United States
Department
of Agriculture

Forest Service

Rocky Mountain
Research Station

Proceedings
RMRS-P-32

May 2004

Breeding and Genetic Resources of Five-Needle Pines:

Growth, Adaptability, and Pest Resistance

IUFRO Working Party 2.02.15
International Conference
Medford, Oregon, USA
July 23-27, 2001

Abstract

Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B. eds. 2004. **Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance**; 2001 July 23-27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 259 p.

This volume presents 29 overview and research papers on the breeding, genetic variation, genecology, gene conservation, and pest resistance of five-needle pines (*Pinus* L. subgenus *Strobus* Lemm.) from throughout the world. Overview papers provide information on past and present research as well as future needs for research on white pines from North America, Europe, and Asia. Research papers, more narrowly focused, cover various aspects of genetics. Throughout the distribution of five-needle pines, but particularly in many of the nine North American species, the pathogen *Cronartium ribicola* J.C. Fisch. continues to cause high levels of mortality and threatens ecosystems and plantations. Studies on genetic resistance to *C. ribicola* are described in papers from different regions of the world. Use of *P. strobus* as an exotic species in Europe and Russia and corresponding problems with white pine blister rust are discussed in several papers. Other papers focus on examining and exploiting patterns of genetic variation of different species.

Key words: five-needle pines, white pines, Cronartium ribicola, genetic variation, conservation, restoration

Pinus L. Subgenus *Strobus* Lemm. Species

Classification of the species as used in these proceedings follows: Price, R.A., A. Liston, and S.H. Strauss. 1998. Phylogeny and systematics of *Pinus*. In Richardson, D.M. (ed.), *Ecology and Biogeography of Pinus*. Cambridge University Press. p. 49-68.

Section *Strobus*, Subsection *Strobi* Loud.

P. armandii Franchet. Armand pine
P. ayacahuite Ehrenberg ex. Schlechtendahl. Mexican white pine
P. bhutanica Grierson, Long & Page. (no English common name)
P. chiapensis (Martinez) Andresen. (formerly *P. strobus* var. *chiapensis*) Mexican white pine
P. dabeshanensis (formerly syn. for *P. armandii*, now separate species)
P. dalatensis de Ferré (Vietnamese common names only)
P. fenzeliana Handel-Mazzetti (Vietnam; no English common name)
P. flexilis James. Limber pine
P. lambertiana Douglas. Sugar pine
P. monticola Douglas ex. D.Don. Western white pine
P. morrisonicola Hayata. Taiwan white pine
P. parviflora Siebold & Zuccarini. Japanese white pine
P. peuce Grisebach. Macedonian pine; Balkan pine
P. strobiformis Engelmann. Southwestern white pine
P. strobus Linnaeus. Eastern white pine
P. wallichiana A.B. Jackson (syn. *P. griffithii* McClelland). Blue pine; Himalayan white pine
P. wangii Hu & Cheng. (no English common name)

Section *Strobus*, Subsection *Cembrae* Loud.

P. albicaulis Engelmann. Whitebark pine
P. cembra Linnaeus. Swiss stone pine; Arolla pine
P. koraiensis Siebold & Zuccarini. Korean pine
P. pumila von Regel. Japanese stone pine
P. sibirica du Tour. Siberian stone pine

Section *Parrya* Mayr, Subsection *Balfourianae* Engelm.

P. aristata Engelmann. Rocky Mountain bristlecone pine
P. balfouriana Greville & Balfour. Foxtail pine
P. longaeva D.K. Bailey. Great Basin bristlecone pine

Breeding and Genetic Resources of Five-Needle Pines:
Growth, Adaptability, and Pest Resistance

Proceedings of the
IUFRO Five-Needle Pines Working Party Conference
July 23-27, 2001
Medford, Oregon, USA

Organizing Committee

Richard A. Sniezko, USDA Forest Service, Chair
Scott E. Schlarbaum, University of Tennessee
Howard B. Kriebel, Ohio State University (retired)
Safiya Samman, USDA Forest Service
Harvey Koester, USDI Bureau of Land Management
Joseph Linn, USDA Forest Service

