Silviculture and the Conservation of Genetic Resources for Sustainable Forest Management

Proceedings of the Symposium of the North American Forest Commission, Forest Genetic Resources and Silviculture Working Groups, and the International Union of Forest Research Organizations (IUFRO)

Quebec City, Canada, September 21, 2003

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2. Plantation de chênes à gros fruits (*Quercus macrocarpa*) à Saint-Nicolas, Québec, Canada / Bur oak (*Quercus macrocarpa*) plantation at Saint-Nicolas, Quebec, Canada (J. Beaulieu)
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FOREWORD

From September 21 to 28, 2003, the XII World Forestry Congress of the Food and Agriculture Organization of the United Nations (UN/FAO) was held in Quebec City, Quebec, Canada. As a side event of that Congress, the symposium on "Silviculture and the Conservation of Genetic Resources for Sustainable Forest Management" was held on September 21. The symposium was sponsored by the Forest Genetic Resources Working Group (FGRWG) and the Silviculture Working Group of the UN/FAO's North American Forest Commission as well as by the International Union of Forestry Research Organizations (IUFRO). The organization was taken on by FGRWG members as one of their tasks. Jean Beaulieu and Eric Teissier du Cros welcomed the participants on behalf of FGRWG and IUFRO, respectively.

The North American Forest Commission is one of the six Forest Commissions established by the UN/FAO. The Silviculture and the Forest Genetic Resources Working Groups are two of the seven working groups set up by the Commission. Their mission is to promote sustainable forest management through improved silvicultural practices, to encourage and promote the conservation of all forest genetic resources, and to promote cooperation among the three North American countries. IUFRO also plays a role in the conservation of genetic resources through its network on breeding and genetic resources. The symposium was a means for both organizations to fulfill part of their mission.

The Proceedings contain the texts of papers submitted to the organizing committee. The authors are entirely responsible for content. The Proceedings also contain a list of participants.

The FGRWG members who took on the responsibility for organizing the symposium with the help of their colleagues were:

Dr. Jean Beaulieu, Chairman, Natural Resources Canada - Canadian Forest Service, Sainte-Foy, Quebec, Canada.
Dr. Ronald C. Schmidtling, USDA Forest Service, Saucier, Mississippi, USA.
Dr. Gil Vera Castillo, INIFAP, Texcoco, Mexico City, Mexico.

ACKNOWLEDGEMENTS

We sincerely thank the Organizing Committee of the XII World Forestry Congress for having provided the conference room for the symposium. Thanks are also extended to the Canadian Forest Service for allocating funds without which it would have been very difficult to gather all the participants at the Delta Quebec Hotel.

We are also grateful for the assistance provided by the staff of the Research Directorate and Policy and Liaison of the Canadian Forest Service - Laurentian Forestry Centre. In particular, we thank Claude Aerni, Marie Deslauriers, Sandra Gravel, Marie Pothier and Micheline Deschambault for their technical help with the organization of the meeting. We also express our appreciation to Isabelle Lamarre, Diane Paquet and Pamela Cheers for their editorial work. Without their strong commitment, the publication of the Proceedings would have been very difficult.

Jean Beaulieu

AVANT-PROPOS

Le XII^{e} Congrès forestier mondial de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (ONU/FAO) a eu lieu du 21 au 28 septembre 2003 dans la ville de Québec, Québec, Canada. Le 21 septembre, un symposium intitulé « Sylviculture et conservation des ressources génétiques pour un aménagement forestier durable » a été organisé en tant qu'événement parallèle à ce congrès. Le symposium a été parrainé par le Groupe de travail sur les ressources génétiques forestières (GTRGF) et le Groupe de travail sur la sylviculture de la Commission des forêts pour l'Amérique du Nord de l'ONU/FAO et par l'Union internationale des instituts de recherches forestières (IUFRO). L'organisation a été assumée par les membres du GTRGF, celle-ci constituant une de leur tâches. Jean Beaulieu et Éric Teissier du Cros ont respectivement souhaité la bienvenue aux participants au nom du GTRGF et de l'IUFRO.

La Commission des forêts pour l'Amérique du Nord est une des six commissions des forêts créées par l'ONU/FAO. Les Groupes de travail sur la sylviculture et sur les ressources génétiques forestières sont deux des sept groupes de travail mis en place par la Commission. Leur mission consiste à promouvoir l'aménagement forestier durable par l'utilisation des pratiques sylvicoles améliorées, à encourager et à promouvoir la conservation de toutes les ressources génétiques forestières et à promouvoir la coopération entre les trois pays de l'Amérique du Nord. L'IUFRO joue également un rôle important dans la conservation des ressources génétiques via son réseau de recherches sur l'amélioration et sur les ressources génétiques. Ce symposium est un moyen pris par ces deux organisations pour remplir une partie de leur mission.

Le compte rendu contient les textes des conférences tels que soumis au comité organisateur. Les auteurs sont seuls responsables de leur contenu. Le compte rendu renferme également une liste des participants.

Les membres du GTRGF qui ont assumé la responsabilité de l'organisation du symposium avec l'aide de leurs collègues étaient:

Jean Beaulieu, Président, Ressources naturelles Canada - Service canadien des forêts, Sainte-Foy, Québec, Canada.
Ronald C. Schmidtling, USDA Forest Service, Saucier, Mississippi, USA.
Gil Vera Castillo, INIFAP, Texcoco, Mexico, Mexique.

REMERCIEMENTS

Nous désirons remercier sincèrement le comité organisateur du XII^{e} Congrès forestier mondial pour avoir mis à notre disposition la salle de conférence où s'est tenu le symposium. Nos remerciements vont également au Service canadien des forêts pour les budgets alloués et sans lesquels il aurait été très difficile de rassembler tous les participants à l'Hôtel Delta Québec.

Nous sommes aussi reconnaissants pour l'aide fournie par le personnel de la Direction de la recherche et de la direction de Politique et liaison du Service canadien des forêts - Centre de foresterie des Laurentides. Nous remercions spécialement Claude Aerni, Marie Deslauriers, Sandra Gravel, Marie Pothier et Micheline Deschambault pour leur assistance technique à l'organisation de la rencontre. Nous exprimons également notre appréciation à Isabelle Lamarre, Diane Paquet et Pamela Cheers pour leur travail d'édition des textes. Sans leur engagement solide, la publication du compte rendu aurait été très difficile à réaliser.

Jean Beaulieu


Session 1

MANAGEMENT AND CONSERVATION OF FOREST GENETIC RESOURCES: ROLES OF IUFRO AND FRANCE ON THE INTERNATIONAL SCENE AND NEED FOR LONG-TERM MONITORING OF GENETIC DIVERSITY IN CONSERVATION NETWORKS

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ABSTRACT

Thanks to FAO's long-time involvement, there is a global commitment on the conservation of forest genetic resources. IUFRO has also played a considerable role with its networks on breeding and genetic resources of species and groups of species. In 1997, after a meeting of the FAO Panel of Experts on Forest Genetics Resources, IUFRO created a Task Force on Management and Conservation of Forest Gene Resources, which is currently working on a State of Knowledge Report on research connected to conservation of forest genetic resources to be delivered at the IUFRO World Congress in Brisbane, in 2005.

In France, activities specific to the conservation of forest genetic resources started in the late 1980s. They were originally part of genetic improvement activities but became somewhat independent in the mid-1990s when a national agreement was signed by several R&D institutions as well as by the forest administration. In 2003, the national programme coordinated by the Commission for Forest Genetic Resources resulted in the establishment, or studies leading to the establishment, of *in situ* and *ex situ* dynamic, or *ex situ* static, conservation networks of seven broadleaves (beech, black poplar, elms, service tree, Sessile oak, wild cherry and wild service tree) and three conifers (maritime pine, Norway spruce and silver fir). Research is also underway in tropical rain forest in French Guyana.

All *in situ* conservation networks are set in managed forests. The long-term effect of management intensity has started being monitored for beech: assessment of genetic diversity and diversity distribution, reproductive regime and gene flow of beech stands with different silviculture treatments started in 2000. In one of them, in northeastern France, an unexpected factor was added: the effect of the 1999 "Lothar" winter storm with its variable impact on the core of a conservation unit.

The conservation of forest tree genetic resources started on a species basis. It will have to be associated with other conservation programmes dealing with ecosystems through which common efforts can be devoted to several species living in the same type of habitat: riparian forest, tropical forest and several other ecosystems with natural or man-made species mixtures. International and intercontinental cooperation is essential to join efforts, avoid duplication and contribute to the reconstitution of lost and endangered germplasm.

IUFRO's role in the conservation of forest genetic resources

The global commitment on conservation of forest genetic resources has been triggered by FAO and particularly by the activity of its Panel of Experts on Forest Gene Resources. At its 1997 meeting, the Panel suggested that state-of-the-art scientific achievements be made to help countries with their respective conservation plans. IUFRO accepted the task and created a Task Force on Management

and Conservation of Forest Gene Resources. Apart from FAO, other Task Force partners are IPGRI and CIFOR. The Task Force has been asked to publish a State of Knowledge Report for the IUFRO World Congress, in 2005 (http://iufro.boku.ac.at/ [Scientific Structure/Task Forces/Gene Resources]). For this purpose, the Task Force is in permanent contact with FAO and meets in Rome at the same time as the FAO Panel does. The Task Force has also been asked to organize sessions during IUFRO World Congresses in 2000 and 2003.

In fact, conserving forest genetic resources is an old story. Forest tree geneticists and breeders, because of their need for large and representative population samples of the species they work on, have gathered a considerable amount of germplasm in common gardens and other collections. This is reflected in the structure of IUFRO Division 2 – Physiology and Genetics, with its specific or multi-specific Working Parties (nine on conifers and six on broadleaves) covering virtually all species submitted to some kind of genetic selection and improvement (http://iufro.boku.ac.at/). "Some kind" means at least provenance testing. These collections have an inestimable value because they sometimes include populations that are no longer present in their natural range. Thereby, they truly contribute to the conservation of forest gene resources.

French achievements in conservation of forest genetic resources

As in many other countries, conservation started as part of selection and breeding. Important samples of major and minor reforestation species have been gathered in provenance and progeny tests covering most ecological conditions of France and of its overseas territories. These tests cover around 2000 ha. They involve 40 conifers (Arbez 1987) and 45 broadleaves (Arbez and Lacaze 1998).

Stricto sensu conservation started in the mid-1980s with a work plan and the establishment of the French Commission of Forest Genetic Resources. Achievements after 10 years were published in a book in French (Teissier du Cros 1999) and English (Teissier du Cros 2001).

In situ conservation networks are in place for beech (*Fagus sylvatica* L.), silver fir (*Abies alba* Mill.) and Sessile oak (*Quercus petraea* (Mattus.) Liebl.), and planned for black poplar (*Populus nigra* L.), maritime pine (*Pinus pinaster* Ait.) and Norway spruce (Picea abies (L.) Karst.). Each conservation unit consists of a core of at least 500 trees at stand maturity, surrounded by a buffer. Units can be submitted to any classical management except coppice and coppice-with-standards. Management constraints are very limited. The most important one is that no exotic provenance of the protected species and no species that may hybridize with it should be used for reforestation in the core and the buffer. In fact, natural regeneration is highly recommended. This type of conservation is called *in situ* dynamic conservation.

A variant of that conservation method is used for wild cherry (*Prunus avium* L.), a scattered species. A conservation unit consists of the mixture of open-pollinated families collected on more than 30 trees in a region. It is literally an *ex situ* conservation technique, but natural regeneration will be applied (*ex situ* dynamic conservation).

Ex situ conservation is applied to black poplar (stool beds and arboreta), European field elm (*Ulmus minor* Mill.), Wych elm (*U. glabra* Uds.) and European white elm (*U. laevis* Pall.) in arboreta and under cryopreservation, and to wild cherry (grafted stool beds) and service tree (*Sorbus domestica* L.) in grafted stool beds and open-pollinated families.

Due to the large area covered by a wild service tree population (*Sorbus torminalis* (L.) Crantz), i.e. more than 400 ha, no firm decision has yet been made for the conservation of this scattered species.

So far all conservation networks focus on one species. This will probably also be true for a number of well-represented species such as Scots pine (*Pinus sylvestris* L.). In the future, however, conservation measures will be applied to entire ecosystems with many species. This is already true for riparian forests that include black poplar, Wych elm and European white elm. It will certainly be the case in tropical rain forest, which covers a large part of French Guyana.

Partnership is highly needed for conservation of forest genetic resources. In France, it involves research, development, management and also administration. A charter was signed in 1997 by several organisations, essentially in the public sector, but also by those representing private owners. The latter type of actors is very important because certain resources exist only on private land.

Stand management and conservation

In the previous section we saw that constraints on management due to the presence of *in situ* conservation units are limited. However coppice and coppice-with-standards management systems are excluded. Why? Because in dynamic conservation, natural regeneration is a key period in the life of a stand. It during this period that recombination takes place, allowing mutations and new gene distribution, resulting in the creation of new genotypes. This process is important because *in situ* conservation involves a long-term commitment, with the predictable or unpredictable role of anthropogenic and natural factors, particularly silviculture and global change. Coppice and coppice-with-standards are essentially regenerated vegetatively. Therefore, there is no chance for recombination and very limited adaptation possibility.

Conservation and stand management

The main issue for silviculturists is how to favour high genetic diversity. The first reaction one may have is that Nature does it well. In many cases this assumption is right. After all, forests were present before man came and began managing them, and they are still here, generally in good conditions. However, here are two case studies showing that management is not a neutral factor in the distribution of genetic diversity. Both case studies are part of an EU project called “Dynabeech: Effects of silvicultural regimes on dynamics of genetic and ecological diversity of European beech forests. Impact assessment and recommendations for sustainable forestry” (EU project QLK5 CT 01210). Basically the project compares the effect of stand intensive management vs. limited management in five pairs of beech stands located in Germany, The Netherlands, Austria, Italy and France.

The French unmanaged beech stand is located in the Sainte-Baume State Forest, 30 km east of Marseille in southeastern France, i.e. in Mediterranean conditions (Latitude: 43°20’ N, longitude: 5°45’ E, elevation: 700 m). Its north slope hosts a magnificent broadleaf and yew stand, probably of man-made origin, with trees reaching a quite unexpected size in Mediterranean conditions, i.e. as much as 37 m in total height with 80 cm dbh. In certain parts of the forest, beech dominates (84%). To protect the public from accidents, management consists in felling dying trees before they fall naturally. Gaps left by felled trees favour natural regeneration. All potentially mature beech individuals in a 2.60-ha plot have been mapped and then genotyped by our Italian colleagues of Dynabeech, using four polymorphic nuclear DNA microsatellites. Autocorrelations between quantitative variables (genotype expressed by allele frequencies) have been estimated using Moran’s index (I_m). This index, computed according to Dewey and Heywood (1988), ranges from -1 to +1. Significant positive values indicate spatial clustering of similar genotypes. Null values indicate even distribution of genotypes. Significant negative values indicate a repellent effect of similar genotypes. Moran’s index is computed using AutocorG (Hardy and Vekeman 2000). When computed on 286 beech trees, I_m revealed a highly significant genetic structure below an average tree distance of 20 m (Figure 1). This result shows that this beech stand seems to be a combination of 20-30 m aggregates composed of related trees. With simulated thinning, starting from an average current distance of 9 m between trees, the genetic structure disappears completely if the

average tree distance exceeds 12 m (180 beech trees left). From a genetic point of view, this result shows that unmanaged stands may not be in the best position to face long-term climate change or any other unexpected biotic or abiotic event when high genetic diversity seems to be needed because trees with common ancestry are left side by side, resulting in inbreeding risk. Management of even-aged stands through regeneration with the shelterwood system and thinning with the F-tree system seems to be a better approach because of the distance factor between trees. This assumption will have to be checked. If gap regeneration is preferred, individual gaps should be large enough to allow long-distance pollen flow (Povillon 2002).

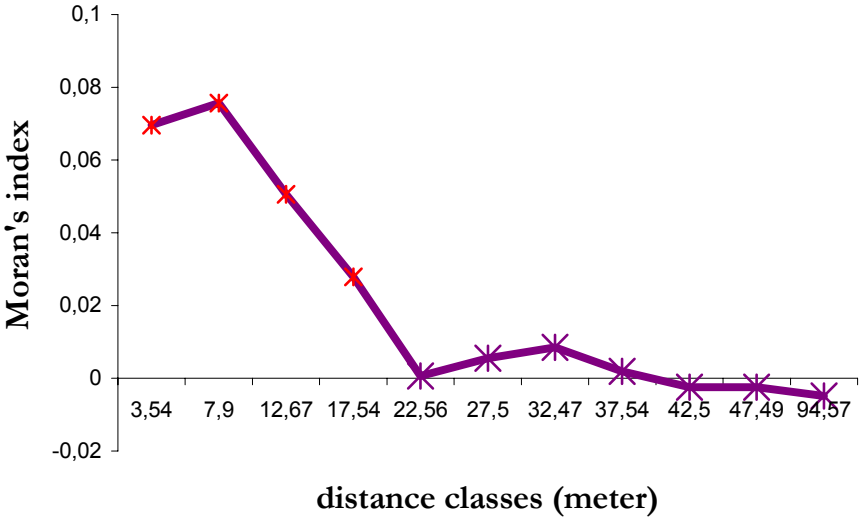

Figure 1. Sainte-Baume: spatial genetic structure (microsatellites) of a 286-individual population. Red symbols indicate significant values with an error risk of 5%. P-values were obtained after 1000 random permutations.

The second French beech plot of Dynabeech is located on the north slope of Ventoux mountain, 60 km northeast of Avignon (Latitude: 44°11' N, longitude: 5°17' E, elevation: 1450 m). All potentially mature beech individuals in a 1-ha plot have been mapped and then genotyped by our Italian colleagues, as in Sainte-Baume. The tree sample includes one old-growth individual with dbh over 96 cm, a potential parent of the generation we analysed, with an average dbh of less than 35 cm. Moran's index reveals significant values for distance classes of less than 4 m (Figure 2). This shows that adjacent trees are genetically related. Among the 41 genotyped individuals, only 13 are spatially isolated. The others form 12 coppice-like clusters. In each cluster, microsatellites have allowed us to distinguish genetically identical individuals (ramets) from the others. To determine if natural ramets were the only reason accounting for the genetic structure, in each cluster, all copies of each clone but one were thinned out *in silico*. When calculated on the remaining 34 individuals, I_m is still significant at very short distances (<5 m), showing a persisting short distance genetic structure (Figure 2). This result shows that clusters including highly related trees probably originate from caches set up by birds or small mammals. Most of the trees in a cluster probably originate from one highly productive seed tree (one of the old-growth trees mentioned earlier). They are perhaps half-sibs. True ramets in clusters are probably the result of natural accidents on the terminal bud. Since there is no deficit in heterozygotes in that generation, beech seems to mate randomly in this plot. The average number of alleles for microsatellites was 9.75, compared with 10.1 on 40 sub-sample trees of the 286 sample trees in Sainte-Baume. This reveals no genetic bottleneck. In other words, the beech genetic background in Ventoux is as rich as in any other beech stand, but in the first generation originating from old growth, genotypes are not distributed randomly. Trees such as the old-growth ones in our sample are probably

more numerous than what we expected, resulting in a sufficient genetic diversity. However, due to uneven distribution of genetic diversity (clusters of related trees), lack of management may lead to inbreeding.

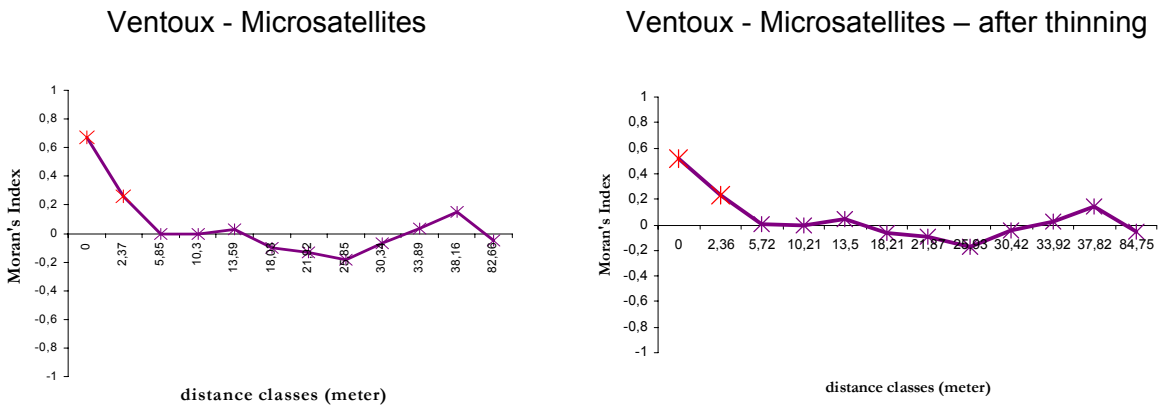

Figure 2. Left: Ventoux: spatial genetic structure (microsatellites) on a 40-individual population. Red symbols indicate significant values with an error risk of 5%. P-values were obtained after 1000 random permutations. **Right:** Ventoux: AutocorG autocorrelogramme (microsatellites) after having thinned out all true clonal copies but one in each cluster. Red symbols indicate significant values with an error risk of 5%.

Need for long-term monitoring of genetic diversity in conservation networks

The Sainte-Baume case study suggests that any stand management consisting in giving more space to individual trees during the regeneration period, thus allowing increased pollen flow, is probably efficient for maintaining a favourable distribution of genetic diversity. This assumption needs to be tested.

Three solutions are available. The first one is to rely on existing spacing experiments such as long-term production tests based on variable stand density. Such tests have been laid out in Europe more than a century ago, some of them under IUFRO auspices. If still existing, they could be revitalised and used for genetic diversity and pollen flow purposes. However, such tests have to coincide with a conservation unit; this may not be the case very often.

The second solution consists in establishing such tests. However, several generations of scientists will be needed to achieve results. This would be part of forest scientists' investment for future colleagues.

The third solution is to look for opportunities. This is the case in France. The storm "Lothar", which caused major damage in forests of northern France and southern Germany just after Christmas 1999, has partly destroyed one beech conservation unit near Nancy, in the Haye State Forest, eastern France. The conservation core covering 16 ha, with an average age varying between 120 and 140 years, can now be divided into three levels of tree distribution: strong damage (<20 trees/ha are left), intermediate damage (20-60 trees/ha are left), limited damage (>60 trees/ha are left). Mature trees – almost a thousand are left in the conservation core – will be mapped, and 350 of them will be genotyped. Demographic studies will be done on regeneration. Some of the mature trees will be used as pollen traps to determine, through the genotype of their open-pollinated progeny, where the fathers are. Periodic assessment of diversity in the regeneration will be needed to determine the evolution of

genetic diversity under several thinning regimes and other possible natural and anthropogenic factors. This long-term test will be related to the Sainte-Baume test as a permanent comparison of the effect of intensive and reduced management.

CONCLUSION

The conservation of forest genetic resources is a multi-dimension process. It needs global and regional coordination through guidelines and networking. Because most species ignore administrative boundaries, each nation has to take care of its own resources, probably in coordination with neighbouring countries. Conservation is a multi-step process. *In situ* and *ex situ*, dynamic and static conservations are complementary parts of this puzzle. Finally, conservation is a considerable undertaking, which needs the scientific background, commitment and partnership of all actors in the forestry sector, public and private funding and space, and time for periodic evaluation.

From a scientific point of view, research activities related to conservation result in an extraordinary, sometimes unexpected, source of knowledge with applications outside its strict limits. Conservation is not an independent and self-sufficient activity. In forest trees, it is closely related to management. It should not be perceived by lay persons and professionals as a “locking-up” of resources, like in a museum. It should be considered part of the daily activities of professionals and specialists. It deserves political support.

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CONSERVATION OF FOREST GENETIC RESOURCES AND SUSTAINABLE FOREST MANAGEMENT IN EUROPE

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ABSTRACT

Considerable efforts have been made to enhance conservation of forest genetic resources and to promote sustainable forest management in Europe over the past decade. The need to enhance genetic conservation emerged from a concern about the impacts of environmental pollution and genetic erosion on forest ecosystems in the late 1980s. The concept of sustainable forest management, in a broader sense, gained ground in Europe after the United Nations Conference on Environment and Development in 1992 paid special attention to the role of forest ecosystems in maintaining and conserving biological diversity. Several Ministerial Conferences on the Protection of Forests in Europe were organised in the 1990s and this was a major political process pushing forward improvements in these two areas. In 1994, the European Forest Genetic Resources Programme was set up as an implementation mechanism of the Strasbourg Resolution (1990) on 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.

In many European countries, however, more resources have been channelled into habitat and species conservation while fewer resources have been allocated to the development of national programmes on forest genetic resources. Less than 30% of European countries have such programmes, which are necessary for implementing gene conservation in practical forestry. Subsequently, these programmes should be better linked with the overall national forest programmes, which are developing policies for the whole forest sector and also allocating resources for implementing sustainable forest management. The pan-European criteria and indicators include genetic indicators in rather operational ways, but there is a need to improve monitoring of genetic sustainability by developing commonly agreed technical terminology. This would facilitate the collection of relevant data and make the data comparable internationally.

Key words: Forest genetic resources, gene conservation, forest management, criteria and indicators

INTRODUCTION

The continent of Europe is characterised by three main types of forest ecosystems, ranging from the relatively open Mediterranean woodlands to more dense temperate and boreal forests. In 2000, the total forest area of Europe was 1,039 million hectares of which Mediterranean, temperate and boreal forest types accounted for 5, 22 and 73%, respectively (FAO 2003). On average, European forests cover about 46% of the continent's land area (FAO 2003). However, the FAO statistics include the whole territory of the Russian Federation in Europe, which gives a slightly biased picture of European forest resources. The actual forest cover is considerably lower than 46% in many European countries.

After the last glaciation, which ended some 10,000 years ago, European tree species have experienced episodes of climatic changes that have likely dominated the evolution of local populations (Namkoong 1998). Natural selection has influenced among-population differentiation through adaptive traits, while long-distance gene flow has been rather efficient in increasing genetic variation within populations in many European tree species. Thus, the natural populations of many widely occurring species demonstrate low genetic differentiation in terms of biochemical markers, but large variations in adaptive traits (e.g., Eriksson 1998; Oleksyn et al. 1998).

Without a doubt, human influence has also played a major role in the evolutionary history of European forests. Large-scale utilisation of forests and trees has been going on for several thousands of years, especially in the Mediterranean region. Today, large areas of continuous forests occur mainly in the less populated areas of northern and eastern Europe, while fragmented landscapes dominate the rest of the continent. Silvicultural practices have been applied for centuries in many European countries (Vallance 2001; Bürgi and Schuler 2003) and common traditions in forest management are now widespread throughout the continent.

In the late 1980s, increasing concern about the impacts of environmental pollution and genetic erosion on forest ecosystems emerged in Europe. Throughout Europe, scientists, managers, policy-makers and others were involved in a public debate regarding the future of the forest, while scientific knowledge available at that time was unable to provide clear and sound answers for several important questions. European countries soon recognised that the problems occurred across the continent regardless of national borders and, subsequently, the countries organised the first Ministerial Conference on the Protection of Forest in Europe (MCPFE) in Strasburg, in December 1990. At this Conference, the participating European countries adopted six resolutions to initiate immediate action to improve the state of the forest without waiting for research to provide all the scientific answers.

One resolution was specifically developed for enhancing conservation of forest genetic resources and after the Strasburg Conference, an intensive consultation process took place to make this resolution operational. A group of experts from four countries was assigned to develop operational recommendations in collaboration with the FAO Forestry Department, the International Plant Genetic Resources Institute (IPGRI) and the European Commission (EC). During this process, it was agreed that a voluntary instrument was needed to promote and coordinate the implementation of the resolution and regional collaboration on the conservation of forest genetic resources. To solve obvious operational problems, it was suggested that activities should be carried out within species-oriented networks focusing only on a limited number of species, which should represent different geographical and ecological conditions in Europe. These operational recommendations and a proposal by IPGRI and FAO for the European Forest Genetic Resources Programme (EUFORGEN) were then endorsed at the Second Ministerial Conference in Helsinki, in June 1993, and EUFORGEN became operational in October 1994.

In addition to highlighting the importance of biodiversity conservation in European forests (Resolution H2), the Helsinki Ministerial Conference also adopted general guidelines for sustainable forest management in Europe (Resolution H1). The development of these non-legally but politically-binding European initiatives was supported by the adoption of the Convention on Biological Diversity (CBD) during the United Nations Conference on the Environment and Development (UNCED) in Brazil, in 1992. The Helsinki Resolution H2 accepted the CBD definition on *biological diversity* and noted that conservation of biodiversity should be an essential operational component in sustainable forest management.

The political agreement on conservation of forest biological diversity as part of sustainable forest management was further strengthened and developed towards a more operational form during the third Ministerial Conference held in Lisbon, in 1998. Lisbon Resolution 2 on *Pan-European Criteria, Indicators and Operational Level Guidelines for Sustainable Forest Management* provided a voluntary framework for developing and promoting sustainable forest management practices at the sub-national level. Later, the Pan-European Forest Certification (PEFC) scheme was developed based on these criteria and indicators to assess the implementation of sustainable forest management in Europe.

The conservation of forest biological diversity and promotion of sustainable forest management have remained at high levels in the political agenda among European countries. In April 2003, the fourth Ministerial Conference in Vienna, Austria, again endorsed several resolutions that are highly relevant for continued efforts in this regard. More detailed information on the outputs of the Ministerial Conferences can be found at www.mcpfe.org.

In this paper, we provide highlights of European collaboration and activities on conservation of forest genetic resources. We also discuss the role of gene conservation in sustainable forest management and how conservation of forest genetic resources is implemented in European countries.

European Forest Genetic Resources Programme

EUFORGEN is financed by participating countries and its Steering Committee is composed of National Coordinators from each country. The role of the National Coordinators is to act as a link between the EUFORGEN Secretariat and national institutions involved in the activities. The Secretariat is hosted by IPGRI, which also takes care of the overall management of the Programme in technical collaboration with the FAO Forestry Department. The Programme operates through networks in which mainly scientists and managers exchange relevant information and develop conservation methods and strategies for selected species. Participating countries implement activities with their own resources and, to some extent, policy-makers have also participated directly in the network.

During the first five-year phase of EUFORGEN (1 January 1995 to 31 December 1999), the operational work was based on five pilot networks for specific species or groups of species, i.e. *Picea abies*, *Quercus suber*, Noble Hardwoods, *Populus nigra* and Social Broadleaves. For the second phase of the Programme (1 January 2000 to 31 December 2004), the scopes of the networks were broadened and some names were changed. Currently, the five networks are called Conifers, Mediterranean Oaks, Noble Hardwoods, *Populus nigra*, and Temperate Oaks and Beech, respectively. As of February 2003, a total of 31 countries were officially participating the EUFORGEN Programme and its activities.

In addition to promoting international collaboration in Europe, EUFORGEN has also contributed to the development of national programmes or strategies on the conservation of forest genetic resources at the national level. National programmes on forest genetic resources are needed not only for meaningful international collaboration but also for implementing gene conservation in practical forestry. It has been recognised that integration of genetic considerations into national forest policies,

involvement of relevant stakeholders and efficient coordination of activities are major challenges for the national programmes on forest genetic resources (see Turok and Geburek 2000).

EUFORGEN Networks

The Conifers Network has identified 52 species as important for gene conservation in Europe. Many of the native conifers in Europe are dominant elements in boreal, Atlantic, alpine and Mediterranean forest ecosystems, while several exotic conifer species are also widely used in a few European countries. Several species, such as *Abies alba*, *Juniperus communis*, *Larix decidua*, *Picea abies*, *Pinus nigra*, *Pinus sylvestris* and *Taxus baccata* are widely occurring in the continent, and 15 or more countries have identified these species as important for gene conservation. Many conifer species are also economically very important and some of them have been utilised extensively in Europe during its long human settlement.

