Milan Lstiburek
NC State University
CB 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-3168
Fax:
mlstibu@unity.ncsu.edu

Pengxin Lu
Ontario Forest Research Institute
1235 Queen St E
Sault Ste Mare, Ontario P6A2E5
Canada
Phone: 705-946-7415
Fax: 705-946-2030
pengxin.lu@mnr.gov.on.ca

John J MacKay
Universite Laval
Centre de Recherche en Biologie For
Quebec, QC G1K 7P4
Canada
Phone: 418-656-2278
Fax: 418-656-7493
jmackay@rsvs.ulaval.ca

Francois Malan
Komatiland Forests Pty Ltd
PO Box 574
Sabie, Mpumalanga 1260
South Africa
Phone: 27137643493
Fax: 27137641239
fsmalan@klf.co.za

Csaba Matyas
University of West Hungary
POB 132
Sopron, Hungary 9401
Hungary
Phone: 36-99-518-395
Fax: 36-99-329-840
cm@emk.nyme.hu

Early McCall
Rayonier
PO Box 819
Yulee, FL 32034
USA
Phone: 904-225-5393
Fax: 904-225-0370
early.mccall@rayonier.com

Kim McCourry
ArborGen
180 Westvaco Rd
Summerville, SC 29483
USA
Phone: 843-851-5092
Fax: 843-851-4595
jssage@arborgen.com

Steven E McKeand
NC State University
Box 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-6073
Fax: 919-515-3169
steve_mckeand@ncsu.edu

Jim McKenna
USDA Forest Service
715 W State St
W Lafayette, IN 47907
USA
Phone: 765-494-3624
Fax: 765-494-9461
jmckenn@purdue.edu

Kim McManus
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843--851-4141
Fax: 843-851-4595
kfmcman@arborgen.com

Tony McRae
Southern Tree Breeding Assoc
PO Box 1811
Mt Gambier, SA 5290
Australia
Phone: 61-8-87-230588
Fax: 61-8-87230660
tmcrae@stba.com.au

Rick Meilan
Purdue University
715 W. State St.
W. Lafayette, IN 47907
USA
Phone: 765-496-2287
Fax: 765-494-9461
rmeilan@purdue.edu

Larry G Miller
Texas Forest Service
Bldg 1042 Agronomy Rd, TAMU 2585
College Station, TX 77843-2585
USA
Phone: 979-845-2523
Fax: 979-845-3272
psieling@tfs.tamu.edu

Lawrence K Miller
Boise Cascade
Cottonwood Fiber Farm, PO Box 500
Wallula, WA 99363
USA
Phone: 509-544-6535
Fax: 509-545-9964
larrymiller@bc.com

Samantha A Miller
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4139
Fax: 843-851-4595
samille@arborgen.com

Olga N. Milliken
ArborGen
4th Floor, 1135 Arawa St.
Rotorua, 3201
New Zealand
Phone: 64-7-350-1421
Fax: 64-7-350-1425
olga.milliken@xtra.co.nz

William R Moody
South Carolina Forestry Commission
POB 21707
Columbia, SC 29221
USA
Phone: 803-896-8863
Fax:
rmoody@forestry.state.sc.us

Susan E Moore
NC State University
Campus Box 8003
Raleigh, NC 27695
USA
Phone: 919-515-3184
Fax: 919-515-6883
susan_moore@ncsu.edu

Andrew Morris
Sappi
Shaw Research Center, PO Box 473
Howick, 3290
South Africa
Phone: 27-033-3302455
Fax: 27-033-3304938
andrew.morris@sappi.com

Daniel Morrow
International Paper
4025 Hwy 178
Jay, FL 32565
USA
Phone: 850-675-0929 x122
Fax: 850-675-0938
dan.morrow@ipaper.com

Alison Morse
University of Florida
118 Newins-Zeigher Hall, POB 110410
Gainsville, FL 32611
USA
Phone: 352-846-0883
Fax: 352-846-1277
ammorse@ufl.edu

Michael Moynihan
Fundacion Chile
AV Parque Antonio Rabat 6165
Santiago, RM 00000
Chile
Phone: 56-2-240-0446
Fax:
mmoynihan@fundacionchile.cl