Sponsors

International Union of Forest Research Organizations (IUFRO)
U.S. Department of Agriculture, Forest Service
U.S. Department of the Interior, Bureau of Land Management
University of Tennessee
Washington Department of Natural Resources
Oregon Department of Forestry
U.S. Department of the Interior, Crater Lake National Park
Sierra Pacific Industries
Inland Empire Tree Improvement Cooperative (IETIC)
White Pine Working Group (IETIC)
Forest Renewal BC

IUFRO Working Party 2.02.15

Preface

An international conference on breeding and genetic resources of the five-needle pines took place in southwestern Oregon, USA, July 23-27, 2001. The scope was worldwide, including 25 species of subgenus *Strobus* found in North and Central America, Europe, and Asia. The conference was held under the auspices of Working Unit 2.02.15 of the International Union of Forest Research Organizations (IRFRO), with the support of the USDA Forest Service and several other forestry organizations. The goals of the conference were to review available knowledge from research on the genetics and genetic resources of this diverse group of pines, and to report current research on genetic diversity and natural hybridization and on the genetics of growth, adaptability, pest resistance, and other traits of interest in applied tree genetics and gene resource conservation.

The five-needle pines are nearly all in three sections of Subgenus *Strobus*. Although there is no universal agreement on the systematics of the subgenus, we have chosen to adhere to the recent classification by Price and others (Richardson 1998), although new phylogenetic research results based on isozyme and molecular analysis do not fully concur with this arrangement. We include two sections, Section *Strobus* and Section *Parrya*, the latter including the foxtail and bristlecone pines, two groups quite different from the rest of the species, of interest for *in situ* conservation of species with an ecological role important in their habitat. Most genetic research to date has been conducted within Subsections *Strobi* and *Cembrae* of Section *Strobus*. Until recently, the greatest attention was given to the approximately 17 species of Subsection *Strobi*, which includes several species of great importance as timber trees, many of which are capable of interspecific hybridization. More recently, research in Subsection *Cembrae*, especially in Siberia, has been focused on genetic diversity and natural hybridization. In addition to two important timber species, this subsection has some species that, although slow-growing, have great importance in horticulture and watershed protection.

The conference had 53 participants from nine countries, including the USA, Canada, Germany, Romania, Bulgaria, Russia, Pakistan, China, and South Korea. Papers were also contributed by nonattending scientists in Japan, Austria, New Zealand, and Russia. Because of the worldwide natural distribution of the five-needle pines, overview papers were invited covering the regions where five-needle pines are of major importance from a forestry standpoint. The exception was the Mexico/Central America region, from which we were unable to secure scientist participation. Research papers addresssed genetics and genecology, blister rust resistance, breeding and propagation, genetic diversity, and gene conservation. In addition to paper sessions, the conference, held in Medford, Oregon, included excursions to seed orchards, research plantations, native stands, Crater Lake National Park, and the Dorena Genetic Resource Center of the USDA Forest Service. Indigenous species of five-needle pines included in the field trips were *P. monticola, P. lambertiana* and *P. albicaulis*.

In Subsection *Strobi*, the white pine blister rust (*Cronartium ribicola*) is the major target of applied research, with the goal of removing this obstacle to survival and growth of natural and artificial stands, especially of sugar pine, western white pine, and eastern white pine. Resistance screening and breeding are proving to be effective strategies for restoring susceptible western North American species of five-needle pines. In eastern North America, rust resistance breeding has been more difficult in eastern white pine, although screening and breeding continues in the USA and Canada. In Europe, *P. strobus* would be a premier species for forestry were it not for the blister rust, which is a serious problem throughout the region, including Russia. For this reason other species are employed in forest planting.

The Balkan or Macedonian white pine has a dual role in the Balkans, critical for watershed protection and to a lesser degree as a timber species. Provenance research has shown that geographic variation in *P. peuce* can be exploited to some extent for optimum productivity, and population research has facilitated gene conservation. The blue or Himalayan pine, *P. wallichiana*, is an important timber tree, especially in India and Pakistan; its wide geographic and altitudinal range requires much more research on population variation and gene diversity for effective conservation of genetic resources. This will only be possible through regional cooperation with support from international organizations.