In 1997, the Conifers Network developed its first guidelines for *in situ* and *ex situ* gene conservation of *Picea abies* (Koski et al. 1997). Currently, the Network is developing more concise six-page technical guidelines for genetic conservation and use that are aimed at practical forest managers. The guidelines will be developed for 12 important species and those for *Pinus pinaster*, *Pinus halepensis*, *Pinus brutia* and *Picea abies* have already been published (Alía and Martin 2003; Fady et al. 2003; Skrøppa 2003). The Network has also initiated the mapping of *in situ* conservation areas for *Picea abies* to create a Europe-wide map. This effort serves as a pilot study and can be used as a model for further monitoring of the state of forest tree gene conservation in Europe.

The Mediterranean Oaks Network promotes gene conservation of *Quercus ilex*, *Q. pubescens* and *Q. suber*. These oaks dominate Mediterranean forests and woodlands, which have been exposed to unsustainable utilisation for thousands of years. Historically, their timber was used to build houses and ships, and the wood was essential for charcoal production and as firewood. Today, Mediterranean forests are no longer an important timber source but they are still heavily exploited for clearing arable land and harvesting acorns to feed valuable breeding livestock. Plantations of *Q. suber* have been established to supply the well-established cork industry, but a large part of the demand is still fulfilled with cork extracted from natural forests and woodlands.

The Mediterranean Oaks Network has facilitated the establishment of international provenance trials for *Q. suber* as little genetic and breeding research had previously been conducted on this species. There is also a lack of basic information on adaptive traits and their geographical distribution patterns. Acorns were collected from seven countries (Algeria, France, Italy, Morocco, Portugal, Spain and Tunisia) as part of a EU-funded project and, subsequently, trials were established in all these countries (except Algeria) during 1997 and 1998. This network of trials holds a unique collection of cork oak genetic material throughout the species' natural range in the Mediterranean basin. The Network is also preparing a technical bulletin and technical guidelines for gene conservation of *Q. suber*. As compared to the EUFORGEN Technical Guidelines, the Technical Bulletins give a broader presentation of the topic and target both scientists and managers.

The Noble Hardwoods Network focuses on broad-leaved species, which only have minor importance in traditional forestry. However, noble hardwoods are important multipurpose trees, which also produce valuable timber, and they are often used in landscaping or other environmental purposes. Many of these species grow scattered in mixed species forests and have low capacity to compete with other tree species. Many European countries lack silvicultural tradition for these species and therefore they tend to be neglected in forest management, although recently they have received more attention. Noble hardwoods are also threatened by uncontrolled seed transfers, illegal cuttings and reduced population size. Furthermore, *Ulmus* spp. are severely threatened by Dutch elm disease.

The Noble Hardwoods Network has identified 32 tree species that are considered important for gene conservation in Europe and has developed long-term gene conservation strategies for many of them (i.e. *Acer platanoides*, *A. pseudoplatanus*, *Alnus* spp., *Castanea sativa*, *Fraxinus* spp., *Juglans regia*, *Malus sylvestris*, *Prunus avium*, *Pyrus pyraster*, *Tilia* spp., *Ulmus* spp. and *Sorbus* spp.). These strategies have been published as part of the Network's meeting reports (see Turok et al. 1998, 1999, 2002) and they are also available through the EUFORGEN Web site (www.euforgen.org). The Network has also promoted *in situ* conservation in managed forests by producing silvicultural management strategies for noble hardwoods (Rotach 1999). Similarly to other EUFORGEN Networks, the Noble Hardwoods Network is also developing technical guidelines for several species. These have now been published for five species, i.e. *Acer pseudoplatanus*, *Alnus glutinosa*, *Fraxinus excelsior*, *Prunus avium* and *Sorbus domestica* (Rusanen and Myking 2003; Kajba and Gračan 2003; Pliûra and Heuertz 2003; Russell 2003; Rotach 2003).

The *Populus nigra* Network has mainly focused its efforts on the species concerned and another poplar species, *P. alba*, was included into the Network activities in 1999. *Populus nigra* is a typical pioneer species that is mainly growing in riparian mixed forests along European and West-Asian rivers. It is threatened by extinction in large parts of its distribution range as a result of habitat destruction and hydraulic engineering practises, replacement by and interspecific hybridization with Euramerican poplars. The direct commercial value of *P. nigra* is low, but it is used as a parental pool in many breeding programmes around the world. More importantly, it has high ecological value in riparian floodplain forests where it helps maintain biological diversity.

Twenty-six European countries have actively participated in the *Populus nigra* Network which has established *ex situ* collections holding nearly 3,000 accessions and made available the related databases (www.populus.it). The Network is also planning to establish an international collection of *P. nigra* provenances based on the material that was recently collected within the EU-funded EUROPOP project (see van Dam and Bordács 2002 for details). The development of a core collection and database for *P. alba* has also been initiated. Other Network outputs include a standardised list of clone descriptors and the dissemination of a set of 15 reference clones from unique stool-beds to participating countries. A standardized list of descriptors for inventories of *P. nigra* stands has also been created as well as an identification sheet in English, French, German, Hungarian, Spanish, Russian and Dutch. The Network has also produced a Technical Bulletin on *in situ* conservation of *P. nigra* (Lefèvre et al. 2001) and Technical Guidelines for the same species (Vanden Broeck, in press). Recently, the Network has initiated the development of a Europe-wide conservation strategy for *P. nigra*, or the so-called "common action plan".

The Temperate Oaks and Beech Network has been working with three species, i.e. *Quercus robur*, *Q. petraea* and *Fagus sylvatica*. These tree species represent the major component of European broadleaved forests. They have high economic and ecological importance and their genetic resources have suffered from drought, air pollution, defoliators, diseases and improper silvicultural techniques. The lack of natural regeneration is a major constraint for *in situ* conservation of oaks in many European countries, while beech does not face the same problem. In addition to assessing the status of gene conservation of the species in various European countries, the Temperate Oaks and Beech Network has facilitated the development and implementation of several EU-funded research projects. The projects have generated a vast amount of new information and the Network is currently preparing a Technical Bulletin on the genetic conservation of oaks and Technical Guidelines for all three species.

All EUFORGEN Networks have also contributed to the development of a "grey literature" database, which includes unpublished reports, theses and other similar documents on forest genetic resources in European countries. Currently, this database contains nearly 2,000 records and is accessible through the EUFORGEN Web site. The Networks have also produced public awareness material, such as brochures, photo collections and posters.

Promotion of sustainable forest management in Europe

After the Ministerial Conference in Helsinki (1993) made a resolution to promote sustainable forest management (SFM) in Europe, a series of expert level meetings were held to develop pan-European criteria and indicators for SFM. During this process, criteria and quantitative indicators were adopted at the first follow-up meeting of the Helsinki Conference in Geneva, in June 1994, and later, descriptive indicators were adopted at the second follow-up meeting in Antalaya, in January 1995. In June 1998, the third Ministerial Conference in Lisbon decided to improve the first set of indicators and, subsequently, four workshops were organised in 2001 and 2002 to consult experts in different parts of Europe. The improved pan-European indicators for SFM were then adopted at the expert level meeting in Vienna, in October 2002. Box 1 shows the criteria, and more details on the improved indicators and operational guidelines can be found at www.mcpfe.org.

Criteria and indicators are tools for assessing national and regional trends in the state of forest resources and their management. After the Pan-European criteria and indicators were adopted, several countries initiated the development and implementation of national level criteria and indicators by modifying the Pan-European criteria and indicators to their specific conditions. Simultaneously, customers buying timber, paper and other wood-based products demanded information on whether the raw material had been obtained from sustainably managed forests. This demand led to the development of national forest certification schemes in many European countries and, in 1999, the Pan-European Forest Certification Scheme (PEFC) was launched to act as a framework for the mutual recognition of credible national or regional forest certification schemes. Currently, national forest certification schemes in Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Latvia, Norway, Spain, Sweden, Switzerland and United Kingdom have been endorsed by the PEFC Council (see www.pefc.org). By August 2003, a total of 48,155,793 hectares had been certified by PEFC in 12 European countries (see www.pefc.org). As a comparison, the competing global forest certification scheme, i.e. the Forest Stewardship Council (FSC), had certified 25,032,514 hectares in 25 European countries under its own principles and criteria by the same point of time (see www.fscoax.org).

Box 1: Pan-European Criteria for Sustainable Forest Management

Criterion 1:	Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles
Criterion 2:	Maintenance of forest ecosystem health and vitality
Criterion 3:	Maintenance and encouragement of productive functions of forests (wood and non-wood)
Criterion 4:	Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems
Criterion 5:	Maintenance and appropriate enhancement of protective functions in forest management (notably soil and water)
Criterion 6:	Maintenance of other socio-economic functions and conditions

The Pan-European Process for developing criteria and indicators for SFM is perhaps the only one among similar regional processes that has properly addressed the issue of genetic diversity (FAO 2002). However, it is still problematic to carry out a sound assessment of the genetic component of SFM, mainly due to a lack of commonly agreed terminology and the fact that standard national forest inventories do not readily provide relevant data for assessing and monitoring this component in all European countries.

Criterion 4 of the Pan-European Criteria is of particular relevance to gene management as it promotes the conservation of forest biological diversity at the ecosystem, species and genetic levels. The improved Indicator 4.6 refers specifically to genetic resources, i.e.:

*4.6 Area managed for conservation and utilisation of forest tree genetic resources (*in situ *and* ex situ *gene conservation) and area managed for seed production.*

The FSC forest certification scheme refers to forest genetic diversity in the following principles and criteria:

Principle 6: Environmental Impact
6.3 Ecological functions and values shall be maintained intact, enhanced, or restored, including: b) Genetic, species, and ecosystem diversity.

Principle 10: Plantations
10.3 Diversity in the composition of plantations is preferred, so as to enhance economic, ecological and social stability. Such diversity may include the size and spatial distribution of management units within the landscape, number and genetic composition of species, age classes and structures.

However, FSC does not provide any specific indicators that are used for assessing whether forest management is sustainable under its other criteria.

In 2002, there was a total of 47,443 hectares in Europe that were reportedly managed for *ex situ* gene conservation of forest trees (MCPFE 2003). This figure is based on the information European countries have provided in their country reports and updates for various EUFORGEN Network meetings. It should be noted that this is likely to be only a rough estimate of the area managed for *ex situ* gene conservation as there is no comprehensive data available from all European countries. Furthermore, not all countries are systematically collecting up-to-date information on *ex situ* conservation areas.

Sound estimates are also difficult to produce for areas managed for *in situ* gene conservation or seed production. One reason for this is that the countries have not yet developed commonly agreed technical terminology, which is mandatory to produce internationally comparable data. At the national level, protected areas or natural parks are often declared as *in situ* gene conservation areas, or “forest gene reserves”, although the protected areas have commonly been established for purposes other than active gene management. Similarly, forest stands are seldom managed solely for seed production purposes and it is common that seeds are collected from different sources, such as *in situ* and *ex situ* conservation areas, other protected areas and production forests, depending on the species-specific demand and seed availability.

Geburek and Müller (2000) listed several issues that need to be considered before declaring an area a forest gene reserve. From the overall management point of view, the most important issues are ownership, management goals and potential for natural regeneration. Secured long-term ownership of an area is necessary for *in situ* gene conservation as it is common that a change in ownership will also alter management objectives. It is possible to manage a forest area for multiple conservation objectives, such as habitat, species and gene conservation. However, silvicultural interventions are often needed as part of active gene management to improve natural regeneration of a given tree species or to keep the existing populations viable, especially in case of rare tree species or if a species will become suppressed by others along successional development. Silvicultural interventions are rarely allowed as part of habitat conservation, thus making it difficult to combine these management objectives. Geburek and Müller (2000) further listed several issues related to genetic conditions that will

set the requirements for declaring forest gene reserves at an even higher level. For example, the reserves should be of autochthonous origin and harbour sufficient genetic variation with respect to both neutral and adaptive genetic markers, in addition to being of sufficient size. It is obvious that European countries need to pay more attention to harmonise terms and definitions before it can be objectively assessed whether forest management is sustainable from the genetic point of view. As Geburek and Müller (2000) pointed out, it makes little sense to declare all possible forest and conservation areas as gene reserves unless the genetic quality of the stands is investigated and documented.

Implementation of gene conservation as part of forest management

European countries have made a lot of progress in promoting sustainable forest management at the pan-European level, as we discussed above. However, the implementation of various recommendations and resolutions in practical forestry remains a national-level responsibility. In 2002, EUFORGEN carried out a survey among national coordinators in its member countries regarding forest management and conservation of forest genetic resources (IPGRI, unpublished). Below, we highlight some results of this survey for which a total of 34 countries provided feedback.

The pan-European criteria and indicators were used for relevant policy formulation with a direct impact on the conservation of forest genetic resources in 6% of the countries, while 61% responded that they will be doing so in the near future. A clear set of guidelines on genetic requirements in forest management was applied in 21% of the countries. Similar guidelines existed in 66% of the countries, but they were insufficiently applied. These results indicate that there has been a delay in many European countries to incorporate genetic considerations to their forest policy formulation. Also, although a fifth of the countries apply genetic guidelines in their forest management, there seems to be a need to enhance the implementation of the existing guidelines in most of the countries.

The existence of a formal national programme on forest genetic resources with well stated objectives and funding is a relatively good indicator fn the level of gene conservation activities in a given country. In the survey, only 27% of the countries indicated having such a programme and in 33% of the countries, an informal programme existed with fairly good coordination of various activities. However, involvement of other relevant stakeholders in the national coordination structure was low. Only 3% of the countries replied that all major stakeholders (e.g., non-governmental organisations, scientists, professionals, forest owners, private sector, etc.) are adequately involved in the decision-making process on forest genetic resources at the national level. In 42% of the countries, only major stakeholders were involved in the national coordination structure.

The 2002 survey did not specifically ask how well the possible national programmes on forest genetic resources are linked with national forest programmes (NFPs), which is considered as the overall planning and implementation mechanism covering the whole forest sector. The concept of a NFP covers a range of approaches and is a framework for addressing forest sector issues in a holistic and multisectoral manner (FAO 1999). Thus, from the implementation point of view, it would be useful if national programmes on forest genetic resources would be closely linked with NFPs and vice versa. Approximately two thirds of European countries have a NFP (FAO 1999), but in many cases, these programmes place very little emphasis on the importance and benefits of gene conservation efforts for the whole forest sector.

Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) have been active in developing and promoting sub-regional collaboration on genetic resources in agriculture (including crops, animals and forest trees) (NCM 2002). All of them except Iceland also have fairly well established national programmes on forest genetic resources (Yrjänä 2003). Similarly, all countries except Iceland have national forest programmes, but it seems that the activities within the two types of programmes are not properly linked with each other. For example, Finland's national forest programme

places very little emphasis on genetic resources (MAF 1999). In 2001, Finland developed a separate national programme on genetic resources in agriculture (including forest trees) (MAF 2001), and although this includes some references to the national forest programme, it remains unclear how these two programmes will interact while implementing their agendas. The link between the two types of programmes is not very closer in other European countries. France has a very strong national programme on forest genetic resources but lacks a NFP (Teissier du Cros 2001; FAO 1999). Netherlands has a NFP but no formal programme on forest genetic resources, but a strategy document has been prepared for it. In Hungary, the national strategy on forest genetic resources has been implemented in 1997 and the country also has a NFP, but there too the genetic component is missing.

Conclusions and recommendations

During the past decade, European countries have achieved a great deal in promoting and implementing both the conservation of forest genetic resources and sustainable forest management. However, it seems that these two efforts are being implemented in a rather separate manner in many countries. The most recent Ministerial Conference on the Protection of Forests in Europe addressed the need for continued international collaboration on forest genetic resources and closer integration of gene conservation in sustainable forest management. This demonstrates the commitment of European countries for international collaboration and their willingness to strengthen the practical implementation of sustainable forest management.

In many countries, biodiversity conservation has received a lot of attention but most of the resources have been channelled into habitat and species conservation while fewer resources have been directed to the national programmes on forest genetic resources or their development. It is obvious that implementation of genetic conservation cannot be effective unless there is a national programme with clear objectives and adequate long-term resources. As only less than 30% of European countries have formal and well-established national programmes on forest genetic resources, countries should continue their efforts in developing these programmes. Furthermore, since the policies and resources for implementing sustainable forest management at the national level are increasingly discussed through NFPs, it would be important to increase the links and coordination between the two types of programmes.

At the international level, the pan-European process on criteria and indicators for sustainable forest management has been able to include the genetic indicators in rather operational ways as compared to similar processes in other regions (cf. FAO 2002). However, to improve the monitoring of genetic sustainability of forest management, a commonly agreed technical terminology needs to be developed to ease the collection of relevant data and make the data comparable internationally. In addition, it might be useful to define minimum requirements for declaring gene reserves or *in situ* conservation areas as this would also facilitate the development of a pan-European network of *in situ* conservation areas for various forest tree species.

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DEVELOPING GENE CONSERVATION STRATEGIES FOR TREE AND SHRUB SPECIES

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ABSTRACT

In 1997 the Gene Conservation Working Group came together in New Brunswick, Canada to begin developing gene conservation strategies for forest tree and shrub species of concern. The process that emerged over a period of time is presented as a model that could be applied in any jurisdiction. The working group consists of industrial, private woodlot, academic, and provincial and federal government representatives, each with a vested interest in sustainable management of forest ecosystems. The model that was developed and followed by the working group consists of several steps. First, a set of criteria was agreed upon. Each native tree and shrub species was tested using these criteria to determine whether gene conservation measures were required. Second, a rating system was applied to define the level of attention needed for each of the species meeting one or more of the criteria. Third, a project was undertaken to find and review all sources of relevant information about each of the identified species and a research program was initiated to address knowledge gaps. Fourth, a field guide was developed to involve volunteers and to assist them in identifying each of the targeted species. The field guide is being distributed to people most likely to encounter the species, such as foresters, biologists, and naturalists. Gene conservation strategies have been developed for several tree species: butternut (*Juglans cinerea*), bur oak (*Quercus macrocarpa*), white elm (*Ulmus americana*) and beech (*Fagus grandifolia*) and members of the Gene Conservation Working Group are responsible for implementing these strategies within their respective realms of activity.

Key words: gene conservation, genetic variability, exotic pathogens, threats, conservation strategies, criteria and rating system, native trees and shrubs

INTRODUCTION

The province of New Brunswick, on the east coast of Canada, is forested over approximately 85% of its land base. New Brunswick falls within the Acadian Forest Region, characterized by tolerant mixed-wood, in a transition zone where hardwood forests to the south meet the predominantly softwood northern forests. Almost all tree species native to New Brunswick have naturally high levels of genetic variability. Less is known about genetic variability of shrub species. All native tree species must interbreed with trees that are not closely related to produce healthy offspring. If the gene pool becomes too small, the species may become locally endangered.

Forest species and ecosystems in New Brunswick have been confronted by various threats during the past three centuries. Human activities continue to change the forest environment at an accelerating rate, probably more rapidly than species are able to adapt. Conservation of genetic variability is increasingly important as environmental uncertainty increases, principally as a result of the greenhouse effect and exotic species invasions.

Forests in New Brunswick have a 300-year history of exploitation for timber and pulp. Areas of the province having rich soil were converted to agriculture; some of these areas have since reverted to forest, but usually not to the original species. Though forest harvest practices have changed over the

years from pure exploitation to a planned sustained-yield approach, the forests of New Brunswick have changed substantially. Certain species were favored for harvest at particular times, and as a result of harvest and other factors, some species have almost disappeared from New Brunswick forests.

What is a gene conservation strategy?

A gene conservation strategy is an action plan that seeks to ensure that genetic variability is preserved and naturally high genetic variability remains. It is not an effort to keep all the genes or all the genetic variants in a species or a population. A gene conservation strategy for a commercial species is usually designed to ensure that the genetic variability is maintained at a level that allows for continued selection for a particular trait, and to ensure that the potential to breed for a new trait, if necessary, is maintained. The primary goal of a gene conservation strategy for a species, which has more of an ecological than commercial importance, is to ensure that the evolutionary potential of a species is retained. In other words, a gene conservation strategy will seek to maintain sufficient genetic variability to allow adaptation to new environmental conditions.

The objective of the work presented here was to identify species of trees and shrubs in the province of New Brunswick that require conservation measures to maintain their genetic viability and to develop gene conservation strategies that are likely to be adopted by forest managers.

The process

In 1997, a group of forest practitioners and researchers in New Brunswick was brought together to begin working on gene conservation. An effort was made to include not only the most knowledgeable people for identifying species of concern, but also the people who would ultimately be responsible for implementing the strategies. Toward that end, naturalists from the New Brunswick Museum of Nature, scientists from the University of New Brunswick, specialists from the provincial and federal government natural resource departments, and representatives from woodlot owner associations and the forest industry were invited to participate in a series of meetings to develop gene conservation strategies. The group became known as the New Brunswick Gene Conservation Working Group (NBGCWG).

The process of developing and the early stages of initiating gene conservation strategies included several steps: identifying which species require attention, assigning priorities to those species, compiling all available information about the present status of species of concern, identifying and initiating required research, publishing a field guide for volunteers and, finally, writing strategies for species for which sufficient information is available.

Identifying species and assigning priorities

The species that were identified are not only those that may be threatened with local extinction or extirpation but also include those that have experienced the loss of a significant number of individuals, or that are believed to have dramatically declined in New Brunswick during the past 300 years.

Eight criteria were used to judge whether or not a species required conservation attention:

1 – Is the species naturally rare in the area?
2 – Is there no or an uncertain viable seed source?
3 – Is there a serious threat from disease or insect pest or from changes in environmental quality?
4 – Is the range or frequency of the species substantially decreasing?
5 – Is the preferred habitat of the species in high demand for other uses?
6 – Do certain harvesting practices prevent the regeneration of the species?

7 – Is there high demand for the species for a special purpose?
8 – Is there a threat of loss of the species due to hybridization and introgression?

An expert opinion process was used to assign criteria. All tree and shrub species native to the province were listed and the NBGCWG discussed, during the course of several open meetings, whether or not any of the criteria applied to the each species. In some cases, a criterion assigned to a species was considered provisional until specific information could be obtained. When all species were evaluated for conservation criteria, priorities were assigned using the same expert opinion process.

The priority rating system was as follows, to identify the type of action required for each species:

0 – species does not need attention
1 – information is inadequate to judge
2 – species requires attention at the level of forestry practices
3 – species requires a gene conservation strategy

Compiling available information and identifying gaps

A project was initiated to compile all available information about the status of species having rating categories of 1 or 3. Sources of information were broad, including data held by provincial and federal departments, New Brunswick Nature Trust, Atlantic Canada Conservation Data Centre and herbaria. All known locations from all sources were plotted for each indicated species, providing estimates of relative frequency in the province. Documented evidence of threats to each of the species was compiled, as well as descriptions of habitat requirements, ecology and history. Criteria and rating assignments were reviewed after evaluating the compiled information, and knowledge gaps were identified. Research projects were initiated for several species to determine regeneration potential, condition of populations with respect to dieback, procedures for seed storage and genetic diversity of natural populations to assist in the development of strategies.

The primary focus in provincial and federal government surveys and sample plots is on commercially important species. Data collected by the herbaria and from ecologically significant areas include all the species of interest, but provide an incomplete coverage of the province. Thus, knowledge is incomplete for several species, even after evaluating all of the compiled data. Researchers at the Canadian Forest Service who are interested in developing gene conservation strategies have limited time and resources for completing surveys, so a project was initiated to develop an identification guide for those species for which current knowledge is insufficient, for distribution to people who spend time in the forest. Target recipients of the guide include technicians working for woodlot owner associations, provincial government and industry, regional nature clubs and any other interested individuals. Addressed forms are included in the guide with encouragement to send information about any occurrences of the indicated species.

Four tree species, i.e. butternut (*Juglans cinerea*), bur oak (*Quercus macrocarpa*), white elm (*Ulmus Americana*), and American beech (*Fagus grandifolia*) were identified as requiring specific gene conservation strategies. The first two species have declined both in numbers and area of distribution since the arrival of European settlers and recently butternut has declined as a result of a fatal, apparently introduced disease. Elm and beech are still relatively common, but most are diseased, infected by fungal organisms that were inadvertently introduced from Europe many decades ago. For species threatened by disease, gene conservation measures focus on maintaining as much diversity as possible within the portion of the gene pool that exhibits resistance to the disease.

Highlights of gene conservation strategies

Butternut

Butternut is common and native in the upper Saint John River Valley and upper Southwest Miramichi River Valley (Hinds 1986), and occasional to common on alluvial soils in the Saint John River Valley near Fredericton.

The most serious threat to butternut is the butternut canker caused by the fungus *Sirococcus clavigignenti-juglandacearum* (Renlund 1971). 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 1995). To date, there is no control for the canker. In Canada, the first report of the canker was in Quebec in 1990 (Innes and Rainville 1996), then in Ontario in 1991 (Davis et al. 1992) and in New Brunswick in 1997 (Harrison et al. 1998). Overall, butternut mortality as a result of the canker exceeds 77% in American forests (Ostry et al. 1994), while in Canada, mortality has been estimated in Ontario to be 80% (Fleguel 1996).

Strategy

Knowledge at this time is deficient for developing a full strategy. The following steps must first be taken:

- survey New Brunswick populations and assess presence of the canker and contribution of the canker to mortality of New Brunswick butternut trees;
- develop an education program for identification of butternut trees, the canker and putatively resistant trees;
- control the movement of seeds (canker spores can be present in seeds).

Bur oak

Bur oak is also found in the Saint John River Valley, primarily around the Grand Lakes complex in central New Brunswick. It occurs in flood plains, usually very close to the water's edge. The range and frequency of the species has substantially decreased and the preferred habitat of the species remains in high demand for other uses. Only eight small (40 to 500 trees) stands remain in the province in addition to occasional isolated trees (McPhee 2000). One small population is currently protected and another is under negotiation. All other populations are threatened by cottage development or harvesting.

Strategy

All populations are on private land, so the gene conservation strategy focuses on private landowners as follows:

- continue education efforts that include distributing a field guide to help landowners and naturalist groups recognize and conserve bur oak;
- ensure that landowners know about conservation options and encourage landowners to choose a conservation option;
- collect seeds for restoration planting and encourage local nurseries, town landscapers and others to use it;
- monitor the status of the remaining populations.

White elm

White elm trees are found throughout New Brunswick, primarily adjacent to rivers and water courses where soils are rich and moist. They are generally not found in the uplands region of the province. The species is threatened by Dutch elm disease throughout the province. Dutch elm disease (DED) is caused by a fungus, *Ceratocystis ulmi* (Buism.) C. Moreau, which is introduced into a tree by the native elm bark beetle (*Hylurgopinus rufipes* (Eichh.)). The disease was first reported in New Brunswick in 1957. No efficient, effective control other than sanitation has been found. The occurrence

of large, healthy older elms in the wild indicates the possibility of the existence of some sort of mechanism in these trees to either prevent or tolerate infections.

Strategy

Due to the nature of the insect/disease combination, the gene conservation strategy focuses on *ex situ* measures as follows:

- survey the province where elm occurs to locate putatively resistant live, healthy, uninfected trees larger than 65 cm DBH;
- collect cuttings in the winter and graft onto white elm rootstock;
- test grafted material for resistance and deploy into a combination genebank and seed orchard for the production of seeds;
- provide seeds to private horticultural nurseries where seedlings will be marketed to the public.

American beech

American beech is widely distributed throughout the province, except for a small area in the extreme northeast of the province. The species occurs wherever tolerant hardwood conditions are found.

The main threat to American beech is beech bark disease, which was introduced more than 100 years ago through Halifax (Shigo 1972). *Cryptococcus fagisuga* is a scale insect that makes the tree susceptible to a beech bark fungus, *Nectria coccinea* var. *faginata* (Houston et al. 1979). Most of the beech in the southern two-thirds of the province is diseased. Many tolerant hardwood stands with a beech component contain some disease-free individuals, but there are no policy regarding conservation of these individuals. Stands with a substantial beech component are commonly clearcut and converted to other species because of the low value of diseased wood. Disease free trees are harvested lost with the diseased ones.

Strategy

A combination of *in situ* and *ex situ* methods are required for beech as follows:

- apply the best management practices in forestry operations that include the removal of diseased trees while leaving putatively resistant ones;
- assess putatively resistant trees for genetic resistance, determine their resistance mechanism and gene action;
- develop vegetative propagation techniques for disease-resistant beech;
- establish a grafted seed orchard of resistant trees.

CONCLUSION

Gene conservation strategies have been written for four tree species in New Brunswick. The strategies will be implemented only if the various landowners and managers accept the need tom implement such strategies, agree with the recommended actions, and can carry them out at low cost. The process followed to develop the strategies was intended to be inclusive to foster buy-in by the range of landowners. Many questions remain to be addressed concerning the full implementation of gene conservation strategies for these four species as well as other species that require conservation attention or for which there is insufficient information to describe the status. Applied research must be a component of ongoing gene conservation efforts.

The process for developing gene conservation strategies that was initiated in New Brunswick could be applied anywhere, even if knowledge is insufficient for many species. Information gaps are highlighted during the process and applied research required to address the gaps can thus be

identified. The key to success is recognition by forest managers of the importance of gene conservation and the political will to fund necessary surveys and biological studies.

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NATURAL POPULATIONS OF DOUGLAS-FIR IN MEXICO: CURRENT STATUS AND NEEDS FOR CONSERVATION

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ABSTRACT

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is one of the most economically important and widely distributed species in western North America. It has been extensively studied over most of its northern range, from British Columbia through the Pacific Northwest and the Rocky Mountains. However, little is known about the status of the southern populations of Douglas-fir in Mexico; yet, they might be particularly important as reserves of genetic resources for the more northern populations in light of predicted changes in global climate. A strong morphological differentiation was found among Mexican Douglas-fir populations, with the geographical region of origin playing an important role in their phenotypic grouping. Populations from Central Mexico were separated from those in the NW and NE regions of Mexico. Sorensen's similarity index confirmed the ecological differentiation between central and northern populations of Douglas-fir. Reproductive success (ratio of sound seed to seed potential) was relatively low (15 to 30%) for most populations, particularly for those from Central Mexico, with 65 to 80% of empty seeds, suggesting high levels of inbreeding, pollination problems, or both. Given this situation, the lack of natural regeneration, and the strong human pressure to change land use, the needs for conservation of these Douglas-fir populations and their valuable genetic resources are discussed.

Key words: *Pseudotsuga menziesii*, genetic resources, differentiation, reproductive success, inbreeding, *in situ* conservation

INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is one of the most economically important forest tree species worldwide. Since Pleistocene times, it has been a major component of forest ecosystems in North America (Hermann and Lavender 1990). The natural range of this species extends from 55° Lat. N Latitude in British Columbia, Canada, down to 17° Lat. N in central Mexico (Little 1979). Recently, a new Douglas-fir population has been reported by Debreczy and Racz (1995) in Sierra Juárez, Oaxaca, Mexico, at 16°30' Lat. N, which now represents the southern limit of the range for *Pseudotsuga* worldwide.

Douglas-fir forms extensive and continuous forests along the coast and the mountain ranges in western Canada and United States. However, in south-western United States and Mexico, species distribution becomes discontinuous and fragmented, creating isolated populations commonly mixed with other tree species (Farjon 1990). Due to changes in environment, isolation, and marginality of Mexican Douglas-fir populations, it might be expected that they have been exposed to strong evolutionary forces, becoming differentiated from other populations in North America.

Paleobotanic records show that Douglas-fir, like many other conifer species, had a broader and more continuous distribution in southern Mexico (Rzedowski et al. 1977; Lozano-García et al. 1993).

However, temperature rising after the last glacial period forced them northward, so populations in the southern extreme disappeared and the distribution in this region became fragmented. As a result of the climate change expected in the near future, a new shift in species distribution is predicted (Melillo 1999; Iverson and Prasad 2002). This situation has several implications for Mexican Douglas-fir populations. On the one hand, they represent an important pool of genetic resources to supply or replace northern populations as climate change occurs, allowing them to adapt to new environmental conditions. On the other hand, the quick pace of climate change will probably increase the pressure on these relictual populations, driving some of them to extinction, particularly those located at the fringes of the species' range in Central Mexico, with no possibility to retreat northward.