Nicholas Muir
Temple-Inland
229 N Bowie St
Jasper, TX 75951
USA
Phone: 409-383-2552
Fax: 409-384-5394
nicholasmuir@templeinland.com

Tim Mullin
NC State University
Campus Box 8002
Raleigh, NC 27695
USA
Phone: 919-515-3644
Fax: 919-515-3169
tim_mullin@ncsu.edu

Olman Murillo
Instituto Tecnologico de Costa Rica
Escuela de Ingenieria Forestal
Cartago, 159-7050
Costa Rica
Phone: 506-550-2511
Fax: 506-591-4182
omurillo@itcr.ac.cr

Zander Myburg
University of Pretoria
Dept of Genetics
Pretoria, 0002
South Africa
Phone: 27-12-4204945
Fax: 27-12-3625327
zander.myburg@fabi.up.ac.za

Jennifer H Myszewski
USDA Forest Service
23332 Hwy 67
Saucier, MS 39574
USA
Phone: 228-832-2747 x212
Fax: 228-832-0131
jmyszewski@fs.fed.us

David B Neale
Institute of Forest Genetics
Dept of Environmental Horticulture
Davis, CA 95616
USA
Phone: 530-754-8431
Fax: 530-754-9366
dbneale@ucdavis.edu

Narender Nehra
ArborGen
PO Box 840001
Summerville, SC 29485
USA
Phone: 843-851-4134
Fax: 843-851-2164
nsnehra@arborgen.com

Charles Dana Nelson
USDA Forest Service
23332 Hwy 67
Saucier, MS 39574
USA
Phone: 228-832-2747 x209
Fax: 228-832-0130
dananelson@fs.fed.us

Teijo O Nikkanen
Finnish Forest Research Institute
Punkaharju Research Station
Punkaharju, FIN 58450
Finland
Phone: 358 10 211 4226
Fax: 358 10 211 4201
teijo.nikkanen@metla.fi

Kim Norris-Caneda
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4145
Fax: 843-851-4595
khnorri@arborgen.com

Mike Oppenheimer
Smurfit-Stone
PO Box 457
Fernandina, FL 32035
USA
Phone: 904-277-5842
Fax:
moppenhe@smurfit.com

Luis Osorio
Smurfit Carton de Colombia
CRA 3 #10-36
Cali, AA219 Cali
Colombia
Phone: 572-6914087
Fax: 572-6914173
luis.osorio@co.smurfitgroup.com

Ronald Overton
USDA Forest Service
Purdue Univ., Pfendler Hall
West Lafayette, IN 47907
USA
Phone: 765-496-6417
Fax: 765-494-9461
roverton@fs.fed.us

Hikmet Ozturk
Forest Tree Seeds/Tree Breeding
Research Inst.
.
Turkey
Phone:
Fax:

John Pait
CellFor
75 5th St NW Ste 321
Atlanta, GA 30308
USA
Phone: 404-526-6176
Fax: 404-526-6179
bwatson@cellfor.com

Yill-Sung Park
PO Box 4000
Fredericton, New Brunsw E3B 5P7
Canada
Phone: 506-452-3585
Fax: 506-452-3525
ypark@nrcan.gc.ca

Dawn Parks
ArborGen
PO Box 840001
SUMMERVILLE, SC 29484
USA
Phone: 843-851-5077
Fax: 843-832-2164
dwparks@arborgen.com

Patrick Pastuszka
INRA
69 route d'Arcachon
CESTAS, 33612
France
Phone: 33-5-57-12-28-16
Fax: 33-5-56-68-02-23
patrick.pastuszka@pierroton.inra.fr

Kitt Payn
NC State University
4339 Avent Ferry Rd Apt 4
Raleigh, NC 27606
USA
Phone: 919-851-8866
Fax:
kgpayn@unity.ncsu.edu

Les Pearson
ArborGen
PO Box 840001
Summerville, SC 29434
USA
Phone: 843-851-4597
Fax: 843-832-2164
lxpears@arborgen.com