Subsection *Cembrae* includes several cold-climate and high-elevation five-needle pines of Eurasia and western North America. Research on the phylogenetics of the Siberian stone pines (*P. sibirica* and *P. pumila*) and *P. parviflora* indicates that the current partitioning of Subsections *Strobi* and *Cembrae* needs revision. Research on within-species genetic diversity in Siberia shows that there are relatively low interpopulation differences within the stone pines, and that natural hybridization occurs between the species. Korean pine also has small genetic distances between populations, even over a wide area; the main diversity occurs within populations. All of these species are nut pines, related to the North

American *P. albicaulis*. The interconnection between subsections is shown by the similarity of ecological role between the high elevation species limber pine, the pines of Subsection *Cembrae*, and the Rocky Mountain bristlecone pine (*P. aristata*), all of which occupy and stabilize habitats not likely to be occupied by other, less tolerant tree species. They are for the most part, less susceptible to blister rust, some much less, than is *P. albicaulis*.

These are but a few of the many findings of recent research brought out in the conference. The extensive worldwide distribution of the five-needle pines, their varied and critical ecological roles in the plant and animal diversity of the world's forest ecosystems, and their aesthetic and economic importance to human society are all indicators of the need for continued worldwide research on these important forest trees. We look forward to continued cooperation and information exchange in the future.

Howard Kriebel
Medford, New Jersey, USA
June 13, 2003

Acknowledgments

The impetus for this volume came from the IUFRO Five-Needle Pine Breeding and Genetic Resources Working Party international conference that was held at the IUFRO XX World Congress in Tampere, Finland, in 1995. Many people and organizations facilitated the planning and undertaking of this conference and the subsequent compilation of this volume. We wish to thank the USDA Forest Service (FS - the International Forestry, Research, and Forest Health groups from the Washington Office as well as the Region 6 Genetics group all provided vital contributions) and USDI Bureau of Land Management (BLM) which served as local hosts for the meeting and field excursions. Thanks to Regional Geneticist Sheila Martinson for opening remarks and support. Along with the editors, Harvey Koester and Joe Linn helped coordinate local arrangements. Many people contributed to success of the meeting including Andy Bower, Jeremy Kaufman, Jeremy Pinto, Bob Danchok, Sally Long, Ryan Berdeen, Laura Berdeen, Jerry Berdeen and Clinton Armstrong from Dorena Genetic Resource Center, Umpqua National Forest (FS); Tom Atzet and Don Goheen (FS); Liang Hsin, Terry Tuttle, Gordon Lyford, Tammy Jebb, Larry Price, Bill Robinson, and Dennis Pyle from BLM; Joel King and the staff (including Smokey Bear) at Prospect Ranger District, Rogue River National Forest. We thank Mike Cloughesy, Cindy Wardles, and Nathalie Gitt of the Forestry Outreach Education office at Oregon State University for support in planning and logistics, and external reviewers S. Aitken, P. Berrang, J. Dunlap, J. Hamlin, R. Hunt, R. Johnson, A. Kegley, B. Kinloch, J. King, S. Kolpak, S. Martinson, D. Oline, and P. Zambino for their technical reviews of the papers. We specifically acknowledge Konstantin Krutovskii (FS) for facilitating communication with Russian scientists and assistance with Russian manuscripts.

The sponsors of the conference and this volume include IUFRO, USDA Forest Service, USDI BLM, Crater Lake National Park, Oregon Department of Forestry, Washington Department of Natural Resources, The University of Tennessee, Inland Empire Tree Improvement Cooperative (IETIC), White Pine Working Group (IETIC), Sierra Pacific Industries, and Forest Renewal BC (British Columbia); this array of sponsors allowed us to invite speakers from throughout the world. The hospitality shown by both the conference hotel (Rogue Regency Inn in Medford), and the town of Jacksonville was truly outstanding. The conference banquet hosted by Dennis and Mary Ann Ramsden in the gardens of the McCully House Inn in Jacksonville on a beautiful southern Oregon evening was truly superb.