Unfortunately, there is little information available about the current situation of Douglas-fir populations in Mexico. There remains controversy on their taxonomic status, and the level and structure of morphologic and genetic variation residing in them is unknown. Except for a few descriptive data from particular sites, information is lacking about the ecological and silvicultural conditions of these populations, particularly in terms of reproductive status. It is well known that small size, isolation and marginal location can strongly reduce variation within populations, increase inbreeding, and reduce reproductive capacity (Mosseler 1998; Saccheri et al. 1998; Mosseler et al. 2000). A low reproductive potential in Douglas-fir populations located in Central Mexico might have strong negative impacts on their long-term viability, so special management practices might be required for *in situ* conservation of these genetic resources.

As the first stage of a broad research project started in 2001, in this paper we report on the geographic pattern of morphologic and ecologic differentiation of Mexican Douglas-fir populations. In addition, indicators of reproductive success are presented for some of them. This information will be useful to identify possible geographic patterns of adaptation for Douglas-fir in Mexico and to establish basic guidelines for germplasm use and movement as well as for management and *in situ* conservation of individual populations.

MATERIALS AND METHODS

Geographic pattern of morphologic variation

A total of 19 populations were sampled throughout the natural range of Douglas-fir in Mexico, including the Sierra Madre Occidental in the northwest (Region I), and the Sierra Madre Oriental in the Northeast (Region II), and Central Mexico (Region III). At each location, vegetative and reproductive samples from 12 to 17 healthy trees were collected. Sampled trees were separated at least 50 m from each other to reduce relatedness, following the environmental gradient within the site. Geographic coordinates and mean altitude of the site were registered with a GPS Garmin® 12XL and an altimeter Thommen ®, respectively (Table 1).

Sixteen cone, foliage, and branchlet traits, considered taxonomically important, were chosen for measurement. They included cone length and diameter, cone scale length and width, bract length, length of center and side prongs, cone length-diameter ratio, scale length-width ratio, bract length-scale length ratio, and center prong-side prong length ratio for cones; needle length, number of stomata lines and sub-epidermis continuity for foliage; and number of resin canals and shape of the transversal section (circular or pentagonal) for branchlets.

A Principal Component (PC) analysis using 14 of these variables (two traits were dropped because of redundancy) was carried out to reduce dimensionality of multi-trait variation. Individual tree scores were calculated using the eigenvector loadings and then used to estimate population centroids in order to identify grouping patterns among them. In addition, a cluster analysis using the Euclidian

distances between populations was done with PROC CLUSTER (SAS Institute Inc. 1998), using the nearest neighbor algorithm. In the cluster analysis, morphologic data of two Douglas-fir populations from the United States, one each from the coastal and the interior varieties, were included as reference.

Table 1. Mexican Douglas-fir populations included in the morphologic variation study.

No.	Location	Coordinates		Elevation (masl)
		Lat. N	Long. W	
	Region I: Northwestern Mexico			
1	Río Chico, Madera, Chih.	29° 37' 03''	108° 09' 42''	1900
2	Cerro La Candelaria, Madera, Chih.	29° 30' 40''	108° 25' 45''	2500
3	Bajío Largo, Guerrero, Chih.	28° 09' 41''	107° 44' 16''	2650
4	Río Verde, Balleza, Chih.	26° 16' 48''	106° 27' 22''	2450
5	Cerro Mohinora, Gpe. y Calvo, Chih.	25° 57' 41''	107° 02' 14''	3050
6	Sierra de la Candela, Sta. María del Oro, Dgo.	25° 27' 51''	105° 33' 42''	2900
7	Mesa de Los Leones, Huachichiles, Dgo.	24° 05' 40''	105° 44' 50''	3000
	Region II: Northeastern Mexico			
8	Santa Anita, Arteaga, Coah.	25° 27' 02''	100° 34' 11''	2400
9	San Antonio de las Alazanas, Coah.	25° 20' 27''	100° 33' 57''	2600
10	Sierra La Marta, Rayones, N. L.	25° 12' 26''	100° 22' 27''	3000
11	Ejido La Providencia, Saltillo, Coah.	25° 09' 36''	101° 11' 57''	2750
12	San Francisco del Javier, Galeana, N. L.	24° 58' 04''	100° 20' 09''	2800
13	La Lagunilla, Galeana, N. L.	24° 56' 42''	100° 16' 02''	2600
14	Ejido 18 de Marzo, Galeana, N. L.	24° 52' 57''	100° 11' 34''	2190
	Region III: Central Mexico:			
15	Rancho El Pardo, Tlaxco, Tlax.	19° 38' 55''	98° 03' 11''	2960
16	Barranca La Rosa, Terrenate, Tlax.	19° 31' 42''	97° 54' 58''	2700
17	La Caldera, Ixtacamaxtitlán, Pue.	19° 30' 23''	97° 52' 10''	2950
18	Axopilco, Altzayanca, Tlax.	19° 27' 42''	97° 46' 18''	2760
19	Apizaquito, San José Cuauhtémoc, Pue.	19° 12' 10''	97° 18' 41''	3350

Floristic similarity index

In addition to the morphologic variation analysis, an evaluation of the ecological affinity among Mexican Douglas-fir populations was done, attempting to identify a geographic trend among them. Botanical and ecological information for several populations representing the three geographic regions described earlier (NW, NE and Central Mexico) was available in the literature (Muller 1947; Maysilles 1959; Valdez 1981; Sánchez-Córdoba 1984; Cornejo 1987; Nájera 1990; Acevedo 1998). Data obtained directly from previous studies by two co-authors of this paper (Domínguez 1986, 1994; Mápula 1995) for other populations were also included in the analysis. Although sampling intensity varied among studies, survey methods were similar. Fixed-dimension plots were established at each site and all plant individuals existing therein were identified and registered to obtain a list of species.

For obvious reasons, this part of the study did not include exactly the same populations as the ones described in Table 1 for analysis of morphologic variation; however, each geographic region was represented by at least four populations. Using the list of plant species (trees, shrubs and herbs) described for each site, Sorensen's Floristic Similarity Index (I_S) between pairs of populations was determined with the following equation (Magurran 1988):

$$I_S = [2 (S_c) / (S_1 + S_2)] * 100$$

Where S_c is the number of species common to both sites; and S_1 and S_2 are the number of species at sites 1 and 2, respectively. With I_S values obtained for all possible pairs of populations, floristic affinity both within and between geographic regions was estimated as the arithmetic mean of I_S for the populations involved in each case.

Indicators of reproductive success

Seed samples from 144 trees representing a total of nine Douglas-fir populations in the three geographic regions were collected during fall 2001. All populations sampled had a good cone crop that year. Three of these populations coincide with those sampled for variation in morphology traits. In each population, 7 to 27 trees ($\bar{x}$=16) with abundant cones and separated at least 50 m from each other were sampled. From each seed lot, a sample of five cones was selected for evaluation of seed production traits, following the methods described by Bramlett et al. (1977). This procedure includes the determination of seed production potential (twice the number of fertile scales), the number of abortive ovules, and the number of developed seeds (empty, filled, and damaged by insects) for each cone. To identify filled seeds from the other categories, X-ray images were used.

From this data, the proportion of aborted ovules, as well as empty, damaged and filled seeds in relation to seed potential was obtained for each cone. The ratio of filled seeds to seed potential represents the efficiency of seed production (Bramlett et al. 1977). The ratio of empty to total developed seeds, which can be regarded as an inbreeding index assuming that empty seeds result from closely related matings, including self-pollination (Mosseler et al. 2000), was also estimated. In addition, average seed size (weight of 100 filled seeds), cone weight and weight of filled seeds per cone were determined. The ratio of filled seed weight to cone weight is considered an indicator of reproductive efficiency (Mosseler et al. 2000). Traits were statistically analyzed to estimate variation among populations in reproductive capacity and efficiency. Variance components were estimated using the REML method of PROC VARCOMP (SAS Institute Inc. 1998). Adjusted means in reproductive indicators for each population were obtained with the LSMEANS option of PROC MIXED (Littell et al. 1996).

RESULTS

Morphological variation among populations

Significant variation ($p < 0.05$) among populations was found for all morphological traits. Except for needle length, the relative amount of variation among populations (between 22 and 92% of total phenotypic variation) was similar or even larger than that among trees within populations (between 6 and 54% of total variation), suggesting a large degree of morphological differentiation among them.

Over 60% of total variation was explained by the first three principal components (PC). They contributed to 28, 20 and 13% of variation, respectively. The next two PC added up to about 20% of the remaining unexplained variation. Traits with the highest relative contribution to the first PC were bract length, center and side prong length, and needle length. The second PC was made up mostly of scale length, scale length-width ratio, and number of resin canals; and the third PC included primarily the traits center-side prong length ratio, cone length-width ratio, and number of stomata lines.

Scatter-plots of centroid population values for the first three PC are presented in Figure 1. Two groups, related to the geographical region of origin, can be distinguished in these plots. The first group includes all the populations sampled in Central Mexico (Region III), whereas the second group is mostly made up of populations coming from NW and NE Mexico (Regions I and II). Populations from Central

Mexico distinguished themselves from the others by having low values in PC 1 and high values in PC 2 and 3, reflecting geographic differentiation in the size of needles, scales, bracts, and prongs; in the shape of cones and scales; and in the number of resin canals and stomata lines. However, there is at least one population from northern Mexico that appears to be more similar to the Central Mexico group than to its own regional group.

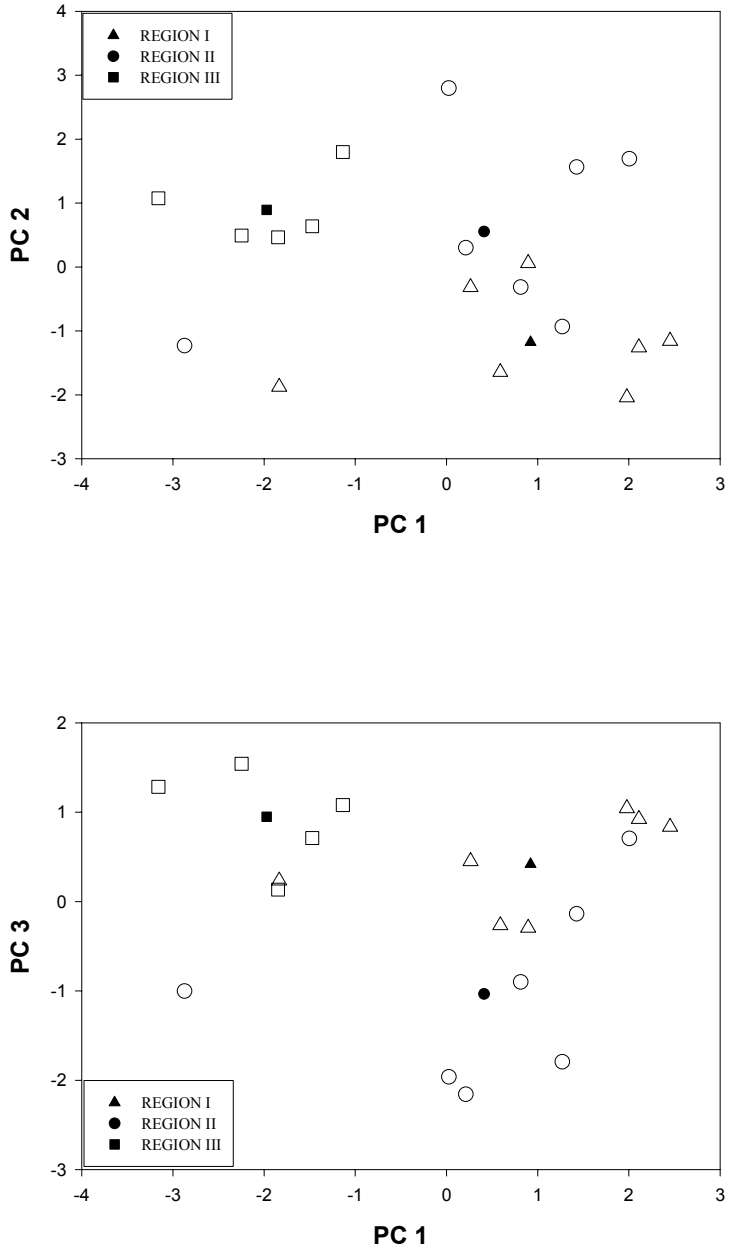

Figure 1. Scatter-plot distribution of Mexican Douglas-fir populations according to the principal components of morphological variation in cone, needle and branchlet traits.

Clustering of populations based on Euclidian distances calculated from original morphological traits corroborated the grouping pattern generated by PC analysis. At a distance of 0.80, three clusters can be distinguished for the Mexican populations considered in the study (Figure 2). The first cluster includes samples from 11 populations, all of them from NW and NE Mexico. The second cluster is made up of seven populations, five of them from Central Mexico and two from the northern regions. The third cluster is represented by only one population (San Francisco), located in the Sierra Madre Oriental, in NE Mexico. All Mexican Douglas-fir populations clustered together before becoming rooted to the reference populations from the interior and coastal Douglas-fir varieties, at an approximate Euclidean distance of 1.0.

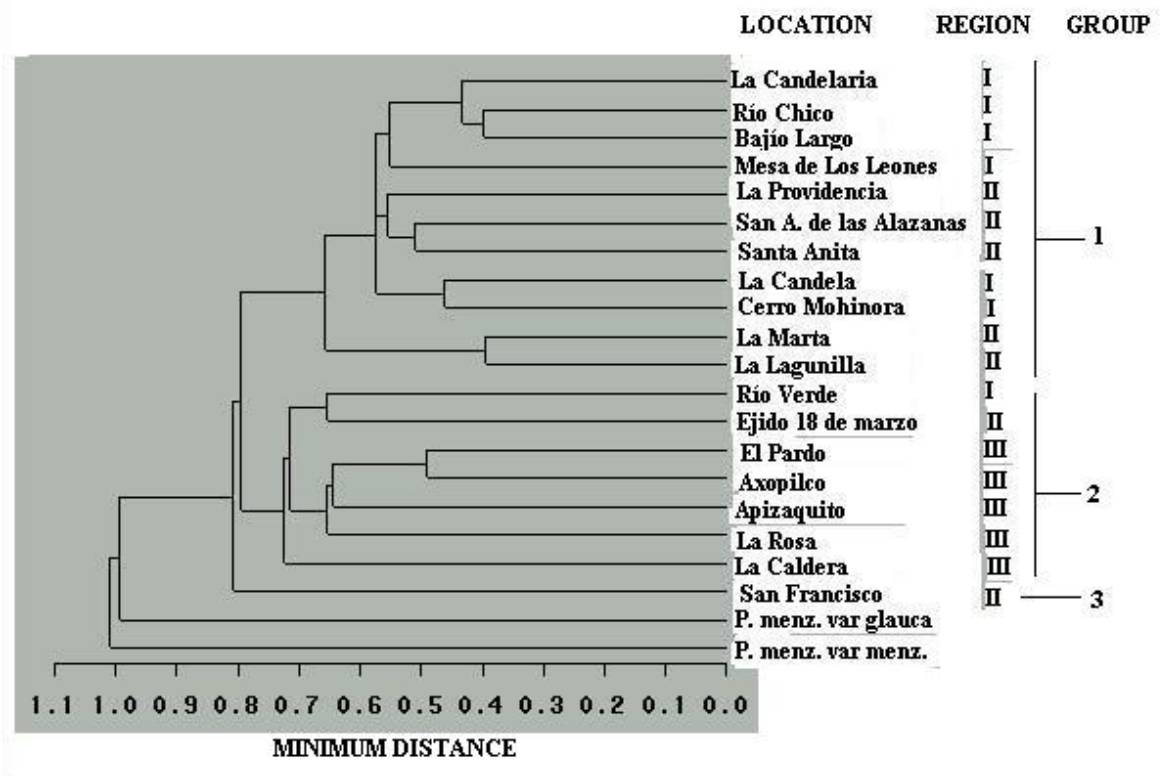

Figure 2. Cluster plot of Mexican Douglas-fir populations based on morphological traits of cones, needles and branchlets. Samples of both coastal and interior Douglas-fir populations are used as reference for the grouping.

Floristic similarity among populations

Sorensen's Similarity Index (I_s) was relatively low on average for all possible pair combinations ($\bar{I}_s$ = 13.7%), but varied widely among them, with extreme values of 2.2 and 36.4%. This situation reflects a large dissimilarity in the botanical composition of plant communities where Douglas-fir could be found throughout Mexico. In NE and Central Mexico, average similarity index between populations was higher within regions ($\bar{I}_s$ > 19.0%) than between them ($\bar{I}_s$ < 7.0%); this was also the case for populations in the NW region, but difference in $\bar{I}_s$ was lower (Table 2).

In addition, $\bar{I}_s$ between NW and NE populations was almost twice as large as $\bar{I}_s$ between Central Mexico and both NW and NE populations (Table 2), indicating a larger floristic distinction in Central Mexico Douglas-fir populations than in those from northern Mexico. Major companion conifer species of Douglas-fir in the NW region are *Pinus ponderosa* Doug., *P. arizonica* Engelm., *P. ayacahuite* var.

brachyptera Shaw, *Picea chihuahuana* Mart., *and Abies durangensis* Mart., whereas in the NE region, *Pinus pseudostrobus* Lind., *P. teocote* Schl. et Cham*., Picea mexicana* Mart., *and Abies durangensis* var. *coahuilensis* Mart. are more common. On the other hand, Douglas-fir becomes associated with *Pinus patula* Schl. et Cham., *P. rudis* Lindl., *P. oaxacana* Mirov*, Abies religiosa* Schl. et Cham., and *Cupressus lindleyii* Klotsch in Central Mexico.

Table 2. Average Sorensen's Similarity Index (%) for Mexican Douglas-fir populations within and between geographic regions.

Geographic region	NW Mexico	NE Mexico	Central Mexico
NW Mexico	14.5	13.2	9.9
NE Mexico		19.4	5.6
Central Mexico			19.8

Reproductive capacity of Douglas-fir populations

Potential number of seeds per cone averaged 56 over all Douglas-fir populations sampled (Table 3), but only 25% of this potential was realized as sound seeds. About 74% of potential seeds either aborted or were empty at time of seed collection; overall, only a small proportion of seeds (< 1%) were damaged by insects. However, significant variation ($p \leq 0.01$) was found among populations for all seed production traits evaluated. Populations contributed to between 21 and 43% of total phenotypic variation found in these traits, indicating broad differences among them in reproductive capacity.

Table 3. Mean values of cone and seed traits related with reproductive capacity in nine Mexican Douglas-fir populations.

Population	Seed potential (No.)	Inbreeding Index†	Seed weight‡ (g)	Reproductive efficiency£
Cerro Mohinora, Gpe. y Calvo, Chih.	55.0	0.405	1.21	44.9
Jamé, Coah.	57.0	0.415	1.20	50.3
Mesa de las Tablas, Arteaga, Coah.	62.8	0.604	1.19	28.5
La Lagunilla, Galeana, N. L.	59.6	0.748	1.17	16.3
Pinal de Amoles, Qro.	57.6	0.681	1.07	27.0
El Carbonero, Huayacocotla, Ver.	46.6	0.565	0.88	21.3
Presa Jaramillo, Mineral del Chico, Hgo.	60.3	0.687	0.95	23.2
Rinconada del Atajo, Singuilucan, Hgo.	45.2	0.811	0.88	18.3
La Caldera, Ixtacamaxtitlán, Pue.	53.0	0.661	0.90	21.5
Overall mean	*55.8*	*0.602*	*1.09*	*29.6*

† Ratio of empty to total developed seeds; ‡ Weight of 100 seeds; £ Ratio of filled seeds weight (mg) to cone dried weight (g).

Potential number of seeds per cone varied from 45.2 to 62.8 among populations, and the average number of developed seeds per cone varied from 29 at El Carbonero, up to 43 at Mesa de Las Tablas, associated both with differences in seed potential and effective pollination. More importantly, however, efficiency of seed production per cone varied widely among populations (from 6 to 23 filled seeds per cone); all populations from Central Mexico had a seed efficiency below 25%, primarily due to the high proportion of aborted ovules and empty seeds found in them. Even though damage by insects was not an important factor in reducing seed efficiency overall, in one population it represented about 10% of seed potential, affecting over one third of sound seeds.

The average ratio of empty to developed seeds was 0.60, but it varied from 0.40 to 0.81 among populations. Assuming that empty seeds are primarily due to self-pollination and mating between relatives (Mosseler et al. 2000), there seems to be a high level of relatedness and inbreeding in Mexican Douglas-fir populations. Average weight of sound seeds in these populations varied from 0.88 to 1.21 g per 100 seeds. In addition, using population values (n=9), a negative correlation (r = -0.53) was found between average seed weight and inbreeding index. Average reproductive efficiency was 29.6 mg g^{-1}, but it also varied three-fold between populations with extreme values.

Most reproductive indicators were significantly correlated with latitude of seed origin; seed efficiency, seed weight, and reproductive efficiency were positively related (r > 0.62), whereas inbreeding index was negatively related (r = -0.68). Thus, Douglas-fir populations in Central Mexico tend to show lower reproductive capacity than those in northern Mexico.

DISCUSSION

Results show that Mexican Douglas-fir populations have differentiated morphologically from the coastal and interior Douglas-fir varieties in the United States sufficiently to become distinguishable using clustering techniques based on multiple traits. Thus, the confusion and discrepancies that have arisen in the past when describing Mexican populations as belonging to different species is not surprising (Martinez 1963). Even though different taxonomic status at the species level is not supported by the morphology data, it seems that these populations can be regarded as a different ecotype or variety from those in the United States, as it has recently been suggested (Debreczy and Racz 1995).

With only a few exceptions, populations from Central Mexico can also be distinguished from those in northern Mexico using multivariate analysis of morphological traits. This variation pattern is probably related to differences in environmental conditions, coupled with the geographic distance between regions. However, the fact that populations in NE Mexico (Region II) were morphologically grouped together with those from the NW (Region I), located over 500 km apart across the desert, instead of joining those from Central Mexico (Region III) in the same mountain range, is difficult to explain. Considering that populations from Regions I and II are located at similar latitudes, it seems possible that a selective force associated with latitude could maintain this geographic pattern of morphological variation. It is well known that natural selection can either differentiate or homogenize populations depending on environmental similarity (Furnier 1997). Ledig et al. (2002) suggest similar reasons as potential explanation for the lack of genetic differentiation between *Picea mexicana* populations in these two geographic regions.

Sorensen's Similarity Index between Douglas-fir communities from different geographic regions is consistent with the degree and pattern of morphological differentiation found between them. Despite the low similarity in botanical composition across all populations, $\bar{I}_s$ was generally larger within regions than between them. In addition, floristic affinity between Region I and Region II was almost twice as large as their affinity with Region III. Sorensen's Similarity Index is a measure of β diversity (Magurran 1988), and thus is an indicator of environmental and ecological differentiation.

Habitat fragmentation for Douglas-fir increases substantially in Central Mexico. In this region, extant Douglas-fir populations are characterized by a patchy distribution of low-density stands, some of them with no more than 50-100 mature individuals and scarce natural regeneration (Domínguez 1986; Mápula 1995; Acevedo 1998). Isolation and small population size are two major factors conducive to genetic drift and inbreeding, which in turn affect genetic diversity, reproductive success and population viability (Mosseler 1998; Rajora et al. 1998). Cone and seed traits used as indicators of reproductive potential for Mexican Douglas-fir populations support this hypothesis. In general, all of them had lower seed production efficiency when compared with published data for Douglas-fir populations in the United

States and Canada (Owens et al. 1991; Webber and Painter 1996) or data for other Mexican conifer species (López and Donahue 1995; Narváez 2000). More importantly, loss of reproductive efficiency was higher in Douglas-fir populations from Central Mexico and it seems to be primarily related to high inbreeding levels, although pollination effectiveness and insect damage had additional impacts on some of them.

The significant latitudinal trend found for most reproductive indicators, including the proportion of empty seeds, and the negative correlation between this trait and seed size are consistent with the hypothesis that the lower reproductive efficiency of Central Mexico populations is due to inbreeding. There is evidence in several conifer species that a reduction in seed and seedling vigor is one of the effects attributable to inbreeding (Sorensen and Miles 1974; Mosseler et al. 2000). However, the effect of extreme environmental conditions on the low reproductive success of Douglas-fir in Central Mexico cannot be ruled out. Populations in this region are exposed to higher temperature and drought stress during spring and summer times than populations located in more northern regions, which might affect pollen production or synchrony.

Reproductive indicators were obtained for only one cone crop, and it is well known that a large year-to-year variation in seed quality exists depending on weather conditions and pollen availability when female strobili are receptive. Although there was abundant cone production in all populations sampled during 2001, data from other cone crops are required to evaluate how consistent the reproductive indicators for these populations are. Until then, inbreeding index and reproductive efficiency values reported here should be taken cautiously.

In addition to the reproductive potential issues, Douglas-fir populations in Mexico are exposed to several human-related threats, including land use changes, illegal harvesting, fire, and grazing; the situation in Central Mexico is particularly severe. On the other hand, the geographic pattern of morphological differentiation found in this study and ongoing common garden tests suggest a wide variation between populations in adaptive traits for Mexican Douglas-fir. Since these traits might become increasingly important as the predicted climate change proceeds, special management plans are required to save the genetic resources of Mexican Douglas-fir. The marginal populations in Central Mexico should have the highest priority for conservation, given the extreme conditions they are facing.

ACKNOWLEDGEMENTS

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PLANNING FOREST GENETIC RESOURCE CONSERVATION UNITS FOR NON-ENDANGERED MEXICAN PINES

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ABSTRACT

Mexico has half of the world's *Pinus* species and a very high deforestation rate (about 670,000 ha/year). Although *Pinus oocarpa* is not considered an endangered species (it has a wide distribution in Mexico and Central America), accelerate deforestation due to the establishment of avocado orchards in Michoacán state, western Mexico, has endangered *P. oocarpa* locally-adapted populations. Designing a forest genetic resources conservation program is very important in this context. However, information is needed about patterns of genetic variation among *P. oocarpa* populations. We established a provenance/progeny test from an open-pollinated seed collection along an altitudinal transect (1100 m to 1500 m) near Uruapan, Michoacán. We found significant differences along the altitudinal gradient among populations on 2.5-year-old seedling height. A quadratic regression model of population altitude *vs.* population average seedling height was fitted and used for altitudinal delimitation of four seed zones. We suggest to establish at least one Forest Genetic Resource Conservation Unit (FGRCU) for each seed zone, at altitudes of 1105 m, 1255 m, 1400 m, 1505 m and 1580 m, in order to capture a representative part of the genetic variation among populations.

INTRODUCTION

Mexico is one of the five countries in the world with the largest richness in biodiversity (Mitteermeier 1988). Regarding forest resources, Mexico has half of the world's *Pinus* species (Styles 1993). Unfortunately, Mexico also has a high deforestation rate, estimated at about 670,000 ha/year (Masera et al. 1997).

Very rare, endemic and highly endangered Mexican pines, such as *Pinus culminicola*, *P. maximartinenzii, P. rzedowskii* and *P. pinceana*, receive much more attention, concerns and conservation efforts from Mexican government and society than widely distributed, economically important and (considered) non-endangered pine species, such as *P. oocarpa* and *P. pseudostrobus*. However, accelerate deforestation has endangered locally-adapted populations of this second group of species.

That is the case of *P. oocarpa*, which is one of the pine species with the largest natural distribution in Mexico and Central America (Perry 1991). Its conservation status is considered at *low risk* (Dvorak et al. 2000). *Pinus oocarpa* distribution is in the low altitudinal range (1100 m to 1600 m) of the pine-oak forest in the western state of Michoacán. In this state, total deforestation amounts to 35,000 ha/year, due to change of land use from forest to agriculture, fruit orchards and grazing, and to forest fires and illegal cutting (COFOM 2001). *Pinus oocarpa* natural stands are severely decimated at present in the region of Uruapan, Michoacán (120 km west of Morelia, the capital city of the state of Michoacán), because avocado orchards are being established extensively in areas of *P. oocarpa* natural distribution. Apparently, climatic requirements for avocado orchards are coincidental to climatic requirements of *P. oocarpa* natural populations. Avocado became an important product for exportation.

Mexico is now the first worldwide country for avocado production (68% of worldwide production), and Michoacán is the first state for avocado production in Mexico (84% of national avocado production) (AALPAUM 2003).

In this context, efforts to establish programs for the conservation of forest genetic resources such as *P. oocarpa* locally-adapted populations are very important. Conservation programs could result in the establishment of a number of Forest Genetic Resource Conservation Units (FGRCU), also called gene resource management units (Ledig 1988; Millar and Libby 1991). A FGRCU is a representative natural stand of any species with a management priority to maintain genetic diversity as well as to allow natural evolutionary forces to mold the population's genetic structure (Ledig 1988, 1992; Millar and Libby 1991).

However, designing such programs requires a minimum understanding of the patterns of genetic variation within and among populations of the species of interest. We examine the patterns of the genetic differentiation for quantitative traits along altitudinal gradients among *Pinus oocarpa* populations in Michoacán, Mexico, and suggest the use of seed zoning as guidelines to decide the placement of Forest Gene Resource Management Units.

METHODOLOGY

Open pollinated cones were collected from approximately 11 randomly selected trees from each of five *P. oocarpa* natural populations distributed along an altitudinal gradient on a southern slope of the Neovolcanic Axis, near of Uruapan, Michoacán state, western Mexico (19°25'12'' Lat. N, 102°06'02'' Long. W, average annual temperature 18.9°C, average annual precipitation 1608 mm). Sampled populations are located approximately at 100 m of altitudinal difference: 1505 m, 1430 m, 1325 m, 1220 m and 1075 m of elevation. Average geographic distance between contiguous populations was approximately 4 km.

We established a field provenance/progeny test on a randomized complete block design with four blocks, five populations, an average of six half-sib families nested within each populations, and three seedlings per plot in Canalejas, near the village of Cuarayo, *Ejido* San José de Cañas, municipality of Ario de Rosario de Rosales, Michoacán, Mexico (1550 m, 19°04'13'' Lat. N, 101°44'21'' Long. W).

We evaluated seedling height at 2.5 years of age (from seed germination). At that age, seedlings had been in the field for two rainy seasons already. We conducted an analysis of variance to test significance among provenances using PROC GLM of SAS (SAS Institute Inc. 1988). We fitted a quadratic regression model of population altitude *vs.* average seedling height. We estimated an interval of confidence (X = 0.2) for population average seedling height. Then, we estimated altitudinal limits of seed zones by crossing the curve of predicted values from the quadratic model to upper and lower interval limit values of the population average seedling height. The seed zone limits were used to suggest the location of Forest Genetic Resources Conservation Units (FGRCU).

RESULTS

Analysis of variance indicated significant differences among populations (P = 0.0368). Population average seedling heights suggest that, in general, there exists a clinal trend, where seedlings originating from populations located at higher altitudes grow more than seedlings from populations located at lower altitudes. However, the population located at the lowest elevation (1075 m, which is the lowest elevation limit of the species' distribution) grows less than the second lowest population (1220 m) (Figure 1).