Sara Matilda Pettersson
Swedish Univ. of Ag. Sciences
Dept. Forest Genetics & Plant Phys.
Umea, 901 83
Sweden
Phone: 46-90-7898280
Fax:
sara.pettersson@genfys.slu.se

Gwenael Philippe
Cemagref
Domaine Des Barres
Nogent-sur-Vernisson, Loiret 45290
France
Phone: 33 2 38 95 03 30
Fax: 33 2 38 95 03 46
gwenael.philippe@cemagref.fr

Michael Powell
Southern Tree Breeding Assoc
PO Box 1811
Mt Gambier, SA 5290
Australia
Phone: 08-8723-0688
Fax: 08-8723-0688
mpowell@stba.com.au

Annie Raffin
INRA
69 route d'Arcachon
CESTAS, 33612
France
Phone: 33-5-57-12-28-30
Fax: 33-5-57-12-28-81
annie.raffin@pierroton.inra.fr

Fred Raley
Texas Forest Service
Bldg 1042 Agronomy Rd TAMU 2585
College Station, TX 77843-2585
USA
Phone: 979-845-2523
Fax: 979-845-3272
psieling@tfs.tamu.edu

Wickneswari Ratnam
Universiti Kebangsaan Malaysia
Sch. Of Env. & Natural Res. Science
Bangi, Selangor 43600
Malaysia
Phone: 6-03-89253357
Fax:
wicki@pkrisc.cc.ukm.my

James H Roberds
USDA Forest Service
23332 Hwy 67
Saucier, MS 39574
USA
Phone: 228-832-2747 x211
Fax: 228-832-0130
jroberds@fs.fed.us

Kay H Roberds
18184 Village Dr.
Saucier, MS 39574
USA
Phone: 228-328-4183
Fax:
khroberds@aol.com

Juan Jose Robledo-Arnuncio
Rutgers University
14 College Farm Rd
New Brunswick, NJ 08901
USA
Phone: 732-932-1122
Fax: 732-932-8746
jrobledo@runbox.com

Eduardo Rodriguez
BioForest SA
Casilla 70-C
Concepcion,
Chile
Phone: 56-41-390438
Fax: 56-41-390439
erodrigu@arauco.cl

Andrew Rodwell
Horizon 2
State Hwy 30
Whakatane,
New Zealand
Phone: 647 322 9058
Fax: 647 322 9040
andrew.rodwell@horizon2.co.nz

Kenneth R Roeder
NC Division of Forest Resources
762 Claridge Nursery Rd
Goldsboro, NC 27530
USA
Phone: 919-731-7988
Fax: 919-731-7993
ken.roeder@ncmail.net

Jose Romero
CellFor
75 5th St NW #321
Atlanta, GA 30308
USA
Phone: 404-526-6177
Fax: 404-526-6179
jromero@cellfor.com

Christopher L. Rosier
Smurfit - Stone Container Corp.
PO Box 457
Fernandina Beach, FL 32035
USA
Phone: 904-277-5826
Fax: 904-277-7742
crosier@smurfit.com

Ola Rosvall
Skogforsk
Box 3
Savar, SE-918 21
Sweden
Phone: 4690-150950
Fax: 4690-150960
ola.rosvall@skogforsk.se

Will Rottmann
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5091
Fax: 843-851-4595
whrottm@arborgen.com

Sairam V. Rudrabhatla
University of Toledo
2801 West Bancroft, Mail Stop 604
Toledo, OH 43606
USA
Phone: 419-530-1587
Fax: 419-530-1539
srudrab@utnet.utoledo.edu

Marc L Rust
Inland Empire Tree Improvement Coop
University of Idaho, CNR
Moscow, ID 83844-1133
USA
Phone: 208-885-7109
Fax:
mrust@uidaho.edu

Mark R. Rutter
ArborGen
POB 840001
Summerville, SC 29484
USA
Phone: 843-851-4596
Fax:
mrrutte@arborgen.com

Jessica Sage
ArborGen
180 Westvaco Rd
Summerville, SC 29483
USA
Phone: 843-851-5092
Fax: 843-851-4595
jssage@arborgen.com

Joe Sanders
University of Georgia
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-542-0917
Fax: 706-542-8356
sanders@forestry.uga.edu