We thank all participants of the conference and all authors of papers. A special thanks to Angelia Kegley (Dorena Genetic Resource Center) who was invaluable with many phases from conference planning to publication. We thank Louise Kingsbury (FS) and her staff at Rocky Mountain Research Station Publishing Services for their patience and for preparing the final publication and the RMRS for distribution of this volume.

Contents

Part I: Regional Overview Papers

P. balfouriana **(foxtail pine),** ***Pinus aristata*** **(Rocky Mountain bristlecone pine) and** ***P. flexilis*** **(limber pine)**

P. aristata and *P. flexilis* photos courtesy of A. Schoettle

P. balfouriana photo courtesy of D. Burton

Genetics, Breeding, Improvement and Conservation of *Pinus strobus* in Canada

G. Daoust
J. Beaulieu

Abstract—The aim of this paper is to present an overview of the research work carried out in eastern Canada over the last 50 years to increase knowledge of the genetics of eastern white pine (*Pinus strobus* L.), the most majestic conifer of eastern Canada. The intent of the paper is also to describe the accomplishments achieved in breeding and tree improvement by a number of private and public sector organizations from different eastern Canadian provinces as well as the activities that are currently underway. Ontario's program, which has been cancelled but which comprised the production of interspecific hybrids from rust-resistant species such as Himalayan white pine (*P. wallichiana* A.B.Jackson), is briefly described. Results of recent studies of population structure of eastern and western North American blister rust (*Cronartium ribicola* J.C. Fisher) are reported. Estimated genetic gain for height 10 years after plantation from a network of three provenance-progeny tests established in Quebec is presented with the origin of the most promising progenies. Other related subjects such as white pine weevil, flower induction, somatic embryogenesis and seed orchard production are discussed. Finally, work presently being carried out for *in situ* and *ex situ* conservation of genetic resources of eastern white pine in eastern Canada are summarized.

Key words: eastern white pine, *Pinus strobus*, genetics, interspecific hybrids, blister rust.

Introduction

Eastern white pine (*Pinus strobus* L.) is the most majestic of all the conifer species growing in eastern Canada. It has a very broad tolerance range and is found on a wide variety of soils ranging from well-drained sands and rocky ridges through sphagnum bogs (Farrar 1995). In Canada, eastern white pine's natural range is limited mainly to the southeast, and extends from eastern Manitoba all the way to Newfoundland (fig. 1). This species is characteristic of Canada's Great Lakes and St. Lawrence Forest Region, where fire plays a primary role in the establishment of extensive stands of eastern white pine (Whitney 1986).

Eastern white pine was overharvested for many decades, owing to the huge size of the mature trees and their prized wood qualities. By the end of the 19th century, the extensive resources of this species had been irremediably decimated in all of eastern Canada, from Ontario to Newfoundland. The subsequent introduction of an exotic pathogen—white pine blister rust (*Cronartium ribicola* J. C. Fisher)—caused major losses in areas that had been naturally and artificially regenerated. Today, with the exception of some zones of white pine in southeastern Ontario and southwestern Quebec, there are only scattered remnants of the beautiful natural stands that once covered eastern Canada. In Quebec, for the year 1998, nearly 90 percent of the volume of wood harvested out of the eastern white pine annual allowable cut of 730,000 m^3 came from the southwestern part of the province (Bouillon 1998).

During the past century, the efforts devoted to reforestation of this species have varied considerably both spatially and temporally. The virulence of pests like blister rust and the white pine weevil (*Pissodes strobi* Peck) are largely to blame for the failures and cutbacks that have occurred in reforestation programs. At present, white pine makes up just a little over 1 percent of the total number of conifer seedlings planted in eastern Canada yearly. Scattered distribution of the species at the landscape level, fire

Figure 1—Natural range of eastern white pine (Wendel and Clay 1990).