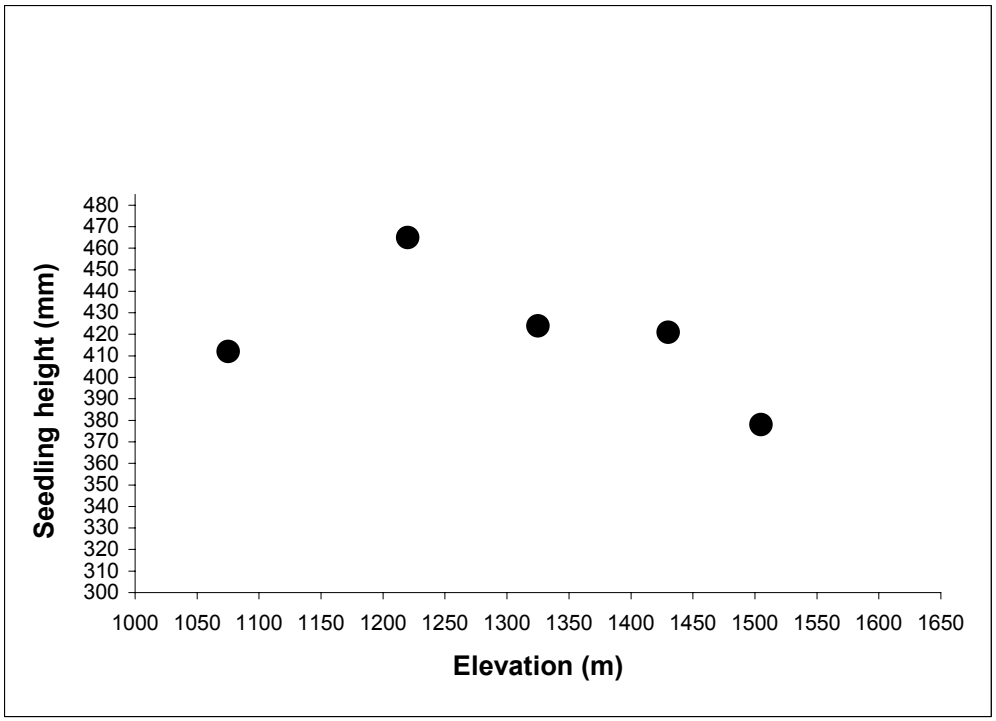

Figure 1. Average seedling height for five *Pinus oocarpa* populations in a 2.5-year-old provenance/progeny test in Michoacán, Mexico.

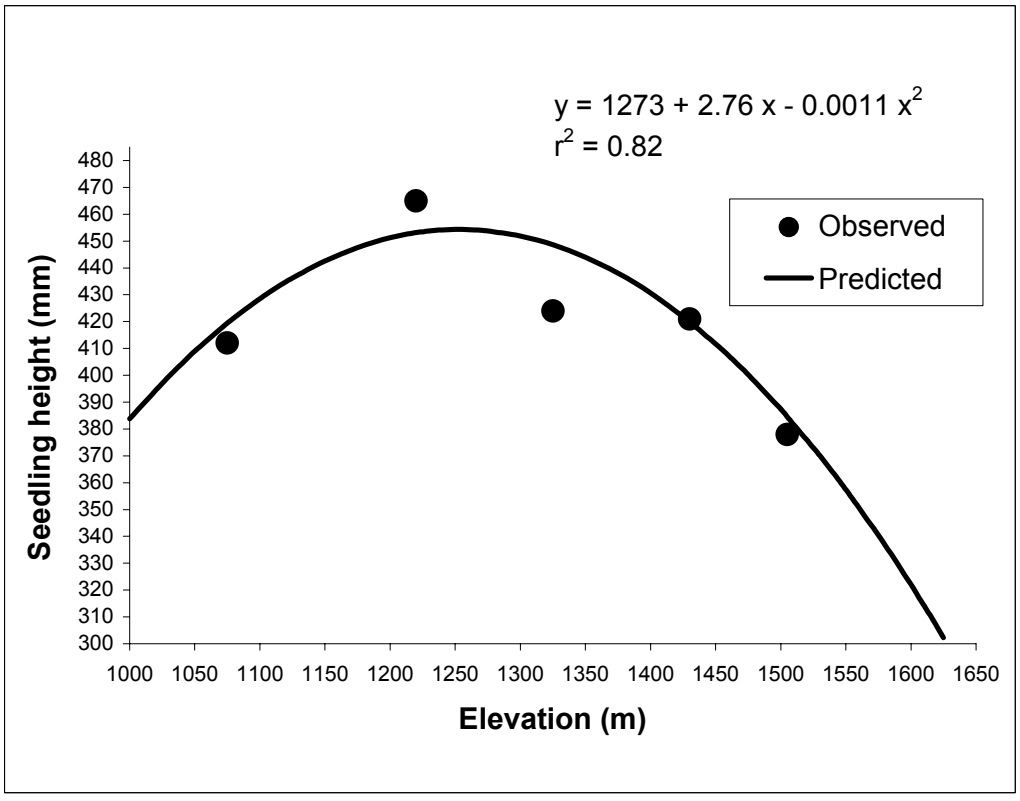

Figure 2. Quadratic model for *Pinus oocarpa* population growth.

Regression of population altitude *vs.* population average seedling height, fitting a quadratic model ($r^2 = 0.8184$), suggests a curve where maximum growth occurs in populations located at 1255 m

of altitude, and growth decline occurs in populations located either at lower or higher elevations (Figure 2).

Table 1. Altitudinal limits and ranges for seed zones and altitudinal suggested placement for FGRCUs for *P. oocarpa* in Michoacán, México.

Altitudinal limits (m)		Range (m)	Seed zone	FGRCU suggested placement (m)
Lower	Upper			
1045	1255	210	1	1105
			1 - 2	1255
1255	1461	206	2	1400
1461	1547	86	3	1505
1547	1613	66	4	1580

Confidence interval (X = 0.2) for population average seedling height was estimated at $\overline{X}$ ± 23.72 mm. The width of the interval is equivalent to 47.44 mm of average seedling height. Using as a starting point the maximum predicted average seedling height (454 mm) as an upper confidence interval limit for population average seedling height, we delimitated successive confidence interval limits downwards on a predicted value curve, and then we found their corresponding altitudinal values. We considered these altitudinal values as limits for four altitudinal seed zones (Table 1, Figure 3). Seed zones have different altitudinal range values, depending on which part of the curve they are situated on. Seed zones on the upper altitudinal part of the species distribution are more narrow (e.g., 66 m of altitudinal range for seed zone 4), whereas they are more wide where the curve slope is less steep (210 m and 206 m of altitudinal range for seed zones 1 and 2, respectively; Table 1).

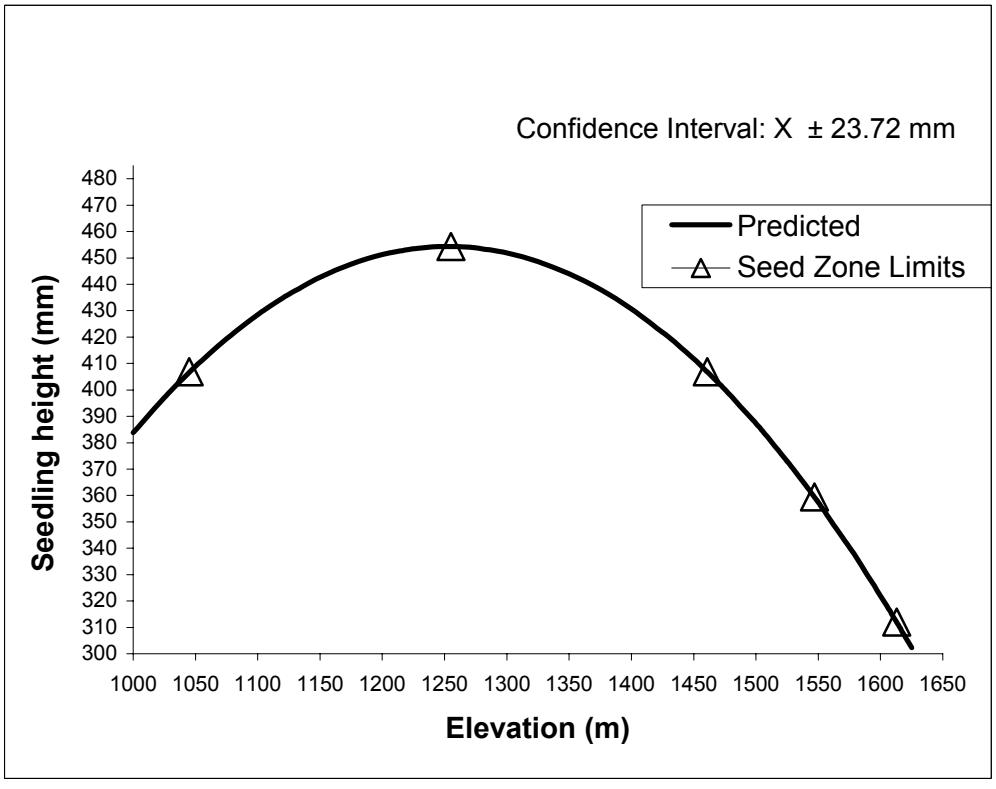

Figure 3. Seed zoning for *Pinus oocarpa*.

We suggest the establishment of at least one Forest Genetic Resources Conservation Unit (FGRCU) in each seed zone. The suggested altitude for a FGRCU location corresponds to the middle part of the confidence interval for population average seedling height (which was used for delimitation of seed zone altitudinal limits) (Table 1, Figure 4). The suggested altitudinal location of a FGRCU does not necessarily correspond to the middle altitude of a seed zone, particularly for seed zones 1 and 2. For this reason, we suggest the addition of an extra FGRCU at the altitudinal limit between seed zones 1 and 2 (1255 m), in order to avoid the excessive altitudinal difference (295 m) between the FGRCU of seed zone 1 and the one in seed zone 2. This additional FGRCU would also be helpful for capturing the likely valuable genetic variation of the population with the highest growth potential (at 1255 m of elevation) (Table 1, Figure 4).

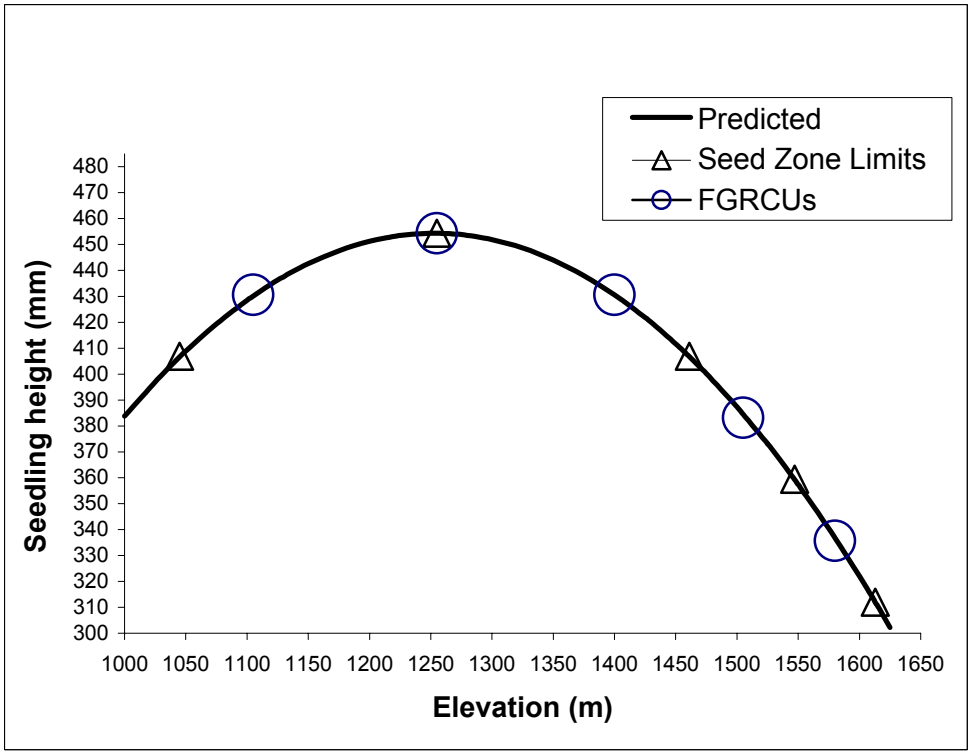

Figure 4. Suggested placement for Forest Genetic Resource Conservation Units (FGRCUs).

CONCLUSION

Populations of *P. oocarpa* differ genetically for growth potential along altitudinal gradients in Michoacán State, western Mexico. Using confidence intervals for population average seedling height, we suggest the delimitation of four seed zones. We suggest to establish at least one Forest Genetic Resource Conservation Unit in each seed zone plus one additional FGRCU at the limit of two of these seed zones, in order to capture a representative amount of genetic variation among populations for quantitative traits. The suggested altitudes for the location of FGRCUs are: 1105 m, 1255 m, 1400 m, 1505 m and 1580 m.

ACKNOWLEDGEMENTS

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EFFECT OF LARGE-SCALE MOVEMENT OF LOBLOLLY PINE SEED ON GENETIC INTEGRITY OF THE SPECIES IN ITS NATURAL RANGE

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INTRODUCTION

Geographic variation

Geographic variation of the major southern pine species has been well studied. Seed collected from different geographic areas vary greatly in their potential for growth and survival depending on where they are planted. In seed source studies of forest tree species, it is often observed that sources from warmer climates grow faster than local sources as long as they are not moved to greatly differing climates. This is true in loblolly pine and is at least partially due to warm-climate sources growing longer in the fall than sources from colder climates (Jayawickrama et al. 1998). There is some advantage to moving loblolly pine seed sources northward to improve yield.

While north/south differences in adaptive traits in loblolly pine are significant, the east/west differences have been much more important economically. The most important early study of loblolly pine seed sources was Philip C. Wakeley's Bogalusa, LA, planting of 1927. There, loblolly pines (*Pinus taeda* L.) grown from local seeds (Livingston Parish, LA) produced about twice the wood volume through age 22 as did trees of the same species grown from Arkansas, Georgia, and Texas seeds (Wakeley and Bercaw 1965). More important, there was a very large difference among sources in susceptibility to fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*). The sources from Arkansas and Texas were very resistant, the Livingston Parish source was moderately resistant, and the Georgia source was very susceptible. Because of the results of this study and other later studies, east Texas and Livingston Parish loblolly seedlings have been moved eastward to areas with severe fusiform rust infection. They have been planted over hundreds of thousands of acres in the southern Coastal Plain, where they have exhibited both substantial rust resistance and good growth rates (Wells 1985).

Gene flow

The Mississippi River Valley provides a barrier to gene flow in the southern pines because pines are not part of the natural vegetation in the moist, alluvial soil of the Valley. Now mostly agricultural, the Valley has historically been home to vast hardwood forests, undoubtedly because of the absence of fire. Of the three southern pine species that occur both east and west of the Mississippi River, loblolly (*Pinus taeda* L.), longleaf (*P. palustris* Mill.) and shortleaf (*P. echinata* Mill.), only in loblolly pine are there important differences between western and eastern seed sources (Schmidtling 2001). This difference between loblolly pine and the other species is probably rooted in the Pleistocene geologic era. During the height of the Wisconsin Ice Age, 14,000 years before present, the South was occupied by a boreal forest. Patterns of genetic variation in allozymes indicate that longleaf resided in one refugium in south Texas / north Mexico and migrated northward and eastward when the ice retreated (Schmidtling and Hipkins 1998). It is probable that loblolly pine originated from two isolated refugia, one in southwest Texas / northeast Mexico, and one in south Florida / Caribbean (Schmidtling et al. 1999). The two populations converged at the Mississippi River Valley at the close of the Pleistocene era. The 100,000-year isolation of the two populations, in differing environments, resulted in the differences we see today.

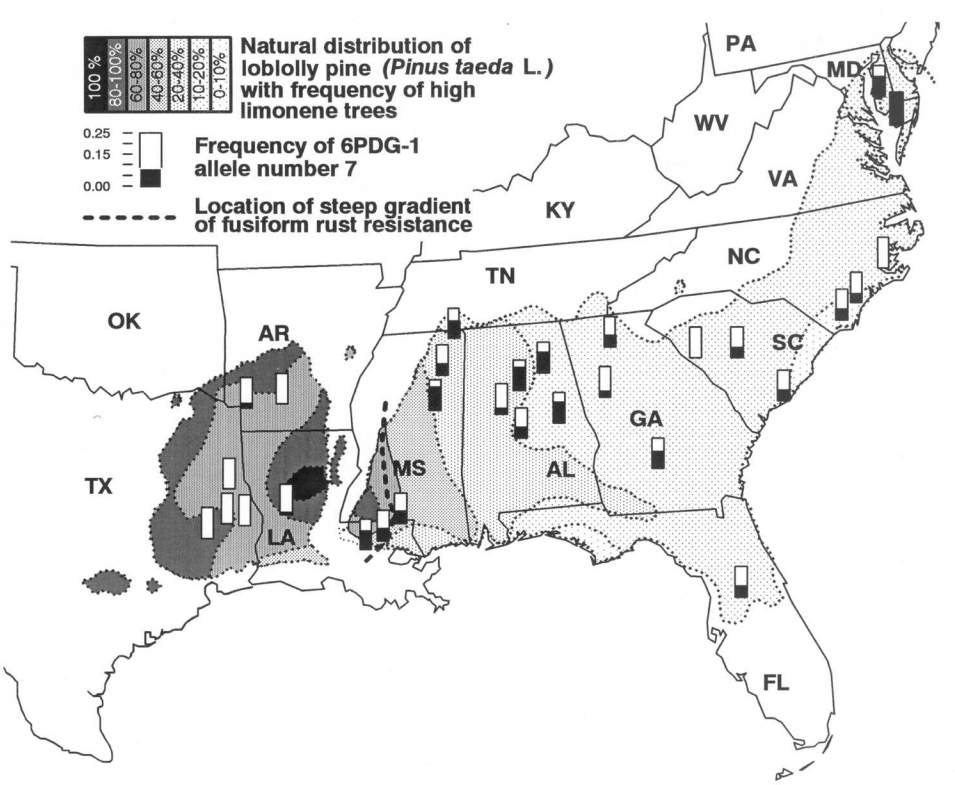

Figure 1. Map of the southeastern United States showing the natural distribution of loblolly pine and the distribution of trees with high limonene content in cortical gum (redrawn from Squillace and Wells 1981). Also shown is the frequency of the slowest migrating allele of the enzyme 6PDG-1 in electrophoresis (from Schmidtling et al. (1999)).

Squillace and Wells (1981) showed that extensive geographic variation exists in the highly heritable monoterpene composition of bud resin in loblolly pine. Especially notable was east-west variation. Variation was largely clinal, but all trees west of the Mississippi River Valley had high beta phellandrine. Limonene content showed the greatest east-west variation. In most of the populations west of the Valley, 100% of the trees had high limonene, whereas populations in many areas of the east coast had zero trees with high limonene (Fig. 1). A re-drawn version of Figure 3 of Squillace and Wells (1981) appears to show that gene flow is occurring eastward across the Mississippi River Valley, especially at the southern end of the distribution.

An eastward gene flow across the Valley would also account for the resistance to fusiform rust (inherent in western seed sources), which is possessed by loblolly pine from Livingston Parish, Louisiana, even though this source is east of the Valley. The distribution of resistance to fusiform rust in an intensive sampling of loblolly pine seed sources across the Valley, showed a pattern very similar to that of limonene in Figure 1, i.e. an indication of gene flow for resistance from west to east, especially at the southeastern end of the western population (Wells et al. 1991).

Allozyme data offers evidence that gene flow is largely one-way across the Mississippi River Valley in loblolly pine (Schmidtling et al. 1999). In electrophoresis of the enzyme 6PGD-1, allele number seven, the slowest migrating allele, has an average frequency of 13% in loblolly pine populations across the south, and ranges from 0 to 25% in individual populations (Fig. 1). There is a striking difference in frequency across the Mississippi River Valley. This allele is absent or very rare west of the Valley, whereas just to the

east of the Valley the allele occurs in substantial frequencies (Fig. 1), suggesting that gene flow from east to west is very low. Prevailing winds during pollination as well as during seed fall are from west to east, which probably accounts for the directionality of gene flow.

The problem

Several long-term tests have shown that loblolly pine from east of the Mississippi River has an inherently faster growth rate than western loblolly. Trees from some eastern sources have grown about 8 feet taller in 25 years than trees from western sources; a substantial difference (Wells and Lambeth 1983).

In the last few decades some forest products manufacturers have planted substantial numbers of loblolly seedlings from Atlantic Coastal Plain sources in southern Arkansas, southeastern Oklahoma, and the Ouachita Mountains of Arkansas and Oklahoma (Lambeth et al. 1984). The short-term economic value of these transfers, at least for a pulpwood rotation, is great. However, the lack of long-term adaptability in growing stock transferred in the westward direction may result in catastrophic losses late in the rotation (Lambeth et al. 1984).

More importantly, even if these trees are harvested completely in a pulpwood rotation, their pollen will affect the seed produced in surrounding native stands. It is not known how the non-local genotypes will affect the native gene pool. In this paper, published allozyme data are used to infer changes in the gene pool that could be attributed to seed transfers.

The data

The loblolly pine populations sampled in Schmidtling et al. (1999) were established during three different time periods. The youngest population was comprised of bulk seed collected around 1980 in 10 natural stands scattered across the natural range of loblolly pine. Three of the natural stands were west of the Mississippi River Valley and seven were east of the Valley. Allozyme data were collected from embryos, giving a genetic snapshot of the populations being established naturally around 1980.

The second youngest population was comprised of seed collected from plantings of the Southwide Southern Pine Seed Source Study (SSPSSS) (Wells and Wakeley 1966). The SSPSSS plantings were established using seed collected around 1950 from 14 natural stands, two from west of the River and 12 from east of the River. Allozyme data were collected from megagametophytes, giving a genetic sampling of the populations being established around 1950 when the original collections were made.

The oldest population was comprised of seed from orchard selections in U.S. Forest Service seed orchards. Only seed collected from clones whose ortets were in existence prior to 1930 were used in this study. Seed from orchard ramets whose donor ortets were established after 1930 were excluded due to the possibility that the donor trees were planted by the Civilian Conservation Corps, and therefore could be non-local in origin. In all, nine seed sources were sampled: two western and seven eastern. Allozyme data were collected from megagametophytes, giving a genetic sampling around 1920 when the original donor trees were established.

Table 1, adapted from Schmidtling et al. (1999), shows east-west differences in various measures of allozyme variability for the three population ages. In general, the younger populations tend to have fewer polymorphic loci and lower expected heterozygosity than the older populations. Western populations also have a tendency to have fewer alleles per locus than eastern populations, which one might expect if gene flow between the populations only occurred from west to east. There is no obvious trend over time in the number of alleles per locus by time of establishment.

Table 1. Allozyme variability of eastern versus western loblolly pine populations from three different eras. Data extracted from Schmidtling et al. (1999).

		Old (Orchard)	Middle (SSPSSS)	Young (Bulk)
Number of Alleles per Locus	E	2.55	2.01	2.33
	W	2.33	1.83	2.08
Percent Polymorphic Loci	E	63.4	53.9	44.4
	W	66.7	45.8	44.5
Expected Heterozygosity	E	0.195	0.180	0.170
	W	0.196	0.140	0.158

The most intriguing trend is in the genetic distance between eastern and western populations. Allozyme data can be used to establish a genetic distance, or index of dissimilarity, to infer relatedness, or genetic divergence. Using the raw data from Schmidtling et al. (1999), Nye's genetic distance was derived using BIOSYS (Swofford and Selander 1989) and plotted versus time of establishment (Fig. 2). Populations from the apparent transition zone, southeastern Louisiana and southwestern Mississippi, were not included in the analysis.

The allozyme data originating from trees established during three different periods, ca. 1920, 1950, and 1980, show a dramatic decrease in genetic distance between western and eastern sources over time (Fig. 2). This decrease appears to be too abrupt to be due to natural gene flow, which has undoubtedly been continuous from west to east since the end of the last glaciation more than 10,000 years ago, a very long time compared to the less than 100 years represented in this study.

An allozyme data set similar to the loblolly data is also available for longleaf pine (Schmidtling and Hipkins 1998). The longleaf populations represented in the data set are very similar to those for loblolly: older seed orchard selections circa 1910, SSPSSS plantations derived in the early 1950s, and recent bulk collections from natural stands (Schmidtling and Hipkins 1998). Again, Nye's genetic distance was derived using BIOSYS (Swofford and Selander 1989). The results for the longleaf data are quite different than for the loblolly data, as one would expect since longleaf expresses little east/west variation and has not been widely planted (Fig. 2). The genetic distance between eastern and western sources is much less overall than for loblolly, and there is no obvious trend over time. Instead, the genetic distance appears to be more or less constant over time, unlike in loblolly pine.

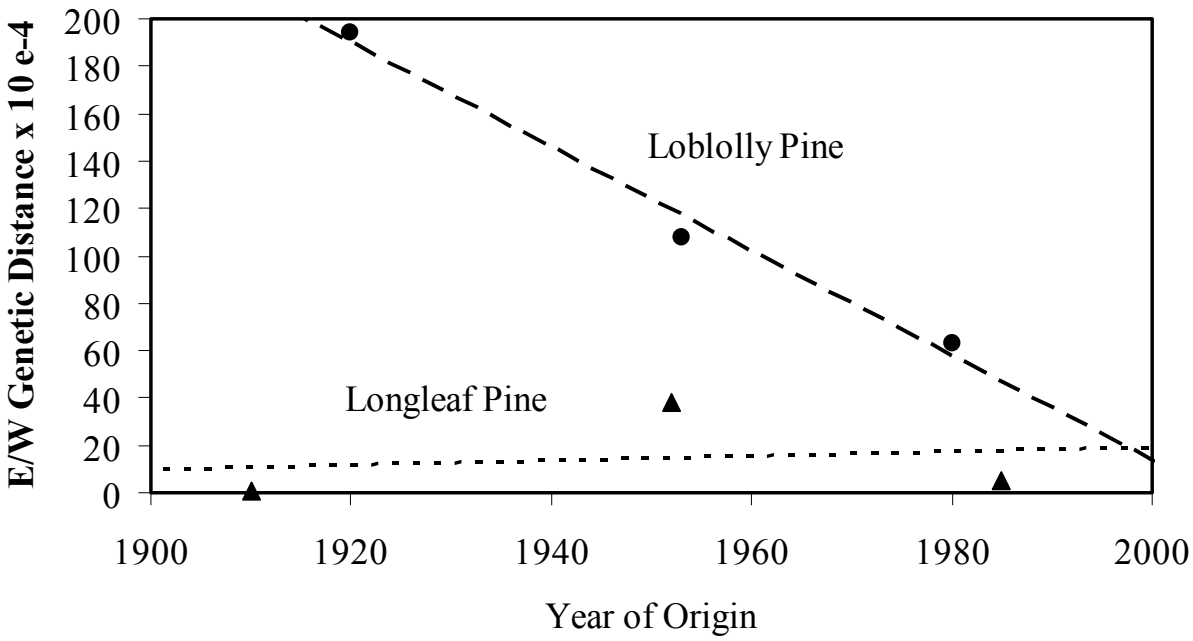

Figure 2. Plot of genetic distance between eastern and western sources versus year of origin for loblolly pine (data from Schmidtling et al. (1999)) and longleaf pine (data from Schmidtling and Hipkins (1998)).

Implications

It appears that the widespread movement of loblolly pine seed sources has a significant effect on native gene pools. Planting non-local sources can increase genetic diversity in local populations but may have important implications for growth and adaptability depending on the direction of seed movement.

Moving western seed sources east of the Mississippi River, which mimics natural gene flow, probably has a positive effect on adaptability since western seed sources tend to be hardier, more fusiform rust resistant, and tend to tolerate crowding better than eastern sources (Schmidtling and Froelich 1993). Eastern sources tend to grow faster than western sources, so moving seed sources from west to east probably has a slightly negative effect on growth.

Moving seed sources from east of the Mississippi River west may be problematical in the long run. During drought, plantings of eastern sources are susceptible to catastrophic failure (Lambeth et al. 1984). Dendrochronological analyses of western plantings of the Southwide Southern Pine Seed Source Study (SSPSSS) have shown that eastern sources tend to keep growing at the onset of a drought, whereas western seed sources cease growth immediately (Grissom and Schmidtling 1997). Since periods of severe drought are common in the western part of the loblolly pine distribution, mal-adapted genes from eastern sources could be incorporated into western populations through pollen shed and cause long-term decreases in adaptability.

This mal-adaptation may become even more important in the future. Most global climate change models predict that in the western part of the loblolly pine range, the climate will become drier than at present (Schwartz 1991). Thus, the incorporation of eastern genes into western populations does not bode well for the future.

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Session 2

ARE OLD-GROWTH FORESTS FROM THE ABITIBI REGION AN IMPORTANT RESERVOIR OF GENETIC DIVERSITY FOR WHITE SPRUCE IN QUEBEC?

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ABSTRACT

Old-growth forests are believed to be potential reservoirs of genetic diversity for keystone tree species. However, few studies have attempted to verify this assumption and none have been carried out for white spruce (*Picea glauca* [Moench] Voss). The level of genetic diversity of 16 populations, including six populations from an old-growth forest sector in Abitibi and 10 populations from second-growth forests in other regions of Quebec, was estimated using allelic variants at eight expressed sequence tag polymorphic loci (ESTP). Trees from two generations were sampled in two locations in Abitibi, i.e. a mature and a young cohort (less than 15 years old). For the two other populations from the same region, only the young cohort was sampled. A total of 23 alleles were detected in the 16 populations. Every allele found in the 10 populations from the second-growth forests was also present in populations representing the old-growth forests in Abitibi. However, one new variant was detected in populations from the Abitibi region. Moreover, the proportion of rare alleles (frequency lower than 5%) observed in the population from the old-growth forests was more than twice that in populations from the second-growth forests. Populations from old-growth forests in Abitibi had a population structure that is remarkably different from that of populations from second-growth forests in other regions. This was however mainly due to the structure observed in the two mature cohort populations. Indeed, contrary to what was observed in the second-growth populations, there was an excess of heterozygotes as compared with the number expected under Hardy-Weinberg equilibrium in both within and total populations in the Abitibi old-growth forest. A cluster analysis performed on the matrix genetic distances between populations made it possible to show that populations from the Abitibi old-growth forests sector made up a quite distinct group from populations of second-growth forests. Based on the results obtained, we recommend that the current protection of populations from Abitibi old-growth forests be maintained for conservation of white spruce genetic resources and further research be conducted on mating systems and adaptive traits of progenies.

INTRODUCTION

Conservation of biological diversity is important to ensure the viability, resiliency and sustainability of forest ecosystems. Canada has acknowledged its importance by introducing a framework of criteria and indicators to help track the country's progress toward sustainable forest development. One of these criteria concerns genetic diversity. Indeed, the conservation of genetic diversity is considered to be an essential component of sustainability to maintain short-term viability of individuals and populations, to maintain the evolutionary potential of populations and species and to provide opportunities for use of genetic resources (Boyle 2000). Knowledge of distribution patterns of genetic diversity is thus essential to develop sound forest policies and management practices.

While old-growth forests are generally considered to form communities that provide unique habitats for forest-dependent plants and animals, there is very little empirical evidence that old-growth forests also serve as reservoirs of genetic diversity or fitness for keystone tree species (Mosseler et al. 2003). If they hold unique hot spots or more diverse gene pools for some tree species as compared with those of second-growth stands, they might play an important role in maintaining the evolutionary potential of these species. This is why special attention must be paid to the evaluation of genetic diversity in these unique ecosystems.

It has generally been assumed that the North American boreal forest was characterized by a forest mosaic composed mainly of even-aged, post-fire stands which has led to the assumption that old forests were relatively rare in that biome (Kneeshaw and Gauthier 2003). However, long intervals (more than 200 years) between fires have been reported, particularly in eastern Canada (Cogbill 1985), where an important proportion of overmature and old-growth stands makes up the landscape (Bergeron et al. 2001). For instance, large areas of Abitibi West in Quebec and the Lake Abitibi Model Forest in eastern Ontario are dominated by forests that last burned more than 240 years ago. In that region, uneven-aged stands, the heterogeneity of which is self-maintained by insect outbreaks and gap dynamics more than 225 years after a fire, are considered as old-growth stands (Kneeshaw and Bergeron 1998, Kneeshaw and Gauthier 2003). They are generally balsam fir - white birch - white spruce stand types. The occurrence of such stands offers an opportunity to test the hypothesis that they are reservoirs of genetic diversity for the keystone tree species present in these stands.

White spruce (*Picea glauca* [Moench] Voss) is a forest tree species found in a variety of ecosystems and climate conditions (Farrar 1995). It is a shade-tolerant species generally associated with trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), black spruce (*Picea mariana* [Mill.] BSP) and balsam fir (*Abies balsamea* L.). It is also a valuable reforestation species in Canada and adjacent northeastern and Lake States (Nienstaedt and Zasada 1990). Most white spruce natural stands were harvested in the past as this tree species is an important source of supply for the pulp and paper and lumber industry. There are breeding programs underway in most Canadian provinces for this species. In Quebec for instance, 20 seed orchards were set up with thousands of superior genotypes over the last 20 years. Moreover, breeding activities are being carried out with two 240-tree breeding populations (Beaulieu 1996). With such large breeding populations, dominant alleles with low frequencies are likely to be captured and maintained (see Yanchuk 2001). However, to capture recessive alleles with intermediate to low frequencies, there is a need to maintain in-situ populations in reserve.