Yoh Sasaki
Akita Technical Forestry Center
Akita, 019-2611
Japan
Phone: 018-882-4513
Fax: 018-882-4443
y-sasaki@pref.akita.lg.jp

Laurie Schimleck
University of Georgia
Forest Resources
Athens, GA 30602-2152
USA
Phone: 706-583-0464
Fax: 706-542-8356
lschimleck@forestry.uga.edu

Scott E Schlarbaum
University of Tennessee
274 Ellington Hall
Knoxville, TN 37996
USA
Phone: 865-974-7993
Fax: 865-974-4717
tennfip@utk.edu

Elaine Schmidtling
Saucier, MS 39574

Phone:
Fax:

Ronald C Schmidtling
USDA Forest Service
23332 Hwy 67
Saucier, MS 39574
USA
Phone: 228-832-2747
Fax:
rschmidtling@fs.fed.us

Joshua R. Sherrill
NC State University
CB 8002
Raleigh, NC 27695
USA
Phone: 919-515-3168
Fax:
jrsherri@ncsu.edu

Kevin Short
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5170
Fax:
kjshort@arborgen.com

Victor Sierra
Forestal Mininco SA
Avda Alemania 751
LOS ANGELES,
Chile
Phone: 56-43-405350
Fax: 56-43-312701
vsierra@formin.cmpc.cl

Tore Skroppa
Norwegian Forest Research Institute
Hogskoleveien 8
Aas, 1432
Norway
Phone: 47-64949067
Fax: 47-64942980
tore.skroppa@skogforsk.no

Peter Smouse
Rutgers University
14 College Farm Rd
New Brunswick, NJ 08901
USA
Phone: 732-932-1120
Fax: 732-932-8746
smouse@aesop.rutgers.edu

Richard Sniezko
USDA Forest Service
34963 Shoreview Dr.
Cottage Grove, OR 97424
USA
Phone: 541-767-5716
Fax: 541-767-5709
rsniezko@fs.fed.us

Kostas Spanos
NAGREF Forest Research Institute
57006 Vassilika
Thessalonia,
Greece
Phone: 30 2310461171(2,3)
Fax: 30 2310461341
kspanos@fri.gr

Per H Stahl
Forestry Research Institute of Sweden
Uppsala Science Park
Uppsala, SE-751 83
Sweden
Phone: 46-18-188570
Fax: 46-18-188600
per.stahl@skogforsk.se

Arne Steffenrem
Norwegian Forest Research Institute
Hogskoleveien *
As, Akershus N-1432
Norway
Phone: 47-64949051
Fax:
arne.steffenrem@skogforsk.no

Leena Suvanto
University of Helsinki
Dept. of Biol. and Env. Sci, POB 65
Helsinki, FI-00014
Finland
Phone: 358-9-191 57740
Fax: 358-9-191 57694
leena.suvanto@helsinki.fi

Robert W Sykes
NC State University
6505 Ebenezer Church Rd
Hillsborough, NC 27278
USA
Phone: 919-225-2930
Fax:
rwsykes@gmail.com

Robert D Thomas
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4589
Fax: 843-851-4595
rdthoma@arborgen.com

Scott Tingey
DuPont Company
Crop Genetics Dept
Wilmington, DE 36780
USA
Phone: 302-695-2630
Fax:
scott.v.tingey@cgr.dupont.com

Becky J Townsend
NC State University
CAMPUS BOX 8003
Raleigh, NC 27695
USA
Phone: 919-515-9563
Fax: 919-515-6883
becky_townsend@ncsu.edu

Claudia Trivino
Fundacion Chile
Laboratorio Biotecnologia Forestal
Valdivia, X Region 0000
Chile
Phone: 56-63-293019
Fax: 56-63-293019
ctrivino@uach.cl

Chung-Jui Tsai
Michigan Technological University
1400 Townsend Dr.
Houghton, MI 49931
USA
Phone: 906-487-2914
Fax: 906-487-2915
chtsai@mtu.edu

Gerald Tuskan
Oak Ridge National Laboratory
PO Box 2008, MS-6422
Oak Ridge, TN 37830
USA
Phone: 865-576-8141
Fax:
gtk@ornl.gov