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 3800, Sainte-Foy, QC, Canada, G1V 4C7. Tel.: (418) 648-5830, Fax: (418) 648-5849, e-mail: gdaoust@cfl.forestry.ca

suppression, competition from other plants and blister rust have affected natural regeneration and have dramatically reduced the presence of the species in the landscape. In some regions such as Anticosti Island, browsing from white tail deer prevents development of advance regeneration. To put harvested stands back into production, forest companies currently rely mainly on silvicultural practices designed to mimic natural disturbances that favour eastern white pine natural regeneration.

Over the past 50 years, a number of private and public sector organizations have made great efforts to learn more about the genetics of this species, establishing breeding programs and increasing knowledge of the most damaging pests. This document provides an overview of the results obtained to date and describes briefly the activities that are currently underway in eastern Canada.

Population Genetics

Population genetics studies help us to understand genetic changes occurring within and among populations. Knowledge obtained can be used to devise breeding strategies adapted to the species life history traits. During the last decade, studies of eastern white pine population genetics were carried out using isozymes as well as DNA markers. Results of these studies showed that there was a high level of genetic diversity in this species, and that about 95 percent of it is located within populations. Population differentiation was reported to be about 2 percent on average for populations sampled in Quebec (Beaulieu and Simon 1994, Isabel and others 1999) while it was about 6 percent for populations located in Ontario and Newfoundland (Rajora and others 1998). This low level of population differentiation means that gene flow is extensive. It was also shown that eastern white pine is a predominantly outcrossing species with a very low selfing rate as reported in a study of its mating system in two populations (Beaulieu and Simon 1995). Finally, while it was shown that the level of genetic diversity in this species is relatively high, a loss of genetic diversity was made clear in the St. Lawrence Lowlands and it is believed to be caused by highgrading that was practiced during the last century (Beaulieu and Simon 1994).

Provenance Testing

Provenance testing of eastern white pine started as early as the 1950s in Ontario, but these first trials were inconclusive and did not include a good representation of provenances (Zsuffa 1985). The first comprehensive information on genetic variation in eastern white pine was derived from a range-wide provenance test initiated by the U.S. Department of Agriculture in 1955. Two out of the 15 provenance tests established were located in southern Ontario (Ganaraska and Turkey Point; fig. 2) and included 12 out of 31 provenances tested in the range-wide study. However, only three provenances of eastern Canada (Ontario, Quebec