Analysis of genetic diversity at allozyme, chloroplast and ESTP loci has already revealed high levels of diversity and typically low population differentiation (Alden and Loopstra 1987; Tremblay and Simon 1989; Furnier et al. 1991; Furnier and Stine 1995; Jaramillo-Correa et al. 2001). However, none of these studies aimed at comparing genetic diversity in white spruce populations from old-growth forests with that of populations originating from major human disturbances. The specific questions we addressed in the current study were: (i) Do white spruce populations from old-growth forests contain greater genetic diversity in nuclear loci than populations from second-growth forests? (ii) Do white spruce populations from old-growth forests in Abitibi make up a distinct gene pool from that of populations from second-growth forests in Quebec and is it worth maintaining them in in-situ reserves?

MATERIALS AND METHODS

White spruce populations and sampling

Thirty sexually mature white spruce individuals at least 30 m apart were sampled in 10 natural populations from second-growth forests in Quebec and in two natural populations from old-growth forests in the Abitibi region (Figure 1). Fifty young-cohort individuals (< 15 years old) were also sampled in the last two populations as well as in two young stands originating from relatively recent forest fires in the same area (Figure 2). The ten second-growth populations were located in three different bioclimatic domains, i.e. the maple - yellow birch, the balsam fir - yellow birch, and the balsam fir - white birch domains (Robitaille and Saucier 1996). Populations from the old-growth forest were located on the shore of Lake Duparquet in Abitibi (79^{o} 20' W, 48^{o} 30'N), in the balsam fir - white birch domain. The Lake Duparquet region was selected because its fire history has been reconstructed using fire scars and age determination of trees that colonize burned sites immediately after a fire (Bergeron 1991). Eight major fires have burned in the forest since 1760 (Figure 2) and three major spruce budworm outbreaks that occurred in the last century (Bergeron 2000). Some of the lakeshore stands were affected by partial cutting in the early 1900s but were spared from industrial clear-cutting that began in the late 1970s.

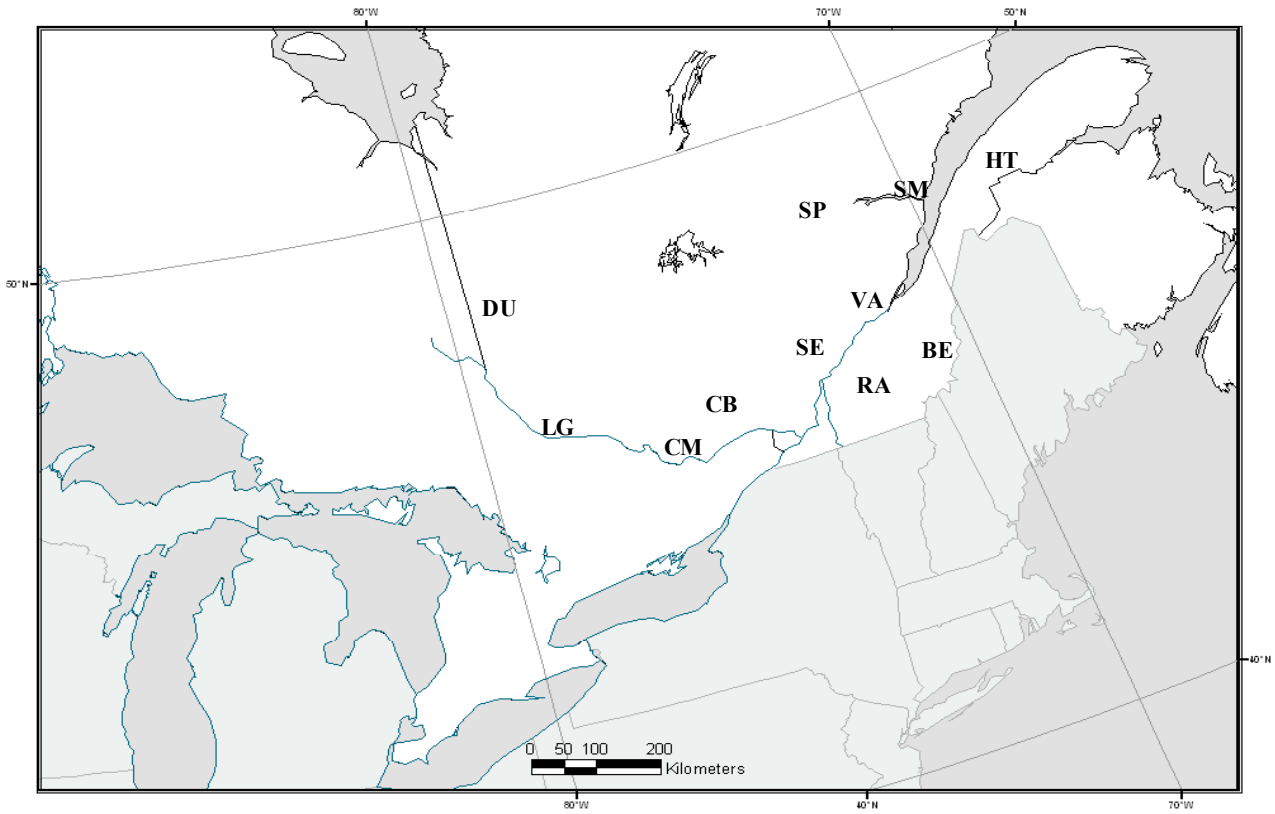

Figure 1. Geographical location of the 16 white spruce natural populations studied in Quebec. Abbreviations: DU = Lake Duparquet (Abitibi), GL = Grindstone Lake, CM = Sainte-Cécile, BT = Boyer Township, SE = Sainte-Émilie, RA = Racine, BE = Beauceville, VA = Valcartier, SP = Saint-Prime, SM = Sainte-Marguerite, HT = Les Hauteurs.

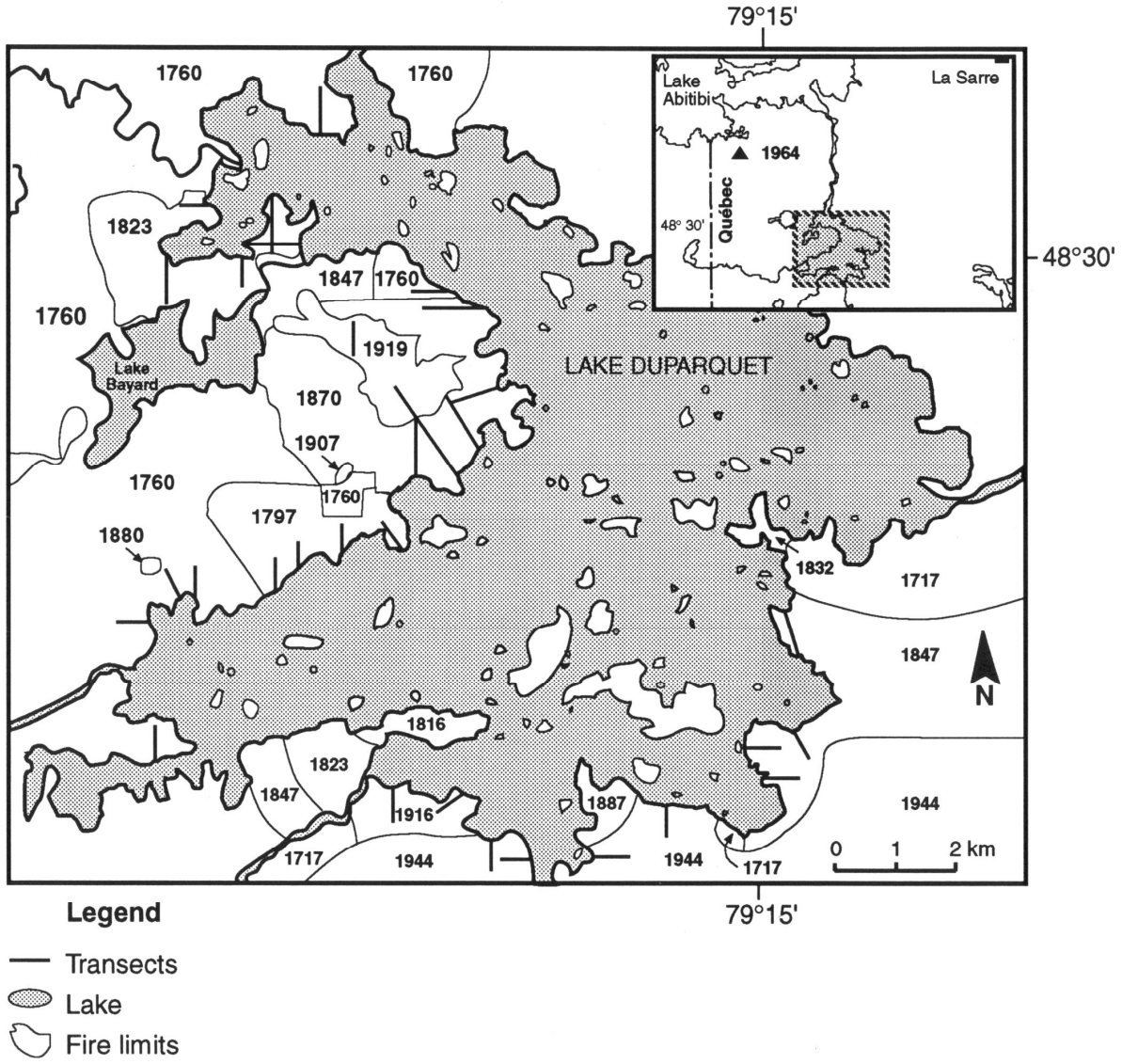

Figure 2. Geographical location of the six white spruce populations sampled in the Lake Duparquet Research and Teaching Forest. Abbreviations: A = Mature cohort – fire 1760, B = Mature cohort – fire 1797, C = Young cohort – fire 1760, D = Young cohort – fire 1797, E = Young cohort – fire 1916, F = Young cohort – fire 1944.

The two old-growth stands were initiated by fires in 1797 (A and C) and 1760 (B and D) and are characterized by a mixed composition of balsam fir, white spruce, eastern white cedar and white birch. The most recent stands date from the 1916 (E) and 1944 (F) fires and are dominated in the overstory by aspen and white birch with an understory of balsam fir and white spruce (Bergeron 2000).

DNA extraction and ESTP assays

Samples collected on trees of the ten second-growth populations have already been used in a previous study aimed at comparing population structure at expressed sequence tag polymorphisms, allozymes and quantitative traits. DNA extraction protocols for these samples and genotype scoring are as described in Jaramillo-Correa et al. (2001). For each tree of the populations from the Abitibi region, 60 mg of fresh needles cut into small pieces were placed into a 1.5 mL microtube containing a 3 mm

tungsten carbide bead. The tubes were soaked in liquid nitrogen for 4 min and then the needles were ground by agitation of the beads with a mixer mill 300 (Qiagen, Mississauga, Ontario). DNA was extracted from needles using a DNeasy Plant Mini Kit (Qiagen, Mississauga, Ontario) according to the manufacturer's instructions. DNA samples were further diluted to 4 ng/µL and subjected to polymerase chain reaction (PCR) using specific primers for eight ESTP loci (*Sb08, SB16, Sb21, Sb29, Sb32, Sb41, Sb58* and *Sb60*). To do so, reaction mixtures containing 20 ng of DNA, 0.12 µM of each primer, 0.2 mM of each dNTP, 0.0375 units/µL of platinum *Taq* DNA polymerase (Invitrogen, Burlington, Ontario) and 1X of both supplied buffer reaction and $MgCl_2$ adjusted at 2 mM were prepared. They were then placed in a Perkin Elmer Gene Amp PCR System 9600 that was programmed for 40 cycles, after a preheating of 4 min at 94°C. The 40 cycles (94°C, 1 min; 55°C, 2 min; 72°C, 3 min) were followed by a period of 10 min at 72°C. The ramp time to annealing and extension temperatures was 4 s/degree. PCR products were electrophoresed through 2% agarose TAE 1x buffer or 3% agarose-synergel gels in TPE 0.75x buffer for 6 h at 130 V. All products were visualized under UV light after ethidium bromide staining. The assayed loci were previously characterized as harbouring exclusively codominant alleles (Perry and Bousquet 1998a, b), and the scoring of genotypes followed previously established rules of inheritance and segregation patterns (Perry and Bousquet 1998b).

Data analysis

Allelic frequencies were determined by gene counting for each nuclear locus in all populations. Hardy-Weinberg equilibrium (HWE) was verified with Fisher's exact test using the program GENEPOP (Raymond and Rousset 1995). Genetic diversity was estimated for every population using five indices: the percentage of polymorphic loci (P), the mean number of alleles per locus (A), the mean number of effective alleles per locus (Ae), the observed heterozygosity (H_O) and the expected heterozygosity (H_E), corrected for small sample size (Nei 1978). Estimates for these indices were obtained using BIOSYS (Swofford and Selander 1989). The proportion of rare alleles (5% criterion as the frequency threshold for rare alleles) was also estimated. Heterogeneity of allele frequencies among populations was tested for each polymorphic locus with Fisher's exact test implemented in GENEPOP (Raymond and Rousset 1995) and using a Markov chain method (Guo and Thompson 1992). Cavalli-Sforza and Edwards arc distances (Cavalli-Sforza and Edwards 1967) were estimated between all pairs of populations in order to determine whether or not white spruce populations were structured geographically. Distance estimates were obtained using BIOSYS and a phylogenetic tree was constructed using the phenetic approach of the unweighted pair group method with arithmetic averages (UPGMA) of the same software. Gene diversity analysis and the estimation of fixation indices (F_{IT}, F_{IS}, and G_{ST}) followed the methods of Nei and Chesser (1983), with adjustments for small samples. The standard deviations for these estimates were obtained by bootstrapping over loci (Weir 1996) using computer software we developed. The number of migrants per generation was estimated according to Crow and Aoki (1984).

RESULTS AND DISCUSSION

All eight ESTP loci analyzed were polymorphic in at least one population (Table 1). The most variable locus was *Sb16* with 5 alleles, whereas both loci *Sb32* and *Sb60* had 4 alleles. The least variable locus was *Sb21* which was monomorphic in most of the populations with one rare allele present in young cohort populations in Abitibi and in Racine. A total of 23 alleles was detected for an average of 2.4 alleles per locus. Most of the alleles observed had been reported previously by Perry and Bousquet (1998b) and Jaramillo-Correa et al. (2001). A new rare allele was detected (frequency lower than 0.05) for loci *Sb32* only. Its length was about 730 bp and was located on gels between alleles A and B. This fourth *Sb32* allele was found in all the populations in the Abitibi region except the young cohort of the 1760 fire population. While rare, this allele appears to be maintained over generations and its absence in the sample of the young cohort of the 1760 fire population is likely due to sampling only. The rare allele D of locus *Sb60*, which had been observed by Jaramillo-Correa et al.

(2001) in the Beauceville population, was also observed in Abitibi but in the mature cohort of the 1760 fire population only. This allele, as well as allele E of *Sb16*, allele B of *Sb21* and allele D of *SB32*, appears to be both rare and local (see Marshall and Brown 1975, for definition). Other alleles also seem rare but are more widespread. This is the case for both allele C of *Sb16* and allele B of *Sb60*. The proportion of rare alleles per population ranged between 0 in Sainte-Marguerite and 0.29 in the young cohort of the population originating after the 1916 forest fire in Abitibi. On average, the proportion of rare alleles in the populations from second-growth forests was less than half of that in the populations from the Abitibi old-growth forests, i.e. 0.108 versus 0.229, respectively. The presence in the Abitibi old-growth forest populations of all the rare alleles scattered among the populations in the second-growth forests of other regions is likely related to the age distribution of trees. Indeed, on one hand the second-growth forest populations regenerated after harvesting and were more or less of the same age. The rare alleles might have been eliminated in some populations in the process. On the other hand, as trees in the Abitibi old-growth forest populations (1760 and 1797 fire areas) are from many generations (age of trees up to 200 years), it is likely that all the rare alleles present were maintained over generations as no major disturbance has occurred over the last two centuries.

At the population level, the percentage of polymorphic loci (P) varied between 87.5 and 100 (Table 2). The mean number of alleles ranged from 2.1 to 2.8 with an overall average of 2.4. The effective number of alleles per locus was quite constant from population to population, varying between 1.5 and 1.6. The average observed heterozygosity varied from 0.262 to 0.387 with a mean value of 0.336, whereas the average expected heterozygosity ranged from 0.316 to 0.375 with an overall average of 0.341. On one hand, the average proportion of expected heterozygotes under the HWE was lower than that observed in the mature cohort of populations from Abitibi old-growth forests (Table 2). It was also true for the 1944 fire population in the same region. For the 10 second-growth populations, the average expected heterozygosity was higher than the observed one in half of them. Loci *Sb16, Sb21, Sb32, Sb58 and Sb60* were in HWE in all populations. Others deviated from HWE (Fisher's exact test, $p \leq 0.01$) in one population only. Hence, *Sb08* significantly deviated from HWE in Les Hauteurs, whereas *Sb29* and *Sb41* were not in HWE at Grindstone Lake and Abitibi 1916 fire, respectively.

Gene diversity average estimates within the populations and in the total population (H_S and H_T) were 0.341 and 0.344, respectively when the 16 populations were considered (Table 3a). While the estimates for the young cohorts of the four Abitibi populations (Table 3c) and for the 10 Quebec second-growth forest populations (Table 3d) when analyzed separately were more or less equivalent to those obtained for the 16 populations, those of the mature cohorts of the two old-growth forest populations were slightly higher (Table 3b), i.e. 0.388 and 0.389, respectively. Fixation indices within populations (F_{IS}) ranged from -0.046 for *Sb41* to 0.083 for *Sb08* with an overall average of 0.014 when data of the 16 populations were analyzed together (Table 3a). This low but positive F_{IS} average value indicates there is a slight excess of homozygotes within the populations when all 16 populations sampled are considered. However, as anticipated from comparisons of expected and observed heterozygosities (Table 2), mature cohorts from old-growth populations (Table 3b) showed a deficit of homozygotes within populations contrary to what was observed in Quebec second-growth forest populations and young cohorts of populations from Abitibi (Table 3c, d). Moreover, six of the eight loci studied had an excess of heterozygotes in the mature cohorts of the old-growth populations compared with the two other groups of populations with a better balance between loci showing an excess of heterozygotes and those with a deficit of heterozygotes. When the six old-growth populations were analyzed altogether, we could still note a slight excess of heterozygotes within populations.

Table 1. Allelic frequencies of eight ESTP loci in 16 white spruce natural populations sampled in Quebec.

Locus/allele	Old-growth forest in Abitibi						Second-growth forest									
	Mature cohorts		Young cohorts				Valcartier	Boyer Township	Grindstone Lake	Sainte-Marguerite	Beauceville	Sainte-Émilie	Racine	Saint-Prime	Les Hauteurs	Sainte-Cécile
	1760‡	1797	1760	1797	1916	1944										
Sb08 / A	0.845	0.883	0.841	0.857	0.853	0.890	0.844	0.813	0.859	0.839	0.823	0.783	0.767	0.833	0.823	0.750
/ B	0.155	0.117	0.159	0.143	0.147	0.110	0.156	0.188	0.141	0.161	0.177	0.217	0.233	0.167	0.177	0.250
(N)†	29	30	44	49	51	50	32	32	32	31	31	30	30	30	31	30
Sb16 / A	0.417	0.250	0.522	0.409	0.480	0.480	0.453	0.516	0.438	0.400	0.241	0.327	0.483	0.433	0.210	0.300
/ B	0.250	0.429	0.278	0.295	0.296	0.260	0.281	0.156	0.344	0.367	0.379	0.365	0.333	0.467	0.435	0.450
/ C	0.017	0.000	0.033	0.000	0.010	0.010	0.031	0.000	0.031	0.017	0.017	0.038	0.000	0.000	0.000	0.033
/ D	0.317	0.304	0.167	0.295	0.194	0.240	0.234	0.328	0.188	0.217	0.362	0.250	0.167	0.100	0.355	0.217
/ E	0.000	0.018	0.000	0.000	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.019	0.017	0.000	0.000	0.000
(N)	30	28	45	44	49	50	32	32	32	30	29	26	30	30	31	30
Sb21 / A	1.000	1.000	0.978	0.990	0.990	0.990	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000
/ B	0.000	0.000	0.022	0.010	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000
(N)†	26	30	46	49	51	50	32	32	32	32	32	30	30	30	31	30
Sb29 / A	0.552	0.672	0.630	0.688	0.684	0.606	0.609	0.609	0.578	0.625	0.679	0.768	0.633	0.700	0.726	0.683
/ B	0.448	0.328	0.370	0.313	0.316	0.394	0.391	0.391	0.422	0.375	0.321	0.232	0.367	0.300	0.274	0.317
(N)†	29	29	46	48	49	47	32	32	32	32	28	28	30	30	31	30

Locus/allele	Old-growth forest in Abitibi						Second-growth forest									
	Mature cohorts		Young cohorts				Valcartier	Boyer Township	Grindstone Lake	Sainte-Marguerite	Beauceville	Sainte-Émilie	Racine	Saint-Prime	Les Hauteurs	Sainte-Cécile
	1760‡	1797	1760	1797	1916	1944										
Sb32 / A	0.054	0.069	0.087	0.112	0.163	0.094	0.172	0.203	0.094	0.161	0.150	0.190	0.133	0.083	0.081	0.117
/ B	0.536	0.466	0.522	0.449	0.439	0.438	0.344	0.547	0.578	0.500	0.567	0.517	0.483	0.417	0.435	0.533
/ C	0.375	0.448	0.391	0.429	0.367	0.458	0.484	0.250	0.328	0.339	0.283	0.293	0.383	0.500	0.484	0.350
/ D	0.036	0.017	0.000	0.010	0.031	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	28	29	46	49	49	48	32	32	32	31	30	29	30	30	31	30
Sb41 / A	0.583	0.583	0.444	0.520	0.520	0.590	0.469	0.359	0.359	0.453	0.403	0.433	0.383	0.417	0.387	0.650
/ B	0.417	0.417	0.556	0.480	0.480	0.410	0.531	0.641	0.641	0.547	0.597	0.567	0.617	0.583	0.613	0.350
(N)†	30	30	45	49	51	50	32	32	32	32	31	30	30	30	31	30
Sb58 / A	0.933	0.950	0.967	0.980	0.960	0.938	0.859	0.906	0.969	0.891	0.852	0.967	0.967	0.967	0.871	0.967
/ B	0.067	0.050	0.033	0.020	0.040	0.063	0.141	0.094	0.031	0.109	0.148	0.033	0.033	0.033	0.129	0.033
(N)	30	30	46	49	50	48	32	32	32	32	27	30	30	30	31	30
Sb60 / A	0.883	0.967	0.978	0.959	0.970	0.960	0.984	0.938	0.984	0.859	0.929	0.900	0.933	0.917	0.952	0.950
/ B	0.033	0.017	0.000	0.000	0.000	0.010	0.000	0.016	0.016	0.063	0.000	0.017	0.017	0.000	0.000	0.000
/ C	0.067	0.017	0.022	0.041	0.030	0.030	0.016	0.047	0.000	0.078	0.054	0.083	0.050	0.083	0.048	0.050
/ D	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
(N)	30	30	46	49	50	50	32	32	32	32	28	30	30	30	31	30

‡ Year of the last forest fire.
† Number of trees sampled.

Table 2. Genetic variability estimates for 16 white spruce populations sampled in two regions of Quebec.

Population	Mean sample size / locus	Mean number of alleles per locus		Percentage of polymorphic loci †	Mean heterozygosity	
		observed	effective**		Direct-count	HWE ‡
Old-growth forest (Abitibi)						
Mature cohorts - 1760 fire	29.0*	2.6	1.6	87.5	0.387	0.358
- 1797 fire	29.5	2.5	1.5	87.5	0.353	0.322
Young cohorts - 1760 fire	45.5	2.4	1.5	100	0.312	0.324
- 1797 fire	48.3	2.4	1.5	100	0.325	0.325
- 1916 fire	50.0	2.6	1.5	100	0.277	0.332
- 1944 fire	49.1	2.8	1.5	100	0.352	0.329
Second-growth forest						
Valcartier	32	2.3	1.5	87.5	0.344	0.354
Boyer Township	32	2.3	1.5	87.5	0.352	0.347
Grindstone Lake	32	2.3	1.5	87.5	0.262	0.316
Sainte-Marguerite	31.5	2.4	1.6	87.5	0.340	0.375
Beauceville	29.5	2.4	1.6	87.5	0.380	0.361
Sainte-Émilie	29.1	2.5	1.5	87.5	0.373	0.349
Racine	30	2.5	1.6	100	0.371	0.357
Saint-Prime	30	2.1	1.5	87.5	0.292	0.325
Les Hauteurs	31	2.1	1.5	87.5	0.286	0.342
Sainte-Cécile	30	2.3	1.5	87.5	0.371	0.338
Overall average		2.4 (0.05)	1.5 (0.01)	91.4 (1.5)	0.336 (0.010)	0.341 (0.004)

* Standard error in parentheses.
** $A_e = 1 / (1 - H_e)$.
† A locus is considered polymorphic if more than one variant is detected.
‡ Unbiased estimate (Nei 1978).

Table 3. Single-locus gene diversity and population structure indices for eight ESTP loci in 16 white spruce populations in Quebec.[†]

a) Sixteen populations

Locus	Heterozygosity			Fixation indices *			Heterogeneity of allelic frequencies [‡]
	H_O	H_S	H_T	F_{IS}	F_{IT}	G_{ST}	
Sb08	0.259	0.282	0.281	0.083	0.078	-0.005	0.759
Sb16	0.663	0.656	0.669	-0.011	0.008	0.019	0.000
Sb21	0.013	0.012	0.013	-0.018	-0.005	0.012	0.287
Sb29	0.435	0.454	0.454	0.042	0.041	-0.001	0.583
Sb32	0.568	0.599	0.601	0.051	0.055	0.004	0.121
Sb41	0.512	0.490	0.499	-0.046	-0.027	0.019	0.003
Sb58	0.124	0.122	0.124	-0.022	-0.007	0.015	0.017
Sb60	0.113	0.111	0.112	-0.020	-0.014	0.006	0.084
Mean	0.336	0.341	0.344	0.014 (0.018)	0.023 (0.014)	0.009 (0.004)	

[†] According to Nei and Chesser (1983) for small sample sizes, [‡] Fisher's exact test p-value (Raymond and Rousset 1995), * Standard deviation estimated with 1000 bootstraps in parentheses.

b) Two populations from old-growth forest of the Abitibi region (mature cohort)

Locus	Heterozygosity			Fixation indices *			Heterogeneity of allelic frequencies [‡]
	H_O	H_S	H_T	F_{IS}	F_{IT}	G_{ST}	
Sb08	0.237	0.238	0.237	0.004	-0.002	-0.005	0.596
Sb16	0.776	0.672	0.682	-0.155	-0.138	0.015	0.079
Sb29	0.534	0.475	0.478	-0.126	-0.117	0.008	0.260
Sb32	0.579	0.583	0.581	0.006	0.002	-0.004	0.769
Sb41	0.567	0.493	0.490	-0.149	-0.157	-0.007	1.000
Sb58	0.117	0.111	0.111	-0.046	-0.053	-0.007	1.000
Sb60	0.150	0.142	0.143	-0.059	-0.049	0.010	0.336
Mean	0.423	0.388	0.389	-0.091(0.032)	-0.088 (0.029)	0.003 (0.004)	

[†] According to Nei and Chesser (1983) for small sample sizes, [‡] Fisher's exact test p-value (Raymond and Rousset 1995), * Standard deviation estimated with 1000 bootstraps in parentheses.

c) Four populations from old-growth forest of the Abitibi region (young cohort)

Locus	Heterozygosity			Fixation indices *			Heterogeneity of allelic frequencies ‡
	H_O	H_S	H_T	F_{IS}	F_{IT}	G_{ST}	
Sb08	0.258	0.242	0.241	-0.067	-0.072	-0.005	0.770
Sb16	0.613	0.649	0.648	0.056	0.055	-0.002	0.456
Sb21	0.026	0.026	0.026	-0.005	-0.011	-0.006	0.818
Sb29	0.421	0.457	0.455	0.079	0.076	-0.003	0.576
Sb32	0.603	0.607	0.606	0.007	0.005	-0.002	0.551
Sb41	0.468	0.499	0.501	0.063	0.065	0.002	0.271
Sb58	0.078	0.075	0.075	-0.037	-0.038	-0.001	0.481
Sb60	0.066	0.065	0.064	-0.023	-0.029	-0.006	0.959
Mean	0.317	0.328	0.327	0.033 (0.018)	0.032 (0.019)	-0.002 (0.001)	

† According to Nei and Chesser (1983) for small sample sizes, ‡ Fisher's exact test p-value (Raymond and Rousset 1995), * Standard deviation estimated with 1000 bootstraps in parentheses.

d) Ten populations from southern Quebec sampled in second-growth forests

Locus	Heterozygosity			Fixation indices *			Heterogeneity of allelic frequencies ‡
	H_O	H_S	H_T	F_{IS}	F_{IT}	G_{ST}	
Sb08	0.263	0.307	0.304	0.143	0.135	-0.009	0.888
Sb16	0.661	0.656	0.670	-0.008	0.013	0.021	0.000
Sb21	0.010	0.010	0.010	-0.036	-0.004	0.032	0.002
Sb29	0.421	0.450	0.449	0.063	0.062	-0.001	0.462
Sb32	0.552	0.599	0.603	0.078	0.085	0.008	0.082
Sb41	0.519	0.485	0.491	-0.071	-0.057	0.013	0.072
Sb58	0.145	0.142	0.145	-0.015	0.003	0.017	0.028
Sb60	0.125	0.123	0.124	-0.009	-0.005	0.004	0.157
Mean	0.337	0.346	0.350	0.027 (0.028)	0.036 (0.024)	0.009 (0.004)	

† According to Nei and Chesser (1983) for small sample sizes, ‡ Fisher's exact test p-value (Raymond and Rousset 1995), * Standard deviation estimated with 1000 bootstraps in parentheses.

Individual F_{IT} values varied between -0.027 and 0.078 when all the populations were considered in the analysis. An average deficiency of 2.3% of heterozygotes was observed in the total population as compared with the expected average value under the HWE (Table 3a). However, mature cohorts of the Abitibi old-growth forest populations were markedly different from other populations as an average excess of 8.8% of heterozygotes was observed when considered to make up one population (Table 3b).

Genetic distances among populations were small, ranging from 0.056 to 0.177, with an average value of 0.110 (Table 4). A cluster analysis made it possible to separate quite distinctly the Abitibi old-growth forest populations from the Quebec second-growth forest ones, despite the high gene flow estimated (Figure 3). Thus, the Abitibi old-growth forest populations appear to make up a distinct gene pool from other Quebec populations sampled in second-growth forests. While the current results do not make it possible to know whether these differences have any effect on viability of individuals and evolutionary potential of the populations, we believe that they are significant enough to justify the protection of the Abitibi old-growth forest populations as reservoirs of genetic diversity for white spruce. Fortunately, the populations studied actually receive some protection as part of the Lake Duparquet Research and Teaching Forest. They are located in a conservation zone that will serve as a benchmark for monitoring management interventions in the Lake Duparquet Forest and elsewhere in the southern boreal forest (Harvey 1999). We suggest that official in-situ reserve status be given to these populations. We also suggest that considering that some inbreeding appears to be present in the young cohort populations, the mating systems of these populations be studied in order to evaluate the selfing rate. Moreover, a study of variation in adaptive traits of these old-growth forest population progenies should be initiated to better document their fitness and capacity to face major disturbances such as climate change.