Theresa Vales
ArborGen
Summerville, SC 29483
USA
Phone:
Fax:
trvales@arborgen.com

Saila Varis
Finnish Forest Research Institute
PO Box 18
Vantaa, 01301
Finland
Phone: 358-10-2112635
Fax: 358-10-2112206
saila.varis@metla.fi

Jerrin Victor
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-4585
Fax: 843-832-2164
jmvicto@arborgen.com

Sara Von Arnold
SLU
PO Box 7080
Uppsala, S 75007
Sweden
Phone: 46-18-673230
Fax: 46-18-673279
sara.von.arnold@vbsg.slu.se

Patrik Waldmann
SLU
Dept. Forest Genetics/Plant Phys.
Umea, 901 83
Sweden
Phone: 46-90-786-85-99
Fax:
patrik.waldmann@genfys.slu.se

Qiuyu Wang
Northeast Forestry University
26 Hexing Rd
Harbin, 150040
PR China
Phone: 86-0451-82190291
Fax:
wqyll@sina.com

Diane Warwick
Tennessee Division of Forestry
185 Tobby Hollow Ln
Knoxville, TN 37931
USA
Phone: 865-594-6432
Fax: 865-594-5430
diane.warwick@state.tn.us

Barbara Wells
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5074
Fax: 843-832-2164
bhwells@arborgen.com

Ross Whetten
NC State University
Dept of Forestry
Raleigh, NC 27695-8002
USA
Phone: 919-515-7578
Fax:
ross_whetten@ncsu.edu

Dayton Wilde
ArborGen
Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5164
Fax: 843-851-4595
hdwilde@arborgen.com

Claire G Williams
Duke University
Box 90658
Durham, NC 27708
USA
Phone: 919-681-7180
Fax: 919-681-7176
claire.williams@duke.edu

Dean R Williams
Forestry Tasmania
GPO Box 207
Hobart, TAS 7001
Australia
Phone: 61-3-62337483
Fax: 61-3-62338102
dean.williams@forestrytas.com.au

Brenda Windsor
ArborGen
PO Box 840001
Summerville, SC 29485
USA
Phone: 843-851-5065
Fax: 843-832-2164
bmwinds@arborgen.com

Jeff A. Wright
ArborGen
PO Box 840001
Summerville, SC 29484
USA
Phone: 843-851-5079
Fax: 843-832-2164
jawright@arborgen.com

Harry Wu
CFFP CSIRO
PO Box E4008
Canberra, ACT 2604
Australia
Phone: 61-2-62818330
Fax: 61-2-62818312
harry.wu@csiro.au

JiYu Yan
International Paper Co.
PO Box 2668
Savannah, GA 31402
USA
Phone: 912-238-6114
Fax: 912-238-7531
jiyu.yan@ipaper.com

Chuanping Yang
Northeast Forestry University
26 Hexing Rd
Harbin, 150040
PR China
Phone: 86-0451-82190006
Fax: 86-0451-82191089
yangcp@mail.nefu.edu.cn

Terrance Z Ye
Oregon State University
321 Richardson Hall
Corvallis, OR 97331-5752
USA
Phone: 541-737-9881
Fax: 541-737-1393
terrance.ye@oregonstate.edu

Tongming Yin
Oak Ridge National Laboratory
PO Box 2008, MS 6422
Oak Ridge, TN 37831
USA
Phone: 865-241-3961
Fax: 865-576-9939
yintm@ornl.gov

Carol Young
USDA Forest Service
1579 Brevard Rd
Asheville, NC 28806
USA
Phone: 828-667-5089 x102
Fax: 828-665-2187
cyoung@fs.fed.us

Qibin Yu
NC State University
Box 8002
Raleigh, NC 27695-8002
USA
Phone: 919-515-5029
Fax:
qibin_yu@ncsu.edu

Barbara Zobel
Raleigh, NC
USA
Phone:
Fax:

Bruce J Zobel
Professor Emeritus, NC State University
PO BOX 37398
Raleigh, NC 27627
USA
Phone:
Fax:
bjzobel@unity.ncsu.edu

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