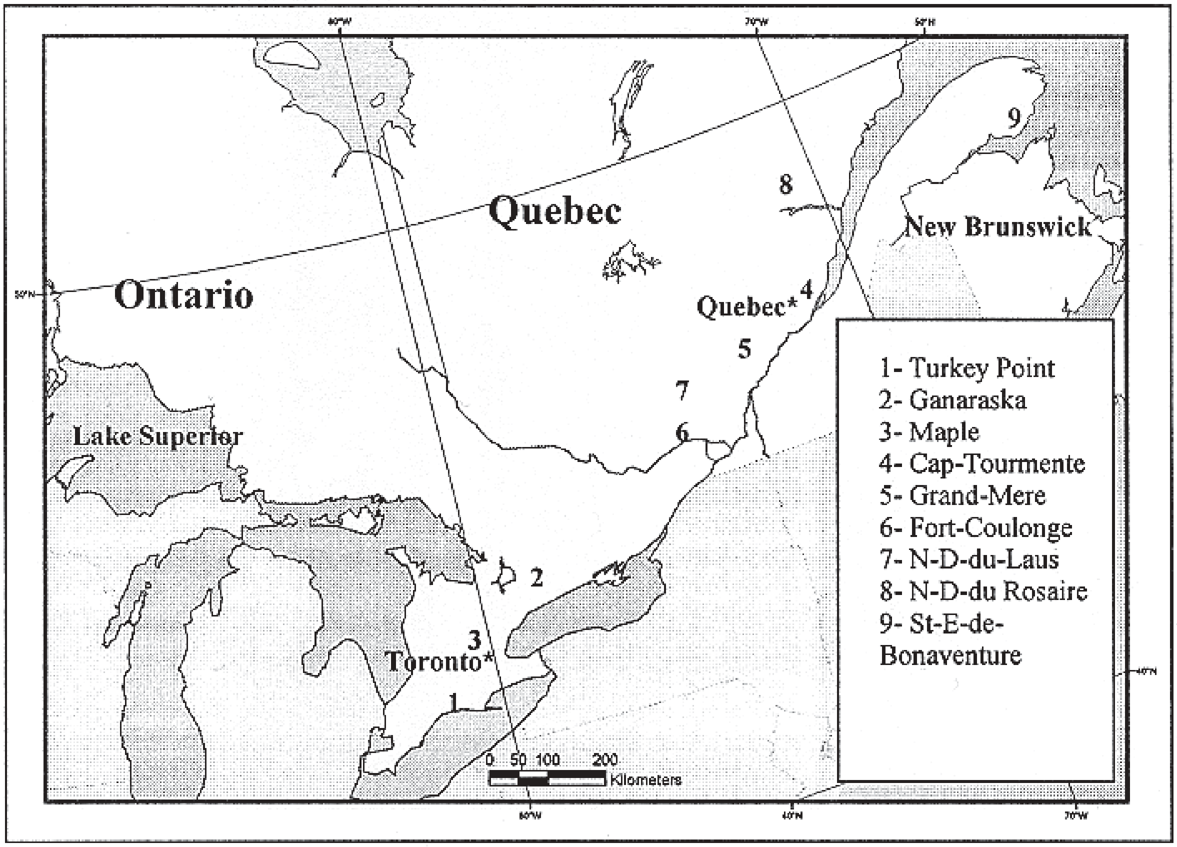


Figure 2—Location of some collections and some provenance-progeny tests in Ontario and Quebec.

and Nova Scotia) were represented. Results observed in these two tests located in southeastern Ontario were reported for 7-year (Fowler and Heimburger 1969) and 28-year (Abubaker and Zsuffa 1991) morphological and growth traits. Significant differences among provenances were found for each of the 12 morphological and growth characters studied. Variation in some characters was more significant than for others. The fastest growing provenances (Pennsylvania, Maine, New York in the U.S.A. and Nova Scotia in Canada) had fewer forked trees, wider branch angles and finer branch diameters and were all from the Atlantic coast. On a broader perspective for provenance testing carried out in North America, Wright and others (1979) demonstrated that trees from the southern Appalachian mountains grew the fastest in the eastern United States and eastern Canada.

The number of provenances originating from Quebec and Ontario tested in the range-wide provenance trial was too small to provide the basic information needed for the breeding program initiated in the 1970s in Quebec (Corriveau and Lamontagne 1977). Hence, a series of three provenance-progeny tests was established in Quebec in 1988. Over 250 open-pollinated progenies belonging to 67 provenances coming from eastern Canada and the eastern United States were tested. Four- and ten-year height data were analyzed by Beaulieu and others (1996) and Li and others (1996). It was shown that there is an extensive variation in eastern white pine and that much of this variation is located within progenies and provenances. Significant differences among provenances and among progenies within provenances were disclosed. Furthermore, the estimates of heritability at the family level were moderate (10-year) to high (4-year), suggesting that tree breeding would be successful. Culling the worst progenies in the nursery could be effective without negatively impacting the expected genetic gain. Nine out of the 10 best provenances identified at 10 years of age were from outside Quebec. Four of them were from the Atlantic coast and the five others were from the Great Lakes region (Ontario in Canada and Minnesota and Michigan in the U.S.A.).

Blister Rust

Introduced in the early 20th century into North America on eastern white pine seedlings imported from Germany, white pine blister rust is now considered to be the most prevalent disease of eastern white pine in eastern Canada. In general, disease incidence increases from west to east and is related to total rainfall and the decrease in mean July and August temperatures. Populations growing on coastal areas are particularly affected by the disease. In Newfoundland, for instance, all the trees in some young plantations, with some as young as 6 years old, were killed by the rust (pers. comm. J. Bérubé 2001). In other provinces, such as Quebec, areas where eastern white pine is particularly susceptible to the rust have been mapped (Lavallée 1986), and ecological characteristics of plantation sites that reduce the risk of infection have been identified (Lavallée 1991). Plantations established in partial shade are generally less severely affected than those established in open fields (Boulet 1998).