Allele frequencies were found to be heterogeneous among populations at *Sb16* and *Sb58* (Table 3a, Fisher's exact test; p-value ≤0.01). Population differentiation (G_{ST}) estimates were close to zero whether data of the 16 populations were analyzed together or on a forest structure basis (Table 3). Such low population differentiation values are not unusual for white spruce populations. Indeed, previous studies have shown that most of the genetic variation was located within populations with reported G_{ST}-values ranging from 0.007 to 0.055 for various classes of genetic makers (see Alden and Loopstra 1987; Tremblay and Simon 1989; Furnier et al. 1991; Furnier and Stine 1995; Jaramillo-Correa et al. 2001; Beaulieu and Deslauriers 2002). Moreover, the average estimate of population differentiation reported here (G_{ST} = 0.009) was lower than the average value observed in gymnosperms, i.e. 6.8% (Hamrick and Godt 1990). ESTP markers have been shown to be essentially neutral in white spruce and populations in migration-drift equilibrium (Jaramillo-Correa et al. 2001). Even though the mature cohort populations of the Abitibi old-growth forests seem to have a genetic structure that is slightly different from that of other populations, it is unlikely that the latter is due to an adaptive response, considering the high gene flow occurring among populations, as is the case for most conifers (Govindaraju 1988). Indeed, the interpopulation gene flow, as estimated by average G_{ST} value (Crow and Aoki 1984) was very high, with the number of migrants per generation (Nm) being equal to 24.2.

Table 4. Estimates of genetic distances (arc distance, Cavalli-Sforza and Edwards 1967) based on data from 8 ESTP loci among 16 white spruce natural populations studied in Quebec.

Populations	Old-growth forest in Abitibi						Second-growth forest in Quebec									
	Mature cohort		Young cohort				Valcartier	Boyer township	Grindstone Lake	Ste. Marguerite	Beauceville	Ste. Emilie	Racine	St. Prime	Les Hauteurs	Ste. Cecile
	1760‡	1797	1760	1797	1916	1944										
Mature - 1760	-															
- 1797	0.087	-														
Young - 1760	0.099	0.107	-													
- 1797	0.086	0.072	0.068	-												
- 1916	0.093	0.082	0.067	0.056	-											
- 1944	0.068	0.069	0.066	0.058	0.052	-										
Valcartier	0.103	0.104	0.075	0.086	0.081	0.073	-									
Boyer Township	0.104	0.123	0.098	0.094	0.100	0.099	0.092	-								
Grindstone Lake	0.106	0.103	0.065	0.095	0.094	0.090	0.088	0.098	-							
Ste. Marguerite	0.084	0.097	0.091	0.095	0.095	0.082	0.082	0.079	0.088	-						
Beauceville	0.103	0.103	0.105	0.099	0.106	0.109	0.088	0.094	0.106	0.084	-					
Ste. Emilie	0.109	0.099	0.093	0.092	0.083	0.098	0.101	0.098	0.099	0.071	0.084	-				
Racine	0.114	0.106	0.072	0.079	0.077	0.083	0.104	0.094	0.095	0.088	0.116	0.089	-			
St. Prime	0.119	0.100	0.082	0.078	0.089	0.095	0.096	0.120	0.103	0.094	0.113	0.098	0.084	-		
Les Hauteurs	0.116	0.083	0.111	0.086	0.110	0.106	0.091	0.112	0.115	0.096	0.067	0.100	0.112	0.090	-	
Ste. Cecile	0.100	0.089	0.086	0.081	0.088	0.090	0.095	0.124	0.103	0.092	0.092	0.077	0.108	0.091	0.099	-

‡ Year of the last forest fire.

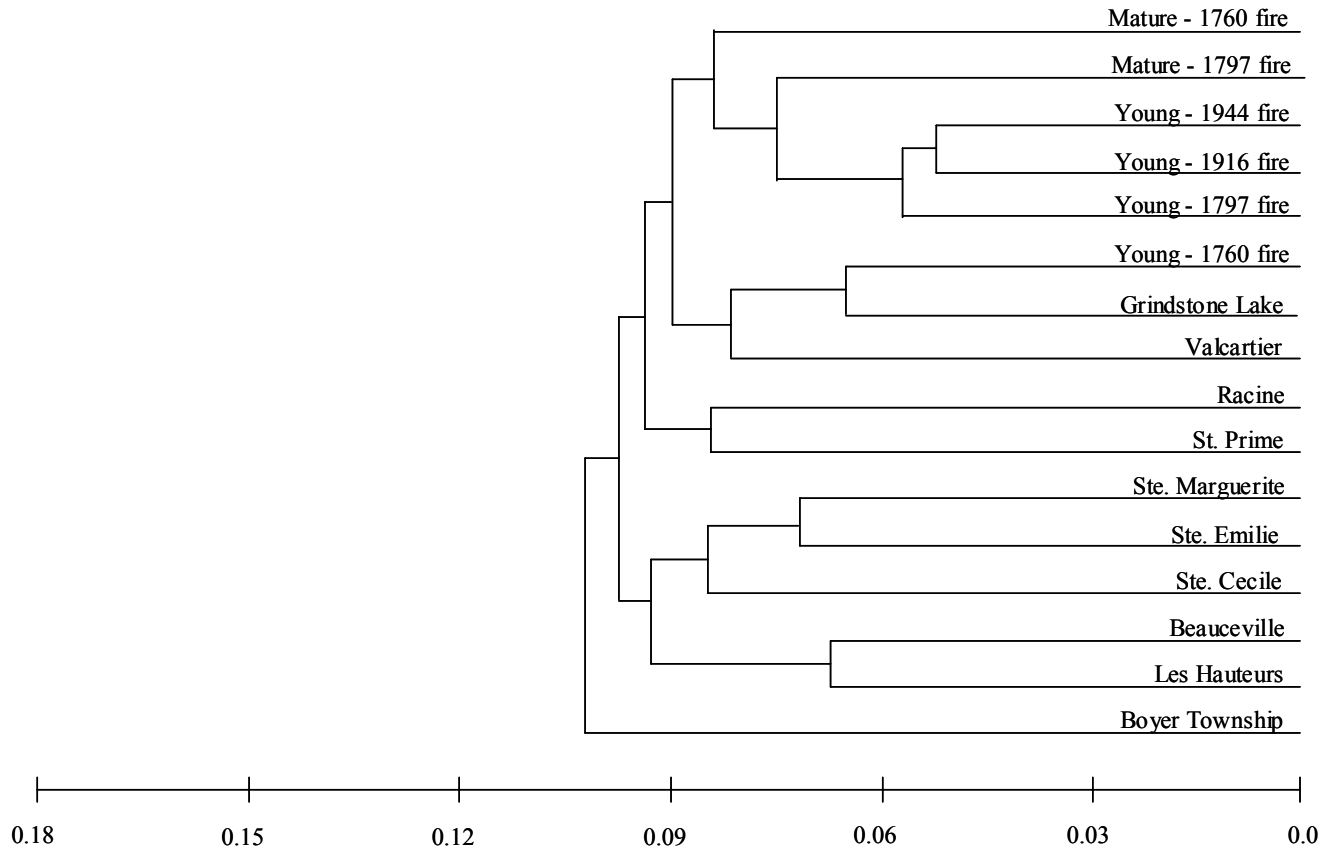

Figure 3. Unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis of genetic distances (arc distances, Cavalli-Sforza and Ewards 1967) between the 16 white spruce natural populations studied in Quebec.

CONCLUSION

In this study, we used eight nuclear DNA markers to study the genetic structure of 16 white spruce populations in Quebec. We found that all the alleles present in populations from second-growth forests were also present in Abitibi old-growth forest populations. Furthermore, a new variant in locus *Sb58* was discovered. We observed that the proportion of rare alleles (frequency lower than 5%) in old-growth forest populations was more than twice that in populations from the second-growth forests, i.e. 22.9% vs 10.8%. Mature-cohort populations from old-growth forests in Abitibi had a population structure that was remarkably different from those of young-cohort populations from the same region and of populations from second-growth forests in other regions. Indeed, contrary to what was observed in the last two groups of populations, there was an excess of heterozygotes as compared with the number expected under the HWE in both within and total populations. Despite the fact that all alleles found in the second-growth forest populations were also present in the Abitibi old-growth forest populations, a cluster analysis performed on the matrix of genetic distances between populations made it possible to show that populations from the Abitibi old-growth forests made up a quite distinct group from populations from Quebec second-growth forests. We recommend that populations from Abitibi old-growth forests be protected for conservation of white spruce genetic resources and further research be carried out to better characterize these populations.

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SILVICULTURAL MANAGEMENT AND THE MANIPULATION OF RARE ALLELES

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ABSTRACT

Because rare alleles provide a means for adaptation to environmental change they are often considered important to long-term forest health. Through the selective removal of trees (and genes), silvicultural management may alter the genetic structure of forests, with rare alleles perhaps being uniquely vulnerable to manipulation due to their low frequencies or associations between rare alleles and harvest selection criteria. We tested this possibility in two ways. First we evaluated the influence of long-term management on the genetics of an eastern hemlock (*Tsuga canadensis* (L.) Carr.) forest within the Penobscot Experimental Forest in Maine. Plots received one of three treatments: 1) a selection cut in which small and poor form trees were preferentially removed in 1957 and 1977, 2) a diameter limit cut in which the largest trees in each diameter class were removed in 1952, 1973 and 1994, and 3) an unmanaged control. Results from isozyme analysis indicated that the number of rare alleles decreased in the selection cut relative to the control. In contrast, rare alleles increased in frequency within diameter limit plots as poor quality trees were preferentially retained. As a separate test, we conducted simulated computer-based harvests within a genetically mapped forest that included isozyme data for 220 eastern white pines (*Pinus strobus* L.) growing in central Vermont. Most harvest scenarios had no discernable impact on stand genetics. However, due to an unequal distribution of rare alleles among tree age and crown classes, harvests that used these parameters as selection criteria altered the frequency of rare alleles relative to random harvests of equal intensity. Similar to the hemlock study, rare alleles either increased or decreased in frequency depending on the selection criteria used. The loss of rare alleles could diminish the potential of populations to successfully adapt to and survive ongoing environmental change, whereas an increase in rare alleles could reduce current stand fitness.

INTRODUCTION

Genetic diversity is essential to the long-term health and survival of biological populations because this diversity is critical for the physiological plasticity and adaptation needed to survive natural and man-made environmental change. This is particularly true for forest trees, which have limited mobility and are likely to encounter environmental change throughout their long life spans. Considering the fundamental importance of genetic diversity to the continued adaptation, health, and long-term productivity of tree populations, a loss of genetic diversity (whatever the cause) can be a serious threat to forest ecosystems and the many products and services they provide. This generalized threat is particularly serious today as a result of escalating human activity which has not only initiated pervasive and sometimes dramatic changes in the earth's environment but has also led to measurable declines in the genetic diversity of at least some tree populations. Although many human influences may work to reduce the genetic diversity within forests stands (e.g., land conversion and forest fragmentation, pollution exposure, climate change, etc.), another factor that could alter the gene pools of woodland ecosystems is intensive forest management.

Forest management represents an anthropogenic force in which sometimes large numbers of trees (and the genes they contain) can be removed from a natural system within a relatively short period of

time. Although probably not consequential to species that vegetatively reproduce, such removals could be important to species reliant upon sexual reproduction. Certainly, tree harvests are critical to supply human resource needs. Indeed, an important element of silvicultural selection is the belief that through the prudent removal of select trees, favorable commercial attributes (e.g., fast growth, good form, etc.) can be fostered in both residual trees (through an improvement of growing conditions) and their progeny (through a manipulation of the gene pool). Still, the long-term impact of tree removal on the genetic base and ecological resiliency of forests is largely unknown.

Can timber harvesting alter forest gene pools and, as a result, the long-term health and productivity of forest communities? Do various types or intensities of harvest differentially impact the gene pool? How might these potential impacts differ among species? The few studies that have examined these questions have provided a mixed indication of the influence of timber harvesting on genetic resources. For example, Neale (1985) and Neale and Adams (1985) found that shelterwood harvesting had little impact on the genetic structure of Douglas-fir (*Pseudotsuga menziesii* Carr.) forests. However, Cheliak et al. (1988) reported that phenotypically selected white spruce (*Picea glauca* (Moench) Voss) possessed on average only 75% of the genes found among randomly selected trees from the same population, indicating that phenotypic selection resulted in gene loss. Indeed, recent data indicates that rare alleles may be particularly prone to loss following silvicultural selection (Buchert et al. 1997; Adams et al. 1998).

We took two approaches toward evaluating the influence of silvicultural selection on the presence and frequency of rare alleles within forest stands. First we assessed the effect of long-term management on the genetics of an eastern hemlock (*Tsuga canadensis* (L.) Carr.) forest within the Penobscot Experimental Forest in Maine. As an additional test, we conducted simulated computer-based harvests within a genetically mapped forest that included isozyme data for 220 eastern white pines (*Pinus strobus* L.) growing in central Vermont. Here we discuss the results of these studies and highlight potential implications of results for short- and long-term forest management and health.

METHODS

Sites

Assessments of the genetics of eastern hemlock were conducted in a long-term silvicultural experiment at the Penobscot Experimental Forest in east-central Maine, USA. This experiment was established in 1952 and included a no-harvest control and two silvicultural treatments: 1) a fixed diameter limit treatment, and 2) a selection treatment. The fixed diameter limit treatment involved a ten-hectare stand that had three harvests: June 1952, May 1973, and January 1994. At each harvest, all merchantable hemlocks with DBH $\geq$ 24 cm were cut, resulting in approximately one-fifth to one-half of all hemlock trees being removed per harvest. The selection treatment was conducted on an eight-hectare stand that had two harvests: May 1957 and January 1977. Harvesting criteria were effectively opposite those for the diameter limit cut: per diameter class, phenotypically superior trees were retained, while smaller, unmerchantable, and poor-risk trees were preferentially removed. Nearly one-third of the eastern hemlock trees of poor merchantability were removed in 1957 and an additional 75% were removed in 1977.

For the simulated harvesting study, the study site was an approximately ten-hectare stand in Jericho, Vermont, USA, dominated by mature eastern white pine. The genetically mapped stand was constructed by first gathering inventory data, including size, age, growth, vigor, crown class, and spatial location for all 232 eastern white pine trees in the forest. These data were combined with genetic data determined from starch gel electrophoresis in a GIS framework to produce a virtual reconstruction of the actual stand's spatial, physical, and genetic components (Nijensohn et al. in review).

Genetic assessments

Eastern hemlock: Seeds were collected in the fall of 1994 from 40 to 50 randomly selected residual eastern hemlock trees from each treatment stand. Starch-gel electrophoresis of megagametophyte tissue was used to assess the genetics of individual trees. A minimum of seven haploid megagametophytes from each tree were used in genetic analyses. A total of 106 trees were examined at 27 gene loci representing 19 enzyme systems. Electrophoretic gel buffers and enzyme recipes were prepared according to procedures described by Jech and Wheeler (1984) and Cheliak and Pitel (1984), including appropriate modifications made in our laboratory. Seeds from individuals of known genotype that have been shown to be highly homozygous were included as standards in each electrophoretic run.

Eastern white pine: Cones were collected from individual trees in the fall of 2000, when almost all trees (220 of 232 trees) produced seeds, allowing for a nearly complete assay of stand genetics. Using the methods described above, starch gel electrophoresis was used to resolve 46 loci (26 of which exhibited polymorphisms) from 19 enzyme systems.

Statistical analyses

Multi-locus genotypes were determined for each tree. Although numerous genetic parameters were assessed, the focus of this manuscript is the effect of actual or simulated harvests on the presence of rare (frequency $P < 0.05$) alleles. Uncommon (frequency $0.25 > P > 0.05$) alleles were also assessed for the study involving eastern hemlock. Here, the lack of replication limited data analysis to assessments of trends among treatment means. For the simulated harvest of eastern white pine, analyses of variance and Tukey-Kramer HSDs were used to test for significant differences in the number of rare alleles among age and crown classes. Harvesting scenarios were developed using phenotypic characteristics (e.g., tree age, DBH, and crown class) as silvicultural selection criteria at various harvesting intensities (e.g., simulating the removal of different proportions of the original stand). Monte Carlo simulations were used to compare the genetics of residual stands following harvest simulations to repeated random harvests of equal intensities (1000 resampling iterations) (Simon 2002).

RESULTS

Hemlock silvicultural trial

Compared to the control stand, the repeated removal of small, poor-formed trees in the selection cut was associated with fewer rare alleles, five alleles no longer detected, and a reduction in the estimated number of the genotypes that could arise from the stand given random mating (i.e., genetic multiplicity; Bergman et al. 1990).

In the diameter limit cut, an apparent loss of rare alleles actually resulted from an increase in the frequency of some alleles that were rare (occurred in < 5% of individuals) in the control, but now occurred at a higher frequency in the treated stand (i.e., were now low frequency alleles that occurred in up to 25% of individuals; Table 2). Even though a fewer total number of alleles were considered rare, the percentage of trees with rare alleles was about double for the diameter limit cut relative to the control. Indeed, compared to the control stand where no trees were homozygous for rare alleles, a remarkable 42% of trees in the diameter limit were homozygous for some rare allele. The concentration of unusual gene forms (rare or low frequency alleles, often in a homozygous form) resulted in a ten-fold increase in the estimated number of genotypes potentially generated by the diameter limit stand compared to the control.

Table 1. The influence of silvicultural selection on the presence and frequency of rare alleles in mature eastern hemlock stands in eastern Maine. Data based on Hawley et al. (in review).

Stand parameter	Treatments		
	Control	Diameter limit	Selection
# sample trees	35	31	40
# rare alleles	9	5	4
# low frequency alleles	2	8	4
% trees with rare alleles	29	68	34
% trees homozygous for rare alleles	0	42	5
Lost alleles relative to control	---	2	5
Genetic multiplicity (millions)	1.26	11.34	0.1

Genetically mapped eastern white pine stand

An important objective in creating this genetically mapped white pine stand was to assess patterns of subpopulation structuring. Indeed, multiple levels of structuring were detected. For example, spatial structuring was evident: trees within 5 m of one another were highly related, and levels of relatedness generally decreased with increasing distance between trees (Nijensohn et al. in review). Temporal structuring (a generation gap) was also evident: trees of similar age showed significant positive relatedness, as did trees 30-40 years apart (Nijensohn et al. in review). In addition, trees in suppressed crown classes exhibited a high concentration of rare alleles, a trend also evident for older age classes.

To test the potential influence of tree removal on stand genetics, a series of computer-based simulated harvests were conducted on this genetically mapped stand. Most harvest scenarios did not significantly alter genetic parameters relative to random harvests of equal intensity. However, due to their association with rare allele frequency, harvests that targeted tree age and crown classes were an exception to this trend (Table 2). For example, when 50% of the stand's oldest trees were preferentially removed, there was a significant reduction in the number of rare alleles relative to random harvests of equal intensity. If wolf trees (large, knot-ridden trees usually avoided in commercial harvests) were excluded from harvest, a significant reduction in the number of rare alleles occurred at even lower harvest intensities (e.g., removal of 43% of the oldest trees). In contrast, selective retention of suppressed trees had the opposite effect, i.e. a significant increase in the number of rare alleles relative to random harvests of equal intensity (Table 2). Similar to results from actual harvests in the eastern hemlock stands (Table 1), results from simulated harvests indicate that silvicultural selection can alter the frequency of rare alleles within forests, and that depending on the criteria used, tree removals can either increase or decrease the presence of rare alleles within a stand.

DISCUSSION

Our results are consistent with recent findings showing that the presence and frequency of rare alleles can be altered by silvicultural selection (Buchert et al. 1997; Adams et al. 1998). Because they already occur at low frequencies, rare alleles are particularly vulnerable to loss. But what are the implications of these alterations to forest productivity and health?

It is possible that rare alleles may represent localized adaptations beneficial to specific locations in space and time. However, evidence suggests that rare alleles are uncommon at the population level for a reason: they have been selected against because they impart some generalized competitive disadvantage. Numerous studies have shown that rare alleles, in both heterozygous and homozygous forms, are associated with weaker phenotypes (e.g., Bergman and Sholz 1987; Cheliak et al. 1988; Bush and Smouse 1991; Adams et al. 1998). As a recent example of this, Zhong et al. (2001) found that Douglas-firs that were susceptible to defoliation by the western spruce budworm (*Choristoneura occidentalis* Freeman) had a higher proportion of uncommon and rare alleles than resistant trees. It was postulated that these alleles remained rare because they were associated with reduced fitness (greater defoliation) (Zhong et al. 2001). In another study, Bongarten et al. (1985) reported a negative correlation between growth rate and heterozygosity for rare alleles.

Table 2. Changes in the number of rare alleles following simulated harvests in the genetically mapped eastern white pine forest in Jericho, Vermont. Monte Carlo simulations were used to detect significant changes (* denotes $0.05 \leq P \leq 0.10$; **denotes $P \leq 0.05$) in the number of rare alleles compared to 1000 random harvests of equal intensity. Data based on Nijensohn et al. (in review).

Trees removed in simulated harvest	Residual n	Mean # rare alleles per tree	Significant Δ rare alleles
oldest 25%	165	0.99	
oldest 50%	110	0.81	decrease*
oldest 75%	55	0.80	
youngest 25%	165	1.10	
youngest 50%	110	1.14	
youngest 75%	55	0.93	
oldest but leave:			
18% wolfs	181	0.96	
43% wolfs	126	0.79	decrease**
68% wolfs	71	0.76	decrease*
understory (U)	218	0.97	
all but Dominant (D)	62	0.82	
all but Codominant (C)	115	0.94	
all but Intermediate (I)	36	1.17	
all but Suppressed (S)	5	1.80	increase*
D & C	43	1.28	
D, C & I	7	1.86	increase*
all but D & C	177	0.90	
Original population	220	0.97	

Because of an apparent association between rare alleles and the negative phenotypes (small size, poor form, etc.) preferentially retained, the diameter limit treatment resulted in a residual stand in which rare alleles become unusually common (Table 1). By repeatedly removing larger competitors, gene forms that were rare (presumably because they imparted reduced fitness) following natural selection in the control plot were allowed to accumulate in the diameter limit stand. This concentration of marginally

fit individuals was associated with reduced woody biomass accumulation relative to other treatments (Hawley et al. in review), which may reflect a reduction in stand fitness under prevailing conditions.

Although they likely impart a fitness disadvantage under current conditions, rare alleles are also assumed to be an important reservoir of adaptive potential for populations. Rare alleles have been important to the survival of a range of biological populations due to the development of resistance following exposure to a variety of selective forces (Table 3). The development of antibiotic, insecticide, and herbicide resistance is a headline-grabbing example of this. Yet, evidence of the importance of rare alleles for the long-term survival of forest trees presented with novel selection pressures also exists. Dutch elm disease (caused by the fungus *Ophiostoma ulmi* (Buisman) Nannfeldt) provides a prominent case in point. Through one outbreak in North America and two in Europe, the majority of elms (e.g., *Ulmus americana* L. and *Ulmus glabra* Huds.) were killed within only a few decades following the accidental introduction of this pathogen native to Asia (Tainter and Baker 1996). Similarly, chestnut blight (caused by the fungus *Cryphonectria parasitica* (Murrill) Barr.) virtually eliminated the American chestnut (*Castanea dentate* (Marsh.) Borkh.) throughout its range within 50 years (Tainter and Baker 1996). Even though disease-induced population (and presumably gene) losses were extreme, rare alleles may yet form the basis of species survival. Rare genotypes resistant to both chestnut blight and Dutch elm disease have been identified and are considered critical to species recovery efforts (Cheng et al. 1997; Griffin 2000).

Examples of dramatic population declines following exotic disease or other novel selection pressures are not merely historic curiosities. The number, intensity, and frequency of stresses (e.g., exotic pests and pathogens, climate change, pollutant exposures, etc.) that tree populations are subjected to seem only to be escalating. Considering the growing burden of anthropogenic stresses that individually or in combination may tax the physiological limits of individual trees, the rich genetic base needed to support population adaptation and survival may be more important now than ever.

Table 3. Examples of organisms for which rare alleles provided the basis for stress resistance that supported population survival despite strong selection pressure.

Organism	Novel selective force	Reference
Bacteria	Antibiotics	Abramson and Sexton (1999)
Insects	Pesticides	Georghiou (1991)
Insects	*Bacillus thuringiensis* toxin in transgenic crops	McGaughey and Whalon (1992)
Herbaceous plants	Herbicides	Heap (1997)
Populus tremuloides Michx.	Air pollution	Berrang et al. (1989)
Ulmus americana L.	Invasive pathogen	Cheng et al. (1997)

Given the likely importance of rare alleles to population adaptive potential, what could mean the legacies of the harvest-induced changes in rare allele frequency we found? The decline in rare alleles (i.e. in the hemlock selection cut for hemlock and preferential removal of the oldest white pine via simulated harvests) could reduce the genetic diversity needed by populations to successfully adapt to and survive ongoing environmental change. Conversely, for the hemlock diameter limit cut and when suppressed trees were preferentially retained in simulated harvests of white pine, residual stands

showed an increase in the frequency of (previously) rare alleles. Although the preservation of rare alleles is of theoretical adaptive benefit, it is unknown whether this hyper-abundance of typically rare alleles imparts any advantage beyond that afforded by the continued presence, but lower frequency, of these same alleles.

Although natural selection tends to remove phenotypes that are generally inferior, the interplay of environmental heterogeneity and stochastic events also allows some gene forms to persist at low frequencies despite apparent fitness costs. Thus, perhaps because it is variable and "incomplete", natural selection preserves rare alleles at levels that balance stand fitness under current environmental conditions with long-term benefits for potential adaptation when conditions ultimately change.

Our data from both actual silvicultural treatments and computer-simulated harvests, as well as studies with different coniferous species (e.g., Buchert et al. 1997; Adams et al. 1998), indicate that silvicultural selection can lead to shifts in the frequencies of rare alleles. But is this potential for alteration meaningful? One factor that could have a substantial influence on the importance of silvicultural manipulations of allele frequencies is the extent to which gene migration ameliorates harvest-induced alterations. In particular, seed and pollen migration from surrounding stands could offset allelic losses provided transport distances were not too great. Although gene flow for species such as eastern white pine are reported to be very high (Beaulieu and Simon 1994), evidence for the same species from our genetically mapped forest indicates that the average distance of genetic influence can actually be quite short, i.e. about 35 m (Nijensohn et al. in review). Furthermore, patterns of species distribution, land use and forest fragmentation, as well as the scale of silvicultural application (e.g., stand vs. forest vs. region), would all likely influence the functional limits of gene migration.

In addition to uncertainties about the influence of gene migration, many basic questions exist concerning the influence of silvicultural treatments on genetic resources. For example, how much can managers increase or decrease the frequency of traditionally rare alleles before the balance between short-term (productivity and fitness) and long-term (enhanced resilience to environmental change) benefits is compromised? Which silvicultural practices most alter genetic reserves? Are some species more vulnerable to genetic manipulation than others? What is the combined effect of management with other anthropogenic factors (e.g., pollution-induced mortality, population losses following exotic pest and pathogen introductions, shifts resulting from climate change, etc.) that may also reshape forest gene pools? These and other questions will need to be addressed to fully evaluate the true vulnerability of forest stands to genetic alteration following silvicultural treatment.

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IMPACTS OF ALTERNATIVE SILVICULTURE SYSTEMS ON MATING SYSTEMS AND GENETIC DIVERSITY OF FOREST TREE SPECIES

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ABSTRACT

Many disparate studies have examined the effects of various management practices on the genetic diversity and mating systems of forest tree populations. The genetic impacts of: a) harvest methods (clear-cutting, shelterwood, patch cutting, seed tree retention), b) stand tending (thinning), and c) regeneration methods (natural vs. artificial) are reviewed and compared. The genetic status of tree populations after silvicultural intervention are compared with unharvested or natural populations. Increasing public concern over clear-cutting initiated most studies, which elucidate the effects of alternative practices simulating natural disturbance regimes. Harvesting and regeneration methods alter current and future stand density, potentially affecting the mating system dynamics of remaining trees. This impacts the genetic constitution of extant and future stands. This review revealed no consistent genetic trends for number of alleles per locus, percentage of polymorphic loci, observed and expected heterozygosity, outcrossing rates, correlation of paternity or F_{IS} following management. Managers must consider each ecosystem type and species individually when developing harvesting and regeneration prescriptions that best approach natural ecosystem processes to optimize genetic gain and diversity as well as resource value. Forest managers must fully understand patterns of reproduction, growth, adaptive potential and plasticity for species under their care to ensure best practices.

Key words: mating system, genetic diversity, silviculture, seed orchard, phenotypic selection, sustainability

INTRODUCTION

Increasing public pressure for multiple uses on forest land has led to a new forest management paradigm (Arnott and Beese 1997; Anonymous 2003; NRTEE 2003). Across Canada, 94% of the land is under provincial (71%) or federal (23%) jurisdiction, of which 117 million ha is operable forest land (FPAC 2003). Forested land is leased for various terms to forest companies, woodlot operators and small businesses (Anonymous 2002; FPAC 2003). Public ownership has led to more input from citizens and interest groups to provide direction regarding priorities, values and appearances for the land over the long term (NRTEE 2003). There is therefore much debate in policy, scientific and public circles around resource sustainability; three Canadian (CSA, FSC, SFI) and one international (ISO) certification agency are currently evaluating and monitoring forestry practices (Anonymous 2002; CSFCC 2002; Anonymous 2003). The widespread move embracing certification implicitly acknowledges the effect of consumer preference on forest management: third-party certification obviates consumer pressure by verifying products that originate from sustainably managed forests.

One interpretation of sustainability is to encourage, or even legislate, forest management practices that imitate natural processes and scales, and which would enable indefinite succession and renewal of resources across the landscape (Anonymous 2002). This perspective has resulted in a huge

increase over the past two decades in the implementation and study of 'alternative silviculture systems,' or 'alternative harvesting methods,' meaning, in this context, harvesting and regeneration methods excluding traditional large-scale, block clear-cuts where virtually all trees within the cutblock were removed (Smith et al. 1996; Sullivan et al. 2001).

One-size-fits-all forestry, although often the least expensive short-term option, may be impractical, aesthetically unattractive, or unsustainable for many species or ecosystem types (Arnott and Beese 1997; Hoberg 2002). Silvicultural systems such as shelterwood, seed-tree, smaller patch cuts, and retention of various stand-level features such as wildlife trees or patches, have become the recommended options for many ecosystems and species (e.g. Anonymous 1995; Rajora et al. 2000; Sullivan et al. 2001). Some harvested areas are reforested with seedlings collected from phenotypically or genotypically selected production populations (seed orchards), while others are left to naturally regenerate via seeds from the parent stand. Ecologists and foresters have noted many qualitative effects of these alternative silvicultural systems, but it is only recently that researchers have begun collecting quantitative data on their genetic impacts (Savolainen and Kärkkäinen 1992). This review synthesizes the effects of various silvicultural systems on mating systems and genetic diversity of various species relative to their natural (i.e. unmanaged) states.

All harvesting methods impact the density and spatial distribution of parent trees. This may alter the distribution and frequency of alleles within the population due to genetic drift: Gillies et al. (1999) found that harvested *Swietenia macrophylla* King populations had significantly less genetic variability than unharvested ones. The mating system of the current and future stand may thus be altered if leave trees are more or less closely related than parents in the unharvested stand. If inbreeding increases, this could result in further allele frequency changes as well as enhanced family structure within populations, which may in turn perpetuate higher levels of inbreeding in future generations. Detrimental effects caused by drift or dysgenic side-effects from phenotypic selection of leave trees can be ameliorated by artificial regeneration. Seedlings produced in seed orchards or seed collected from a variety of nearby stands tend to have higher genetic diversity of common alleles (frequency > 0.05) than natural regeneration from a harvested stand, and can increase stand-level outcrossing rates as they mature (El-Kassaby and Ritland 1996; El-Kassaby 2000).

Mating systems

Families and genera have characteristic mating system dynamics (Hamrick et al. 1992; Mitton 1992). Most temperate forest trees have mixed mating systems dominated by outcrossing, but some mating among relatives, or even selfing, are frequently tolerated (e.g. Yazdani et al. 1985; Mitton 1992). Parameters such as the percentage of selfing or the correlation of paternity may vary from year to year (El-Kassaby et al. 1993) with crown position (El-Kassaby et al. 1986), male and female fertility, weather, latitude, or density (Mitton 1992). This inherent flexibility provides an adaptive buffer since environmental and demographic conditions fluctuate over a tree's lifespan. The mating system of a species is strongly influenced by genetic factors, and selfing capacity is a heritable trait which can be partially gauged by the correlation of outcrossing rates between parents and offspring (Ritland 1990).

Many tree species have evolved mechanisms to reduce selfing: phenological cycles temporally stagger male and female fecundity within the same tree (e.g. *Alnus rubra* Bong., Briggs et al. 1978; *Tsuga heterophylla* (Raf.) Sarg., Edwards 1976). Male and female reproductive organs can also be spatially isolated within the same tree: e.g. *Abies* spp. (Owens and Molder 1977) have male strobili on bottom branches and female cones at the top of the crown. *Taxus* spp. (Bolsinger and Jaramillo 1990) and *Salix* spp. (Pitcher and Knight 1990) are dioecious. Some species use a combination of these mechanisms, such as partial physical separation combined with phenological differentiation (e.g. *Pseudotsuga menziesii* (Mirb.) Franco, Hermann and Lavender 1990; *Pinus contorta* Dougl. ex Loud.,

Lotan and Critchfield 1990). Seed orchards provide a controlled environment where physiological cues can be manipulated to alter phenological cycles to maximize the number of (non-self) pollen donors.

There are some exceptions: *Pinus resinosa* Ait. and *Thuja plicata* Donn ex D. Don, due to severe and repeated range-wide bottlenecks during glaciation, have evolved through frequent selfing and consanguineous mating, and have very low genetic diversity compared to most other long-lived woody perennials (Hamrick et al. 1992; Echt et al. 1999; O'Connell 2003). Populations of red cedar are highly homogeneous, and the genetic consequences of density reduction via harvesting are negligible (El-Kassaby et al. 1993). The best silvicultural system for these species would depend more on ecological factors, such as seed characteristics, and factors that affect seed germination ecology (e.g. frost pockets), than on the resulting stand-level genetic diversity.

While a species' inherent tolerance for selfing influences paternity, density may also have a role. Studies have shown that when provided with a mixture containing selfed, related, and unrelated pollen, most forest species will have the highest proportion of viable seed sired by unrelated, or outcrossed, pollen (Mitton 1992; Savolainen and Kärkkäinen 1992). Some species, for example *Tsuga heterophylla*, have developed pollen competition mechanisms during fertilization (Colangeli and Owens 1989), enabling outcrossed pollen to out-compete self pollen prior to fertilization. Other species such as *Abies* spp. and *Pinus sylvestris* L. feature post-fertilization mechanisms whereby inbred progeny fail to germinate, or grow poorly during the seedling and juvenile stages, often due to a buildup of homozygous recessive deleterious alleles or genetic load (Owens and Molder 1977; Savolainen and Kärkkäinen 1992). These alleles need not be immediately lethal: their combined impact on fitness is quantified as "lethal equivalents" (Falconer and Mackay 1996). Fixation indices, or proportional heterozygote deficiency, calculated for primarily outcrossing long-lived perennial species tend to decrease with age, approaching Hardy-Weinberg equilibrium at successional maturity (e.g. Tigerstedt et al. 1982; Yazdani et al. 1985; Mitton 1992; Macdonald et al. 2001).

Farris and Mitton (1984) found that selfing increased with lower densities in *Pinus ponderosa* Dougl. ex Laws. *Pinus caribaea* Morelet var. *caribaea* revealed the opposite trend: when density was reduced by thinning or harvesting, the multilocus outcrossing rate decreased from complete outcrossing to accommodate some selfing (Zheng and Ennos 1997; Table 1). El-Kassaby and Jaquish (1996) also found variability in the mating system of *Larix occidentalis* Nutt. associated with density in unharvested and seed-tree populations, but correlations were inconsistent, possibly obscured by natural variation in outcrossing rates. They did find significantly higher proportions of full-sib progeny with higher densities (Table 1). *Pseudotsuga menziesii* outcrossing rates did not significantly differ between old-growth and shelterwood harvested stands (20 to 30 stems·ha^{-1} retained), all of which were nearly completely outcrossing regardless of density (Neale 1985; Neale and Adams 1985; Table 1).

Clear-cut, advance regenerated stands of *Picea mariana* (Mill.) B.S.P. were compared to natural stands originating after wildfires (Perry and Bousquet 2001). Both stand types revealed similarly high outcrossing rates. Differences among the correlation of paternity (r_p) were not statistically significant, but were slightly higher in the natural (17%) than in clear-cut (13%) stands, suggesting a slight decrease in mating among relatives following typical stand management (Table 1).

El-Kassaby et al. (2003) compared old-growth and stands subject to clear-cutting, seed-tree retention (15 to 25 stems·ha^{-1}), shelterwood (30% basal area removal), and patch cutting (50% basal area removal). The correlation of paternity in *Abies amabilis* (Hook.) Nutt. decreased with lower density since the species does not screen out selfed pollen, and related pollen was able to compete more successfully for receptive ovules (Table 1). Outcrossing rates reflected some biparental inbreeding, but were only marginally higher in the patch cut than in the other treatments (Table 1). Outcrossing rates of *Tsuga heterophylla* in the same study did not significantly differ among treatments (El-Kassaby et al. 2003; Table 1). Full-sib progeny were found in the seed-tree and shelterwood but not in unharvested

and patch cut stands, indicating an increase in consanguineous mating with decreased density. The differences were attributed to pre-fertilization pollen competition excluding selfed pollen in *T. heterophylla*, unlike *A. amabilis*.

Table 1. Summary of management impacts on forest tree species mating system parameters.

Species	Treatment	[1]t_m	[2]F_{IS}	[3]r_p	Marker	Reference
Abies amabilis	Natural	0.801		0.165	Isozymes	El-Kassaby et al. 2003
	Seed tree	0.798		0.105		
	Shelterwood	0.796		0.140		
	Patch cut	0.837		0.123		
Eucalyptus siebera	Fire-origin mature*		0.005		CDNA RFLP	Glaubitz et al. 2003
	Clearcut*		0.019		& microsatellites	
	Seed tree + burnt*		-0.014			
	Seed tree + site prep*		0.016			
Fagus crenata	Unmanaged		0.042		Isozymes	Takahashi et al. 2000
	Seed tree regeneration		0.055			
Larix occidentalis	Unmanaged*	0.848	-0.023	0.083	Isozymes	El-Kassaby and Jacquish 1996
	Seed tree*	0.894	-0.137	0.011		
Picea mariana	Fire-origin	0.989	0.04	0.167	Sequence-Tagged	Perry and Bousquet 2001
	Clearcut-origin	0.993	0.02	0.132	Sites (STS)	
Pinus caribaea var. *caribaea*	Unmanaged	0.984			Isozymes	Zheng and Ennos 1997
	Seed tree	1.009				
	Clearcut	0.894				
	Seed orchard	0.985				
Pinus contorta var. *latifolia*	Unmanaged		0.047		Isozymes	Macdonald et al. 2001
	Post-harvest		0.037			
Pinus echinata	Unmanaged*		0.059		Isozymes	Rajora et al. 1998
	Seed tree*		0.100			
	Artificial regeneration		-0.133			
Pseudotsuga menziesii var. *menziesii*	Unmanaged		0.007		Isozymes	Adams et al. 1998
	Group selection		0.000			
	Shelterwood		0.011			
	Natural regeneration		0.004			
	Planted regeneration		0.008			
Pseudotsuga menziesii var. *menziesii*	Unmanaged*	0.98			Isozymes	Neale and Adams 1985
	Shelterwood*	0.95				
Tsuga heterophylla	Unmanaged	0.946		-0.062	Isozymes	El-Kassaby et al. 2003
	Seed tree	0.922		0.043		
	Shelterwood	0.921		0.098		
	Patch cut	0.925		0.053		

[1]t_m is the multilocus outcrossing rate; [2]F_{IS} is the coefficient of co-ancestry, or degree of inbreeding; [3]r_p is the correlation between the genotypes of parent trees and their progeny; *Arithmetic mean of >1 replicate, where replicates were separately reported in original study.

Genetic diversity

Various markers have been used to quantify genetic diversity in forest trees: from phenotypic data (e.g. height, phenology, foliage colour) to genotypic data (e.g. isozymes, RAPDs, AFLPs, microsatellites (SSRs)). For the purposes of quantitative comparison, this review will focus on genotypic data obtained using molecular markers. Each marker has unique resolution, assets and drawbacks, thus they have complementary applications.

The mating system of a species is intimately related to its population genetic structure and diversity. Long-lived species such as most forest trees, especially wind-pollinated conifers, tend to grow in genetic neighbourhoods, where proximity is correlated with relatedness (Tigerstedt et al. 1982). This results from seed and/or pollen dispersal physics, where more seed and pollen fall near the maternal tree. Seeds or seedlings often have more rare alleles than their parents, expressing low-frequency deleterious alleles which eventually impede their survival or reproduction (Marshall and Brown 1975; Yazdani et al. 1985; Cheliak et al. 1988). Seedling populations also typically have more homozygotes than the parental population, gradually shifting to heterozygote excess in mature stands (Tigerstedt et al. 1982; Yazdani et al. 1985). This is reflected in genetic diversity statistics by increased heterozygosity and decreased number of alleles per locus.

A. Harvesting

Fire origin and harvest origin *Picea mariana* stands were compared using codominant sequence-tagged-site (STS) markers (Perry and Bousquet 2001). Allelic diversity and richness were similar for both stand origin types, negating concerns around stand management practices that encourage asexual reproduction in this species by layering (Table 2). Rajora and Pluhar (2003) confirmed these results using other DNA-based markers (RFLPs and SSRs), finding no differences between pre- and post-harvest stand genetic diversity and richness across the landscape (Table 2). *Fagus crenata* Blume also showed no genetic diversity or allelic richness differences in unharvested vs. second-growth (80-year-old) stands, although harvesting significantly increased population genetic structure and linkage disequilibrium by limiting the number and distribution of potential parents (Takahashi et al. 2000; Table 2). It was suggested that this founder effect may disappear over several generations of random mating. Parker et al. (2001) also noted an increase in population genetic structure of *Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg. with decreased parent tree density in the *clausa* variety, but not in the *immuginata* variety; differential responses were attributed primarily to the disturbance history and consequent stand demographics.

Parents and advance regeneration were compared among unharvested, clear-cut, patchcut, shelterwood and seed-tree silvicultural systems in mixed stands of *Abies amabilis* and *Tsuga heterophylla* (El-Kassaby et al. 2003). While a few low-frequency alleles were lost in the parental populations following harvest in both species, both generations had similar numbers of low-frequency ($p \leq 0.05$) alleles (Table 2). Parent-offspring comparisons revealed no significant differences in *A. amabilis* genetic diversity or richness. *Tsuga heterophylla* offspring had significantly higher expected and observed heterozygosity than parent trees in patchcuts, and higher expected heterozygosity than parents after seed-tree harvesting. Overall, *A. amabilis* showed no significant differences in genetic parameters from any silvicultural treatment (Table 2). Shelterwood harvesting resulted in lower genetic diversity in *T. heterophylla* than in the other treatments, despite having the most abundant advance regeneration, least residual logging damage and highest seedfall (Arnott and Beese 1997). It was suggested that artificial regeneration from a variety of local populations may augment the gene pool and offset genetic drift due to harvesting (El-Kassaby et al. 2003).

Glaubitz et al. (2003) similarly found no significant differences between unharvested, clear-cut, fire-origin seed tree and harvest-origin seed tree in *Eucalyptus sieberi* stands (Table 2). The species' genetic robustness to management was attributed to its distribution, mating system and life history

traits. *Pseudotsuga menziesii* exhibited no significant genetic differences between parent trees following clear-cuts, patch cuts (0.2 ha) and shelterwood harvest (leaving 15-30 stems·ha^{-1}) (Adams et al. 1998; Table 2). Several low-frequency alleles were lost after shelterwood treatment in both the parents and offspring. It was surmised these effects could be easily offset by retaining stems of all size classes, or by leaving more mature trees. Neale (1985) also found no systematic allele frequency distribution differences between shelterwood and natural stands of *P. menziesii*, or among parents, embryos and seedlings (Table 2).

Table 2. Summary of management impacts on forest tree genetic diversity parameters.

Species	Treatment	[1]H_o	[2]H_e	[3]A	[4]P	Marker	Reference
Abies amabilis	Unmanaged	0.079	0.103	1.80	50.0	Isozymes	El-Kassaby et al. 2003
	Patch cut	0.062	0.082	1.53	40.9		
	Shelterwood	0.069	0.081	1.63	45.5		
	Seed tree	0.078	0.084	1.57	39.4		
Eucalyptus sieberi	Fire-origin mature*		0.507	7.96		cDNA RFLP & SSRs	Glaubitz et al. 2003
	Clearcut*		0.501	8.60			
	Seed tree + burnt*		0.492	7.67			
	Seed tree + site prep*		0.484	7.72			
Fagus crenata	Unmanaged	0.193	0.203	3.3	78.0	Isozymes	Takahashi et al. 2000
	Seed tree	0.189	0.200	3.3	78.0		
Picea glauca	Unmanaged		0.381	1.89	88.7	RAPDs	Rajora 1999
	Natural regeneration		0.389	1.84	83.8		
	Artificial regeneration		0.297	1.72	72.7		
	Phenotypic selections		0.259	1.67	66.7		
Picea mariana	Fire-origin	0.25	0.26	2.75		STS	Perry and Bousquet 2001
	Harvest-origin	0.25	0.26	2.76			
Picea mariana	Fire-origin mature	0.220	0.307	2.54	75.8	Isoyzmes	Rajora and Pluhar 2003
	Fire-origin natural regeneration	0.200	0.290	2.45	75.0		
	Post-harvest natural regeneration	0.237	0.320	2.56	77.3		
	Post-harvest artificial regeneration	0.230	0.315	2.51	72.7		
Picea sitchensis	Unmanaged		0.183	1.8	66.9	Isozymes	Chaisurisri and El-Kassaby 1993
	Seed orchard		0.229	2.8	100.0		
Pinus contorta var. *latifolia*	Unmanaged*	0.46	0.73	12.2		SSRs	Thomas et al. 1999
	Clear-cut + natural regeneration*	0.46	0.72	11.5			
	Clear-cut + artificial regeneration*	0.46	0.75	11.5			
Pinus contorta var. *latifolia*	Unmanaged	0.164	0.174	1.93	38.5	Isozymes	Macdonald et al. 2001
	Post-harvest	0.144	0.158	1.87	34.3		
	Post-fire	0.155	0.172	1.89	37.3		
	Artificial regeneration	0.138	0.149	1.83	35.0		
Pinus echinata	Unmanaged*	0.113	0.120	2.18	83.4	Isozymes	Raja et al. 1998
	Seed tree*	0.108	0.120	2.13	80.7		
	Artificial regeneration	0.197	0.177	1.90	74.2		

Species	Treatment	[1]H_o	[2]H_e	[3]A	[4]P	Marker	Reference
Pinus monticola	Harvest-origin		0.081	1.3	26.7	Isozymes	El-Kassaby and Benowicz 2000
	Commercially thinned		0.067	1.1	13.3		
Pinus strobus	Unmanaged*	0.126	0.148	2.31	75.0	Isozymes	Buchert et al. 1997
	Post-harvest*	0.132	0.150	1.74	53.8		
Pinus strobus	Unmanaged*	0.522	0.607	9.43	92.3	SSRs	Rajora et al. 2000
	Post-harvest*	0.510	0.586	7.00	92.3		
Pseudotsuga menziesii var. *menziesii*	Unmanaged		0.171	2.14	52.6	Isozymes	El-Kassaby and Ritland 1996
	1st generation seed orchard		0.172	2.28	62.5		
	1st generation rogued seed orchard		0.173	2.24	66.4		
	2nd generation seed orchard		0.163	2.25	56.3		
Pseudotsuga menziesii var. *menziesii*	Unmanaged		0.197	2.86	90.2	Isozymes	Adams et al. 1998
	Patch cut		0.200	2.85	88.5		
	Shelterwood		0.191	2.76	88.5		
	Natural regeneration		0.190	2.75	87.0		
	Artificial regeneration		0.203	2.89	95.5		
Pseudotsuga menziesii var. *menziesii*	Unmanaged*	0.206	0.218	2.6	89.5	Isozymes	Neale 1985
	Shelterwood*	0.216	0.232	2.8	95.0		
	Regeneration*	0.195	0.214	2.7	95.0		
Pseudotsuga menziesii var. *menziesii*	Harvest-origin*		0.159	2.4	54.6	Isozymes	El-Kassaby and Benowicz 2000
	Commercially thinned*		0.161	2.4	54.6		
Thuja plicata	Harvest-origin		0.068	1.29	19.05	Isozymes	El-Kassaby and Benowicz 2000
	Commercially thinned		0.087	1.29	23.80		
Thuja plicata	Unmanaged		0.055	1.1	11.1	Isozymes	El-Kassaby et al. 1993
	Seed orchard		0.058	1.2	11.1		
Tsuga heterophylla	Unmanaged	0.098	0.113	1.60	57.1	Isozymes	El-Kassaby et al. 2003
	Patch cut	0.073	0.080	1.57	47.6		
	Shelterwood	0.085	0.095	1.75	58.7		
	Seed tree	0.086	0.095	1.57	49.2		
Tsuga heterophylla	Harvest-origin*		0.056	1.6	14.2	Isozymes	El-Kassaby and Benowicz 2000
	Commercially thinned*		0.044	1.2	17.5		

[1]H_o is observed heterozygosity; [2]H_e is expected heterozygosity; [3]A is mean number of alleles per locus; [4]P is mean percentage of polymorphic loci. *Arithmetic mean of >1 replicate, where replicates were separately reported in original study.

Pinus strobus L. was examined after removing 75% of the old-growth stem density in two stands (Buchert et al. 1997). Neither observed nor expected heterozygosity markedly differed following harvest, although the percentage of polymorphic loci and number of alleles per locus were reduced by 20-25% in both stands (Table 2). No alleles with frequency >0.05 were lost. Low frequency alleles (frequency <0.05, Marshall and Brown 1975; Macdonald et al. 2001) and private alleles suffered drastic reductions in the remaining old-growth seed trees (30-32% lost): the lower the allele frequency, the higher the losses due to drift caused by logging. Both allozyme and microsatellite markers showed

similar results, although losses of low-frequency and localized alleles were more severe for highly variable microsatellites (Rajora et al. 2000) than for protein-based allozymes (Buchert et al. 1997). Similar results were observed for *Eucalyptus consideniana* Maiden (Yerchuk), where seed tree harvesting reduced lower allelic richness and evenness relative to clear-cut stands, primarily due to the loss of low-frequency, localized alleles. These detrimental effects were detectable using codominant isozyme and microsatellite markers, but not dominant RFLPs (Glaubitz et al. 2003; Table 2).

In some cases, there is evidence of adaptive traits in forest trees associated with or directly detectable using molecular markers (growth and reproductive traits for various species: Bush and Smouse 1992; decline susceptibility in *Fagus sylvatica* L.: Müller-Starck 1985; pollution tolerance in *Picea abies* (L.) Karst.: Bergmann and Scholz 1987). It is difficult to detect any fitness effects of heterozygosity alone at most loci or linked loci quantified by nearly-neutral markers. Allelic diversity represents the long-term potential of an individual or population to adapt via recombination. Homozygous trees or homogeneous populations with low genetic diversity are restricted to responses based on plasticity or demographics, such as immigration, or mutation, which are usually low at loci influencing adaptive traits. Most studies of the fitness consequences of rare alleles document deleterious, not positive, effects in their current environment or adaptive landscape (e.g. Cheliak et al. 1988; Bush and Smouse 1992). However, populations or individuals harbouring low-frequency alleles may represent the evolutionary potential of a species. Individuals may be homozygous at many loci and still be genetically diverse: one must consider allelic richness as well as heterozygosity, or allelic evenness. These alleles may also provide information on historical patterns of population migration, selection pressures, or the footprint of a long-changed environment (Millar and Westfall 1992). Reflecting on the past, we are constantly reminded that historical events may recur, and this is no less so when viewed in genetic, ecological and scientific perspectives.

B. Stand Tending

Macdonald et al. (2001) found no detrimental impacts of thinning on genetic diversity parameters of *Pinus contorta* var. *latifolia* Dougl. ex Loud. Another study of species growing in two commercially thinned second-growth *P. menziesii* var. *menziesii* plantations found some allelic losses in *Tsuga heterophylla* and *Pinus monticola* Dougl. ex D. Don. after thinning, but *Thuja plicata*, *Abies amabilis* and *Pseudotsuga menziesii* lost no alleles in one plantation and only one allele in another (El-Kassaby and Benowicz 2000). Heterozygosity was not affected by thinning in either plantation (Table 2).

C. Regeneration

Natural regeneration in fire-origin *Pinus contorta* stands had significantly higher heterozygosity and allelic diversity than artificially regeneration: low-frequency alleles accounted for most of the differences, and were also absent or reduced in frequency in seed orchard seedlings (Macdonald et al. 2001). These differences were not detected among mature trees in post-harvest and post-fire stands (Table 2). Microsatellites (SSRs) revealed significantly lower expected heterozygosity in *P. contorta* natural regeneration than artificial regeneration following clear-cutting and site preparation (Thomas et al. 1999; Table 2). An assessment using dominant RAPDs showed similar, but less pronounced effects: seedlings had higher expected heterozygosity than parent trees.

In *Pseudotsuga menziesii* seed orchards, El-Kassaby and Ritland (1996) found a different trend: allelic richness and diversity were higher in the seed orchards than in the original populations. Several rare and private alleles were lost in the advanced generation seed orchards, but were partially offset by new allelic gains (Table 2). Adams et al. (1998) found no significant genetic differences between *Pseudotsuga menziesii* and either planted or natural regeneration following clear-cut, patch cut (0.2 ha) and shelterwood harvest (leaving 15-30 stems·ha^{-1}) (Table 2). As El-Kassaby and Ritland found out, seed orchard *P. menziesii* seedlings in this study were more genetically diverse than the natural advance regeneration, since orchard genotypes originated from a wider area (Adams et al. 1998).

El-Kassaby and Benowicz (2000) summarized genetic diversity parameters for phenotypically selected seed orchard populations vs. natural populations of *Picea abies* (L.), *P. sitchensis* (Bong.) Carr., *P. glauca* x *engelmannii* (Engl.), *Thuja plicata* and *Pseudotsuga menziesii*. In all cases, seed orchards had equal or higher genetic and allelic diversity of common alleles than their natural counterparts based on their greater sampling breadth (Table 2).

Cheliak et al. (1988) studied a *Picea glauca* (Moench.) Voss. seed orchard. Observed and expected heterozygosity were similar to those of wild populations, but 25% of the original allelic richness was lost, most often via low-frequency and local alleles (Table 2). Rajora (1999) found *P. glauca* genetic and allelic diversity decreased in the following order: old-growth ≥ naturally regenerated second-growth > managed plantations ≥ seed orchards (Table 2); rare and localized alleles accounted for the vast majority of these differences.

Management implications

Customized silvicultural prescriptions are already implemented for each cutblock or polygon in a management area. Choosing the most appropriate option depends on the species mixture, each species' inherent ability to adapt to future changes, such as climate change, pollution or disease epidemics, plus population density, management and social pressure (Müller-Starck 1985; Scholz 1987; Buchert et al. 1997). Conservation of low-frequency alleles, which may have adaptive value for traits such as pathogen resistance or biochemical activity, is a goal that managers can achieve within a larger management framework. Collecting seed from the parent stand for reforestation, retaining advance regeneration, supplementing with locally sourced planted seedlings, or preserving individual or small groups of high-quality parent trees may easily be incorporated into silvicultural plans. Glaubitz et al. (2003) remark that genetic diversity underlies all other scales of biological diversity, underlining the need to conserve genetic diversity as a prerequisite for sustainable and healthy organisms, ecosystems and landscapes.

There will always be tradeoffs between short-term operational costs and sustainability. Once forestry crews have gained experience with alternative silvicultural systems, they often are able to increase efficiency and may help decrease operating and planning costs (Arnott and Beese 1997; Howard and Temesgen 1997). When community members can participate actively in managing their legacy, both the intangible and tangible benefits from the forest landbase can be sustained.

Overall, these studies show similarities and differences for each species in different ecological contexts. The genetic consequences of forest management depend on inherent adaptation and life history characteristics. Site conditions and stochastic factors, and their effects on species' genetic potential, make local experience a critical component of sound management.

For example, McDonald (1966) found differing responses when a group selection system was used to form various sized gaps to regenerate *Pseudotsuga menziesii*, *Pinus lambertiana* Dougl., *Pinus ponderosa* and *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. There were no significant differences among the proportions of regeneration for each species among different opening sizes, but the large-seeded *P. lambertiana* had less regeneration in larger gaps, due to poor dispersal and/or seed predation. The other small-seeded species had proportionately larger wings and achieved more complete stocking as gap size increased. After one year, seedlings showed similar trends, except that *A. concolor* had fewer surviving seedlings in larger openings due to its smaller seeds and early protection requirement, despite its high fecundity.

Growth attributes and physiology of *Abies amabilis* advance regeneration were compared to those of planted seedlings at several distances from the edge of a clear-cut (Hawkins et al. 2002). Advance regeneration was smaller and had higher root to shoot biomass ratios and net photosynthesis

than planted seedlings, but these differences disappeared after five years. *Abies amabilis* and *Tsuga heterophylla* planted seedlings had the lowest three-year growth increments under shelterwood stands compared to clear-cut, seed tree or patch cut stands (Mitchell 2001), revealing light as the limiting factor, since nutrient availability and photosynthesis were similar across all treatments. Future stand composition and dynamics depend on interacting factors which vary with regeneration method and site (Sullivan et al. 2001).

Increasing the genetic potential of a stand may be very simple: five-year seedling survival was compared for *Tsuga canadensis* (L.) Carr. among substrates (Greene and Johnson 1998). Mineral soil, followed by exposed humus, resulted in the best survival relative to initial seedling density and area. These seedbeds also accounted for 99% of postfire *Pinus banksiana* Lamb. natural regeneration (Greene and Johnson 1998). Enabling more seeds to germinate and reducing environmental selection pressure on germinants and young seedlings by creating optimal microsites may enable individuals containing low-frequency alleles to survive in the stand.

Each species has characteristic reproductive habits and has developed an ecological niche; this may be represented genetically by co-adapted gene complexes. While some conclusions may broadly apply to groups with similar life history traits (e.g. shade-tolerant climax conifers), a single prescription is not appropriate for the existing variety of situations and requirements. By making up-to-date research easily understandable and available to forest managers and the public, decisions based on sound scientific studies can be implemented across the landscape. Open houses, community mail-outs, workshops, interpretive or guided tours, internet forums, extension pamphlets, guidebooks, notices in local media and participation in local planning facilitate cooperation where creative and innovative practices can arise.

Canada has ratified many international conventions to maintain biodiversity (Anonymous 2003). The Canadian National Forest Strategy, revised every five years, has committed Canada to manage forests for multiple uses while maintaining and enhancing biological and ecological integrity (Anonymous 2002; Anonymous 2003). The National Round Table on Environment and the Economy (NRTEE), comprised of a panel representing government, aboriginal, industry, community, academic and other groups, has recommended that Canada maintain and increase its commitment to sustainability via stewardship and monitoring (NRTEE 2003). Canada contains some of the most spectacular natural landscapes and attributes in the world, many of which are irreplaceable. It is in the best interests of all stakeholders, i.e. business, communities, governments, aboriginal people, and all citizens to adopt sustainable forest management principles based on sound data.

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SHOOT PHENOLOGY STUDIES TO DETERMINE GROWTH CYCLES IN TWO *PINUS RADIATA* D.DON PROGENY TESTS IN NORTHERN SPAIN

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ABSTRACT

Phenology is the study of periodicity phenomena in nature. Knowledge about timing and duration of certain life cycle events provides valuable information about the condition of trees and the effects of climate fluctuations and changes on them. Possible changes in the timing of phenological phases can subsequently be explained in relation to environmental factors.

The objectives of phenological monitoring are:

- To determine the course of the annual development stages;
- To explain possible changes in the timing of these stages in relation to environmental factors;
- To utilise this knowledge in interpreting observed changes in tree conditions (growth, crown condition, etc.).

Two radiata pine half-sib family-test field trials were established in 2001 in northern Spain. Each test consists of 58 open-pollinated families with 25 repetitions per family. The chosen design was 25 randomised complete blocks with single-tree plots.

Phenologic observations were made twice per month during ten months, being of special interest the dates of bud opening and winter bud formation, which consequently fix the duration of the vegetative growth period and the different development capacities of the families. We found differences between families and also between sites.

INTRODUCTION

The annual development cycle has a crucial role in the adaptation of forest trees (Alía et al. 1994). Shoot elongation patterns have been studied to gain insight into the mechanisms underlying the adaptation of forest tree species to their environments and to accelerate breeding programs (Baley and Feret 1982; Bridgwater et al. 1985; Kaya and Isik 1997; Isik et al. 2002).

The timing of spring bud flush in radiata pine is an important adaptative trait (Bollmann and Sweet 1976). This phenological trait plays a critical role in initiating the annual growth cycle early in the spring when soil moisture is high, yet late enough to avoid spring frost damage. Frost damage to new shoot tissue can delay growth, cause stem defects and, in severe cases, kill young trees (Jermstad et al. 2001).

The amount and types of shoot elongation that occur during the growing season are integral components of the annual sequence of developmental events in seedlings.

Two models of shoot growth in conifers are recognised: fixed and free growth. In fixed growth, stem units (stem + leaf primordium) are formed only after a bud forms in mid- to late summer. The age of fixed growth species can often be determined by counting branch whorls – they have one flush (whorl) per year. Otherwise, free growth implies that stem units are formed while elongation is taking place. These trees may also form a bud in late summer and stack primordia that will elongate the following spring.

Somewhere between fixed and free growth species are the recurrently flushing species that may exhibit free growth during the growing season so long as conditions are favourable. When conditions are bad, recurrent flushing species may produce a temporary, or resting, bud. While conditions are poor, primordia are stacked under the resting bud. When conditions are once again favourable, this resting bud flushes. Recurrent flushers may produce several flushes during the growing season, usually with the first flush being the longest.

Radiata pine produces multiple cycles, i.e. annual height growth occurs as a series of discrete stages called cycles or flushes (Harrington 1990; Kaya et al. 1994). Elongation of the first cycle is primarily related to temperature (Boyer 1970; Bridgwater 1990). As the first cycle elongates, a new bud forms and begins to elongate at about the time that maximum elongation occurs in the cycle during which the new bud was formed (Griffing and Elam 1971). This rhythmic pattern of bud formation and elongation occurs until one to several “summer shoots” (cycles not performed the previous year) have elongated (Lavender 1985; Birchler et al. 1998).

Lanner (1976) points out that year to year extension can be more effectively influenced by modifying primordium numbers than by modifying internode elongation between those primordia.

The objectives of this study are to describe the shoot phenology variation of radiata pine families tested at two sites in northern Spain, to determine the course of the annual development stages, to explain possible changes in the timing of these stages in relation to environmental factors, and to utilise this knowledge in interpreting observed changes in tree conditions, such as growth and crown condition.

MATERIALS AND METHODS

Plant material, trial site and design

Two-year-old seedlings were planted in two progeny tests in northern Spain. Progenies were sown in 2000 from open-pollinated seeds obtained from 50 plus trees in plantations in northern Spain. All parent mother trees were selected for superior growth and form as part of a first-generation breeding population. Therefore, the genetic estimates determined from their progeny performance refer only to selected stock in this region (Cotterill and Zed 1980; Espinel and Aragonés 1997).

We also included as controls progenies from six parent clones selected in Basque Country and two lots of commercial seeds (one lot from interior trees and another one from coastal trees).

The trials were established in May 2001 on moderately fertile sites in the villages of Daneiro and Benade. Both sites previously supported radiata pine plantations. Preparation and silviculture were as usual: the cleared sites were ploughed and ridged before planting.

The design was a randomised complete block design with 25 repetitions of single-tree plots with 3 x 3 m spacing. Figure 1 shows the geographic origin of plus trees selection zones, the location of progeny trials and that of a little grafting seed orchard.

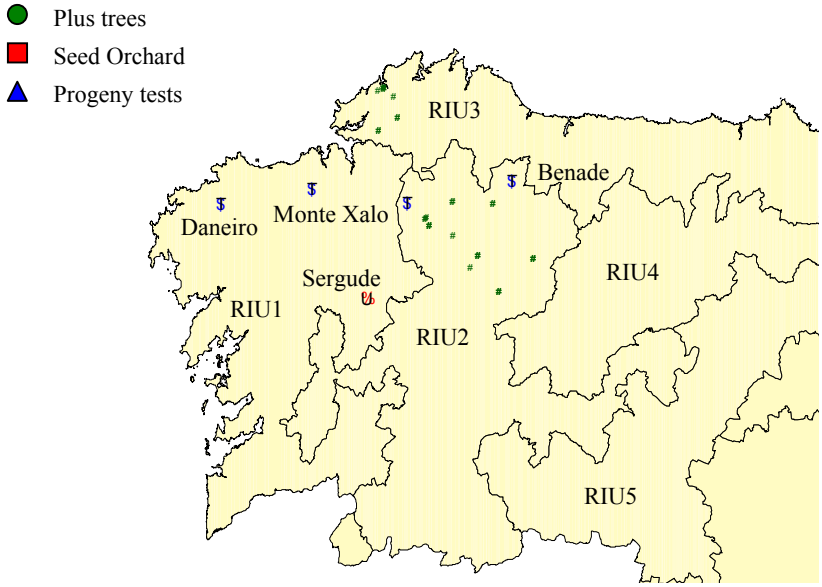

Figure 1. Selected plus trees, progeny tests and seed orchard locations.

Phenology measurements

Shoot phenology was measured periodically (twice per month) from October 2002 to August 2003. Terminal bud flush was measured at both sites, using a refined scoring system that recorded the stage of bud flush (see Figure 2):

- Phase 0. Dormant bud, not flushed. The entire structure is wrapped in, or protected by, cataphylls.
- Phase 1. Bud still closed, but swollen (meristematic activity started).
- Phase 2. Bud burst. The bud is opening, but we cannot distinguish any new needle.
- Phase 3. Bud open, needles 0-5 cm long.
- Phase 4. Bud open, needles 5-10 cm long.
- Phase 5. Bud open, needles 10-15 cm long.
- Phase 6. Bud open, needles 15-20 cm long.
- Phase 7. Bud open, needles 20-25 cm long.
- Phase 8. Bud open, needles >25 cm long.

Statistical analysis

Test-site means, coefficients of variation among families, and the measure of the skewness in the frequency distributions of families were calculated using PROC UNIVARIATE by SAS Institute Inc. (1985) (data not shown). The terminal bud flush scores for each date were generally normally distributed.

The following phenological parameters have been derived for each seedling: Date1, date of height growth initiation (day when buds reach phase 2); Date2, date of height growth termination (day of bud set); Dur, duration of terminal bud elongation; and NOB, total number of new terminal buds formed from October 2002 to August 2003.

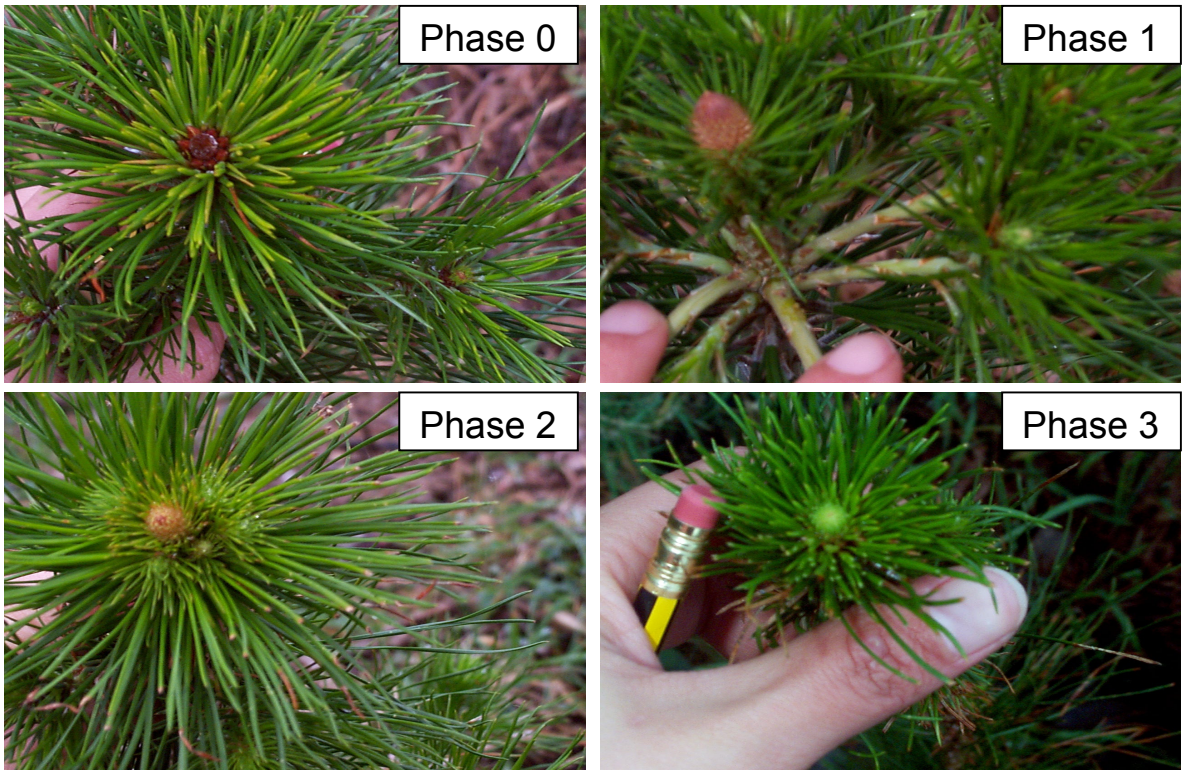

Figure 2. Different stages of terminal bud flush cycle.

Individual tree data were analysed using PROC GLM by SAS Institute Inc. with Type IV sums of squares, appropriate for hypothesis testing when there are missing values (SAS Institute Inc. 1985), with the following random model:

$$Y_{ijk} = \mu + f_i + b_j + e_{ijk}$$

Where,

y_{ijk} = value of the *k*th tree in the *i*th family, *j*th block;
μ = experimental mean;
f_i = effect of the *i*th half-sib family;
b_j = effect of the *j*th block;
e_{ijk} = error.

Variance components were estimated using PROC RANDOM with family- and block-like random effects every 15 days, for each measurement data.

Genetic parameters

The offspring of an open-pollinated family were assumed to be half-sib and therefore the individual heritabilities (h_i^2) were estimated as:

h_i^2 = additive variance / variance of individual phenotypes = $\sigma_A^2 / \sigma_p^2 = 4\,\sigma_f^2 / (\sigma_f^2 + \sigma_e^2)$;
σ_f^2 = component of variance due to family;
σ_e^2 = component of variance due to within-plot error.

Approximate standard errors of individual heritabilities (σ_h) were estimated according to Wright (1976):

$$\sigma_h = (1-h_i^2/4)\,[1+ (NB-1)h_i^2/4]/[(NB/2)(NB-1)(F-1)]^{1/2}$$

Where,

h_i^2 = heritability;
N = number of trees per plot;
B = number of replications;
F = number of families.

Phenotypic correlations between different traits in the same site were estimated as Pearson correlation coefficients.

RESULTS

Within-site variation in shoot phenology

The seasonal pattern of initiation and subsequent extension growth of the annual shoot of 58 families at Benade and Daneiro is shown in Figure 3 (a and b), which presents data from 1450 trees in each location, scored at intervals of 15 days during the 10 months of the experiment. Figure 3 illustrates a continuous pattern of initiation and extension throughout the year, with normally more than one complete cycle of growth. We represented the cumulative average rainfall and temperatures at each date to determine the environmental influence over initiation and cessation dates. At the Benade site, half the families extended their leading shoots before spring. Nevertheless, height growth initiation in Daneiro took place, for most families, around the same date in spring.

The summary of ANOVA analysis for all the variables measured in each site is shown in Table 1. Differences among families were statistically significant for all traits investigated, except for Date2 in both sites and the duration of the first shoot elongation in Daneiro.

Table 1. Summary of ANOVA.

Site	Variable	Family[1] Mean Square	F	Pr>F	Block[2] Mean Square	F	Pr>F	Error Mean Square
Benade	Date1	7.9214E13	1.98	**<0.0001**	1.4562E13	3.64	**<0.0001**	3.9977E13
	Date2	7.0837E12	1.02	0.4446	9.1504E12	1.31	0.1438	6.9735E12
	Dur	10998.8551	1.93	**<0.0001**	14880.7263	2.61	**<0.0001**	5696.462
	NOB	1.4169	1.96	**<0.0001**	1.02	1.41	0.0903	0.7229
Daneiro	Date1	8.1179E13	2.05	**<0.0001**	7.4827E13	1.89	0.0062	3.9673E13
	Date2	1.4616E13	0.92	0.6490	2.4262E13	1.52	0.0512	1.5927E13
	Dur	1305145.74	1.04	0.4008	1175059.18	0.93	0.5542	1257586
	NOB	0.9954	2.20	**<0.0001**	0.5782	1.28	0.1691	0.4533

[1]Degrees of freedom: 57
[2]Degrees of freedom: 24
Traits: **Date1**: date of height growth initiation (day when buds reach phase 2); **Date2**: date of height growth termination (day of bud set); **Dur**: duration of terminal bud elongation; **NOB**: total number of new terminal buds formed from October 2002 to August 2003.

Individual heritabilities with their standard errors for all traits studied are shown in Table 2. Individual heritabilities of growth traits were moderate with low standard errors for all traits in each location, except for Date2. In Daneiro, there was also a very low heritability for Dur. These heritabilities suggest that moderate genetic gains should follow individual selections for date of initiation of shoot growth and for the number of terminal buds formed during the same year in Galicia (northern Spain).

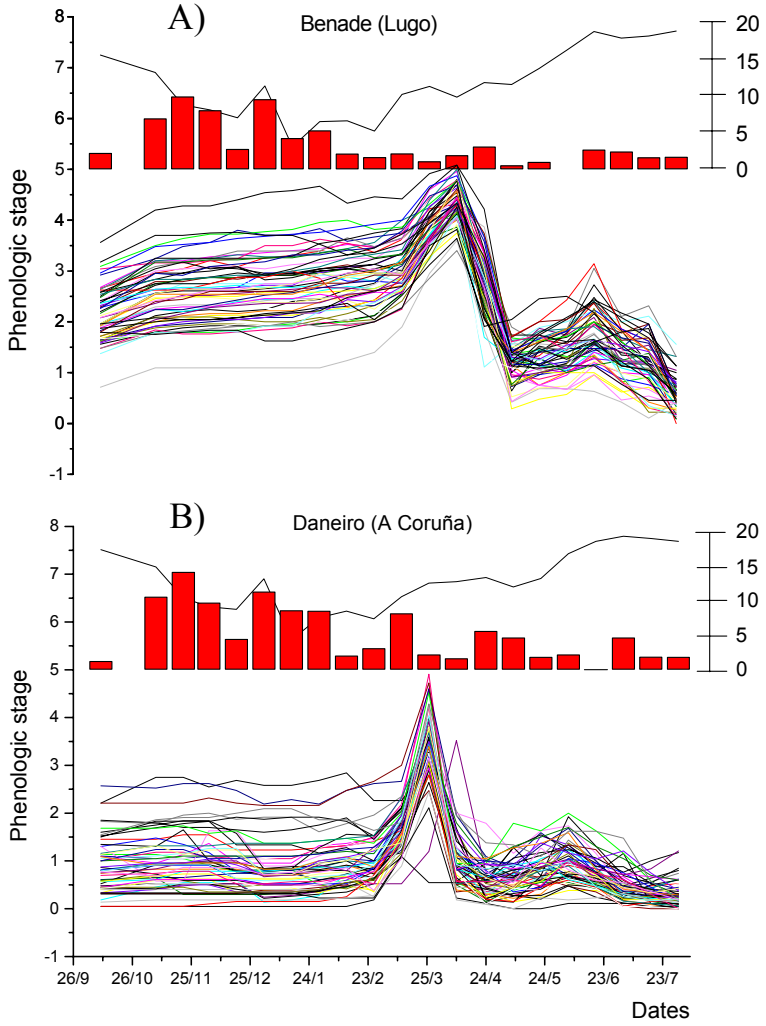

Figure 3. Seasonal pattern of shoot phenology mean values of radiata pine at two sites: A) Benade (Lugo) and B) Daneiro (A Coruña). The upper line shows average precipitation (mm) and the columns show average temperature (°C).

Table 2. Individual heritabilities and their standard errors for the traits measured in the Benade and Daneiro progeny tests.

Site	Trait	Heritability	Error
Benade	Date1	0.16	0.01
	Dur	0.17	0.01
	NOB	0.17	0.01
Daneiro	Date1	0.21	0.02
	Dur	0.01	0.01
	NOB	0.17	0.01

Phenotypic correlations between different traits at the same site are presented in Table 3. We found moderate and positive phenotypic correlations between Date1 and Date2 at both sites (0.17508 for Benade and 0.1919 for Daneiro). There were moderate to strong adverse phenotypic correlations between Date1 and NOB at both sites (-0.22767 for Benade and -0.47121 for Daneiro). Date1 had a strongly negative phenotypic correlation with Dur (-0.89865) and a positive correlation with NOB (0.208079) in the Benade test site, but no correlation between those traits was found in Daneiro. Date2 had a moderate negative phenotypic correlation with NOB in Daneiro (-0.26261), but no such correlation was found in Benade.

Table 3. Phenotypic correlation for all traits in the Benade (above the diagonal) and Daneiro (below the diagonal) progeny tests. The significance of the correlation appears in brackets.

Trait	Date1	Date 2	DUR	NOB
Date1		0.17508 (<0.0001)	-0.89865 (<0.0001)	-0.22767 (<0.0001)
Date2	0.1919 (<0.0001)		0.22460 (<0.0001)	-0.0039 (0.8957)
Dur	-0.0913 (0.0021)	0.00051 (0.9864)		0.20879 (<0.0001)
NOB	-0.47121 (<0.0001)	-0.26261 (<0.0001)	0.03875 (0.1924)	

DISCUSSION

Radiata pine is the third most important forest tree species in Galicia. Together with *Pinus pinaster* and *Eucalyptus globules*, it supports 90% of the Galician forestry industry. It is obviously desirable to know as much as possible about its growth cycles because of their consequences on tree form and branching habit. This knowledge is a prerequisite for the selection of traits to be considered in the breeding programme aiming to increase wood production and quality of *Pinus radiata* in Galicia (for the optimal timing of periodic measurements, sampling and silvicultural treatments).

Total annual height growth for polycyclic species is a function of both the number of cycles and the length of the cycles. The number of cycles elongated annually and the length of these cycles vary according to three factors, namely age, vigour and genotype (Greenwood 1984), and according to environmental conditions, such as rainfall, temperature, soil moisture, nutrition, solar radiation and day length (Boyer 1970).

There was considerable family variation in shoot growth patterns of *Pinus radiata*. Some families grew in height all year without setting any bud, but the majority of families grew episodically, setting buds intermittently for short periods. We observed that one to four internodes were produced during the period of observation. These results are consistent with other studies of radiata pine in Australia (one to three internodes; Fielding (1966)) and New Zealand (one to five internodes; Bannister (1962)).

It seems that elongation of the first cycle is primarily related to the progressive increment of temperature from winter to spring (Boyer 1970). The components of the first cycle are laid down during late summer and autumn as a series of primordia inside a bud in which they overwinter (Bollman 1976).

Due to the high unusual temperatures of the last winter, many families were already elongating the apical shoot before the spring, but a period of sudden and strong frosts caused a reversible growth termination (Figure 3). Low temperature inhibition of growth in *Pinus radiata* is usually almost immediately reversible and thus it does not represent true dormancy (Jackson et al. 1976).

The capacity for a second flush is an adaptation of sources from dry sites to intermittently favourable water conditions during the growing season, i.e. an ability to cease growth during periods of drought and to resume growth when soil water becomes available (Kaya et al. 1994). Our results were consistent with this hypothesis: we observed the formation of a new terminal bud in early spring due to an unusual drought period in Galicia. The annual rainfall regime seems to be the principal factor of formation and elongation of new buds (Figure 3; Kaya et al. 1994).

We found statistical differences between families for the date of initiation of height growth, but no differences in the date of cessation were found (Table 1). These results showed again that Date2 is highly influenced by environmental conditions. Nevertheless, Date1 has a significant genetic effect and is little influenced by the environment, so we can affirm that there exists earlier and later flushing families.

The statistical differences between families found in the number of new terminal buds formed (Table 1) and its moderate and negative phenotypic correlations with Date1 (Table 3) reported that early flushing families have more possibilities to develop a high number of new buds than the late ones due to the capacity of early flushing families to make full use of a long growing season. The early flushing families cannot be included into the progeny selected for the second generation of a breeding program because they will be more susceptible to frost damage and they will have a very large number of nodes that will produce a decrease in wood quality. On the other hand, the late flushing families cannot be included in advanced breeding programs due to their slow height increment.

In conclusion, the results indicate that early family evaluation for shoot growth patterns of radiata pine seedlings must be limited to the date of height growth initiation and to the number of new terminal buds formed. The result on the possibility of early selection in branch habit agrees with the findings of Williams (1987) about the possibilities of early selection in growth which showed that for 18-month-old loblolly pine seedlings, the length of annual shoots after the first whorl of branches produced at first budset (cyclic growth) was the best indicator of eight year heights of sibs in field tests.

Shoot growth patterns should be an important consideration in radiata pine breeding due to their repercussion on tree form, seed source selection and gene conservation programs.

The next step will be the phenotypic and genetic correlation studies between the new annually buds formed and the total number of whorls of branches in each family.

ACKNOWLEDGEMENTS

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USEFUL TREE SPECIES FOR URBAN AREAS OF THE TROPICAL REGION OF NORTH AMERICA

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ABSTRACT

This paper provides information on silvics and silviculture of three tropical tree species that presently grow in areas of tropical North America and provide several tangible and intangible benefits. This information will enable urban dwellers to select, establish and culture species well adapted to local environments, and manage them for maximum yield. The avocado (*Persea americana* Mill. [Lauraceae]) is an attractive tree that grows up to 40 m in height, in sun or light shade, in areas with good drainage. It produces nutritious, globular fruits 5-15 cm long, and the leaves are also used in cooking. The wood is easy to work and has a variety of uses for furniture, interior construction, flooring, boxes, crates and firewood. The mamey (*Mammea americana* L. [Clusiaceae]) is a handsome tree with dark green leathery leaves that grows up to 60 ft in height. It prefers deep, rich soil, but is adaptable. The fruit is nearly round and 10-20 cm in diameter, and is eaten fresh or cooked as a sauce, made into preserves or jam, or is used in pies and tarts. The wood is used for cabinetwork, decorative features, rafters, fence posts or fuel. The tree also has medicinal uses and can be used as a windbreak or shade tree. The guanábana (*Annona muricata* L. [Annonaceae]) is a low-branching, bushy but slender tree, 7.5-9 m in height, that produces oval fruits 10-30 cm wide and up to 15 cm long. The flesh is eaten fresh or is made into drinks or ice cream. The ground seeds or a leaf decoction can be used as a pesticide.

INTRODUCTION

Trees can help to create a more pleasant and livable atmosphere in urban areas by making houses, other buildings and pavement more attractive, and can provide additional benefits as well. They can help reduce temperature in tropical areas by providing shade and by intercepting solar radiation. During the process of transpiration, trees evaporate water from their leaves and function as natural air conditioners. Trees can also help to reduce air pollution because leaves absorb gaseous pollutants and trap particulates on their surfaces. Trees serve as privacy screens and can decrease the force of wind, reduce noise levels and block bothersome lights at night. They provide food and shelter for birds and other animals that otherwise might not occur in urban areas (Schubert 1979). They may also provide fruit, wood, medicine, insecticide and other products.

The objective of this paper is to provide essential information on silvics and silviculture of three broadly useful tropical tree species that presently grow in areas of tropical North America. This information will be useful to urban dwellers so that they may select species well adapted to local environments, establish and culture them, and manage them in order to yield a maximum return. An extensive literature has accumulated about tropical tree species of the region, but it may not be easily available to the anticipated audience.

Persea americana Mill. (Lauraceae)

Synonyms: *Laurus persea* L., *Persea gratissima* Gaertn., *P. persea* Cockerell
Common names: avocado, alligator pear (U.S.), aguacate (Mexico and Central America), avocatier (Martinique), avocet (Martinique)
Description. The avocado tree may be as tall as 40 m in height, or it may be short and bushy. The trunk may be 30-60 cm in diameter, with large branches. The leaves are alternate and variable in shape, lanceolate, elliptic, ovate or obovate, and are dark green on the surface and whitish underneath. The flowers are borne in panicles near the tips of the branches. They are small, pale green or yellowish with a pubescent ovary, and lack petals. The fruit may be pear-shaped, oval or nearly round, 7-33 cm long and up to 15 cm wide. The skin ranges from yellow-green to dark green to purplish to almost black, and may be smooth or pebbled, and the flesh is yellow (Fournet 1978; Morton 1987). Cultivated avocados are separated into three races: the Mexican, the Guatemalan and the West Indian (Seddon and Lennox 1984; Morton 1987; Ospina 2002).

Habitat (geographic extension, topography and soils). The avocado probably originated in southern Mexico, but it grows from northern Mexico to Chile and in the Caribbean islands, and has been introduced to many parts of the tropical and subtropical world. The avocado grows at elevations from sea level to 2400 m. The West Indian race is the most cold sensitive, and requires a tropical or near tropical climate and high humidity. The Guatemalan race is a little more cold tolerant and the Mexican race can withstand temperatures as low as -4^{o} C. Windbreaks are necessary where winds are strong, otherwise wind interferes with pollination and causes loss of fruit (Morton 1987).

The avocado will tolerate a variety of soils, from clay to sand to loam and limestone. Ideal pH is between 6 and 7, but the species grows on limestone soils in Florida that range from 7.2 to 8.3. Good drainage is the most important requirement as the species cannot withstand excessive water or temporary water logging. The water table should be at least 0.9 m below the surface and there should not be an impervious layer or the trees will die in a few years. Salt is not well tolerated (Samson 1996).

Life history. Seedlings begin to bear fruit within 4-5 years and will continue to produce fruit for 50 years or longer. Only around 5% of flowers will produce fruit (Ospina 2002). Bees are the principal pollinators but they prefer citrus, so it is better not to plant an avocado tree too close to citrus trees (Samson 1986). Avocados will not ripen while on the tree. Homeowners usually consider the entire crop ready to be harvested when a few fruits fall from the tree, but fruits may be in various stages of development, so the largest should be picked first. Avocados will ripen in 1-2 weeks at room temperature. Fruits should be collected with a short piece of stem attached, using ladders and scissors or knives, and should not be pulled off the tree or allowed to fall on the ground (Morton 1987).

Nursery practices and establishment. The seeds lose viability within 2-3 weeks when removed from the fruit. The seeds should be soaked in water for 24 hours before planting and should germinate in about 21 days (Ospina 2002). People in urban areas pierce the seed slightly with a toothpick on two sides to hold it on the top of a glass with water touching the base of the seed. After roots and leaves are well formed, the seedling is planted. Grafting is used in commercial plantations, but cuttings are difficult to root (Morton 1987). Avocado roots are sensitive to transplanting, and seedlings should be handled carefully. A hole at least 0.6 m deep and wide should be dug and soil should be put in a mound. Mulch is helpful and the seedling should be watered until roots are well established. Weeds should be controlled and small amounts of fertilizer should be applied every two months but not at flowering time or until the fruits are firmly set. Nitrogen is most important but the fertilizer mix needed depends on the type of soil (Morton 1987). Trees should not be severely pruned (Samson 1996).

In Mexico, the avocado weevil (*Heilipus lauri)* tunnels into seeds, and various other insects will sometimes attack the species. In some areas, various fungus species cause root rot (Morton 1987).

Tomatoes and eggplants should not be planted near avocado trees because they may pass *Verticillium* fungus to avocado. The stems of young trees may be whitewashed or covered with cardboard to protect against sunburn and to lessen damage by rodents (Samson 1986).

Uses. Avocado trees are cultivated in family gardens and in commercial plantations. The fruit and leaves are sold in public markets. The fruits are eaten and the leaves are used as a condiment (SEMARNAT 2003). The leaves are boiled with other plant parts and are taken to prevent dysentery. The ground seed, mixed with seed of mamey and boiled with arnica, is used to combat stomach problems and gastroenteritis (SEMARNAT 2003). The wood is attractive and easy to work and is used in joinery, furniture, interior construction, and other uses (Chudnoff 1984).

Figure 1. Avocado (*Persea americana* Mill., Lauraceae) tree with fruit.

Mammea americana L. (Clusiaceae)

Synonyms: *Mammea emarginata* Moc. & Sesse ex DC
Common names: mamey, mammee-apple, abricot, abricot des Antilles (Martinique)
Description: Mamey is a handsome evergreen tree up to 25 m in height with white flowers and edible fruit. The leaves are elliptical or elliptical-ovate and are glossy green and leathery. The white flowers are 2 cm wide and are borne on the twigs, usually in the back of the leaves. The globular fruit has a thick skin and yellow-orange pulp (Hargreaves and Hargreaves 1965; Fournet 1978; Schubert 1979).

Habitat (geographic extension, topography and soils). Mamey is native to the West Indies but is planted in the tropical areas of Mexico, Central America and the Caribbean area (Francis 1989; Navarette-Tindall and Orellana Nunez 2002). The species grows best in humid to very humid areas with annual rainfall of 1500-3000 mm. Mamey occurs in tropical to near tropical areas and cannot withstand freezing temperatures (Morton 1989). Mamey grows best on deep rich soils, but is adaptable and will tolerate sandy and clayey soils. It grows well in limestone areas of Jamaica and Puerto Rico (Francis 1989; Barbeau 1990).

Life history. Flowers can be male, female or hermaphroditic (Francis 1989). Male flowers are grouped, but hermaphrodic flowers occur singly. Flowers occur on branches and branchlets that are more than 1 year old (Barbeau 1990). In the West Indies, the tree flowers from May to October and the fruit takes more than a year to mature. Young mamey trees begin to flower and produce fruit when they are between 8 and 13 years of age, and produce annually. A mamey tree can live for 100 years (Francis 1989). The fruit is ripe when there is a slight yellowing of the skin. The tips of the spines break off easily when the fruit is ripe. The fruit should be clipped with a small portion of the stem attached, and should not be allowed to fall from the tree or it will be easily bruised (Morton 1887).

Nursery practices and establishment. Seeds germinate in 2 months or less and come up readily in the organic material under the parent tree. Vegetative propagation allows the gardener to avoid planting a male tree and provides earlier fruiting. Cuttings may be used, but grafting onto self-seedlings is also successful. The mamey needs little attention except protection from cold during the first few years after planting. The species is very resistant to pests and diseases (Morton 1987). Seedlings grow quickly and 1-year-old seedlings may reach 50 cm in height. Weeding during the first year is helpful in avoiding competition for light and nutrients (Navarette-Tindall and Orellana Nunez 2002). The species tolerates shade and seedlings can survive for several years in dense shade (Francis 1989).

Uses. The fruit can be eaten fresh or in pies and preserves but should be used in moderation because it can cause stomach upsets. The flowers are attractive to bees (Schubert 1979). All parts of the plant have insecticidal properties. The tree is often planted as an ornamental around houses and in parks and along roads (Francis 1989). Mamey makes a good windbreak. It grows more slowly than other species used for this purpose, but is superior (Barbeau 1990). The wood is hard and strong and is easy to work, but because of its lack of stability, it is not good for the manufacture of furniture. It is used for fuel (Morton 1987), turned objects and posts, and in the past it was used for tobacco pipes (Francis 1989). The wood is used for fence posts because it is resistant to decay, but it is very susceptible to termites (Morton 1987).

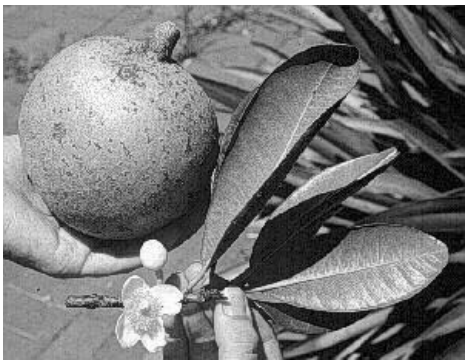

Figure 2. Flower and fruit of mamey (*Mammea americana* L., Clusiaceae).

Annona muricata L. (Annonaceae)

Synonyms: none
Common names: guanábana, soursop, corossolier, corossol
Description: Guanábana is a bush or a small tree up to 6 m in height. The alternate leaves are oblong, oblong-lanceolate or oblong-obovate, and are shiny on the upper surface. The flowers may occur anywhere on the trunk, branches or twigs. Flowers are fleshy, up to 4.5 cm in width, with three outer yellow-green petals and three inner yellow petals. The large fruit is often irregular in shape but may be more or less oval, and is covered with soft spines. It is 10-30 cm long and 15 cm wide and may weight 4.5-6.8 kg. The flesh is white (Fournet 1978; Seddon and Lennox 1984; Morton 1987).

Habitat (geographic extension, topography and soils). Guanábana occurs naturally in the West Indies and in northern South America, and today it is found from southern Mexico to Peru and Argentina (Seddon and Lennox 1984; Morton 1987; Rathore 1996). The species is very intolerant to cold, and is killed by only a few degrees of frost. It occurs from 244-300 m above sea level, in areas with moderate humidity, full sun and protection from strong winds. It grows best in deep, rich, well-drained, semi-dry soil, but also occurs on limestone soil (Morton 1987). Waterlogging causes the tree to decline (Rathore 1996).

Life history. Guanábana flowers and fruits almost continually, but there is a main season of ripening in Mexico from June to September (Morton 1987). Guanábana depends on cross pollination because the flowers are protogynous, i.e. pollen is shed only when the stigma of the same flower is no longer receptive. Hand pollination can be helpful in increasing the number of fruits (Samson 1986). The species produces a crop of 12 to 20 or 24 fruits per tree. In areas with high humidity, the trees only produce a few fruits of poor quality, which may rot at the tip. Fruits should be picked when still firm but slightly yellow-green in color, because if a fruit falls it will be damaged. Fruits should be handled carefully and will ripen in a few days at room temperature (Morton 1987).

Nursery practices and establishment. Guanábana is not established as a commercial fruit and is usually found only in gardens and home orchards (Rathore 1996). The species is usually grown from seed, which should be sown and then kept moist and shaded. The seeds germinate in 15-30 days. Some varieties can be reproduced by cuttings or shield-budding. Seedlings are planted in the ground when they are 30 cm or more in height and at the beginning of the rainy season. The tree grows rapidly and will begin to produce fruit at 3-5 years of age. Mulching helps to avoid dehydration of the roots during dry seasons. The species is subject to attack by various insect pests (Morton 1987). Although pruning is not generally done, some amount of training and pruning of guanábana should be helpful because floral buds are produced on new shoots, but it should be done when the plant is about to begin growth, as at the beginning of the rainy season. Removal of leaves causes new growth (Rathore 1996).

Uses. The fruits are divided into three types: sweet, subacid and acid. The sweet fruit are eaten raw and the others are used in drinks and desserts, especially ice cream (Seddon and Lennox 1984; Morton 1987). The ground seeds or a leaf decoction are used as a pesticide against head lice and other insects. The juice of the ripe fruit is used as a diuretic, and pulverized immature fruits are a remedy for dysentery (Morton 1987).

Figure 3. Guanábana (*Annona muricata* L., Annonaceae) tree with fruit.

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