Recently, Et-touil and others (1999) studied the genetic structure of nine populations of blister rust in eastern Canada. They found that most of the total gene diversity ($H_T = 0.386$) was present within populations ($H_w = 0.370$), resulting in a low level of genetic differentiation among populations ($F_{st} = 0.062$). No statistically significant genetic differences either among provinces or among regions were revealed. The eastern Canadian provinces are considered to make up one large white pine blister rust epidemiological unit. Furthermore, gene flow between the populations is high and trees tested in this unit could be infected by inoculum travelling hundreds of kilometres.

Comparing eastern Canadian blister rust populations with western ones, Hamelin and others (2000) found that the populations clustered into two distinct clades, one from the east and one from the west. Furthermore, the genetic differentiation was very high (F_{st} = 44 percent). Results of this study suggest the presence of a barrier to gene flow between blister rust populations from eastern and western North America but that there may be zones in central North America where the two populations can bridge. It will be important to determine if the genetic differences between eastern and western populations could also be translated into differences in adaptation, virulence, or any other traits having a high impact in terms of this host-pathogen pathosystem.

White Pine Weevil

The white pine weevil (*Pissodes strobi* Peck) is the native insect that has the greatest impact on the quality of white pine trees growing in plantations. Studies conducted on this insect and its relationship with the species have focussed primarily on management techniques (Stiell and Berry 1985) and on the environmental variables of plantation sites (Lavallée 1992, Lavallée and others 1996) with the goal of reducing tree susceptibility and the consequences of infestation. In eastern Canada, few genetic studies have been carried out on family or individual resistance to the weevil but it was demonstrated by Ledig and Smith (1981) that there is genetic variation for weevil resistance in eastern white pine. With regard to the susceptibility of different provenances, Abubaker and Zsuffa (1991) showed that there was a significant difference among the 12 North American seed sources tested at two locations in Ontario. However, the variance that could be attributed to the provenances accounted for only 11.5 percent of total variance. Although it is possible to select specific phenotypes (narrow crown, slender leader and resin flow) for their resistance to the weevil, this phenotypical resistance appears to vary widely and even disappear depending on the environment and the conditions at the plantation site (Zsuffa 1985). In 1996, a farm-field test comprising 14 open-pollinated families with some putatively resistant families was established at the Valcartier Forest Station in Quebec and will make it possible in the near future to study family variation for resistance to the weevil (pers. comm. R. Lavallée 2001).

Breeding and Improvement

History and Present Status in Ontario

The first breeding program for eastern white pine in eastern Canada was initiated in Ontario by C. Heimburger in 1946. The main goal of this program, which was a major effort for that time, was to develop varieties resistant to white pine blister rust. The program included selection in natural stands and the propagation of white pines free of symptoms of the disease, as well as the production of interspecific hybrids from rust-resistant species such as Himalayan white pine (*P. wallichiana* A.B.Jackson (syn. *P. griffithii* McClelland). For the eastern white pine, any significant resistance expressed after blister rust inoculation was particularly notable in the progenies coming from healthy parents. Zufa (1971) found that the percentage of diseased trees in progenies of both healthy and diseased parents was similar and very high. No major genes for resistance were found.

A great deal of effort was put into developing hybrids. At the Maple Research Station (fig. 2) , 17 soft pine species were tested and the breeding effort resulted in about 100 hybrids (table 1). The program was successful, resulting in the development of rust-resistant interspecific hybrids (Zsuffa 1981). Most notable were the eastern and Himalayan white pine hybrids, because of their superior growth, high level of resistance to blister rust and ability to transmit these characteristics to future generations (Heimburger 1972, Zsuffa 1976). However, selections of resistant material based

Table 1—List of the interspecific five-needle pine hybrids produced by the Ontario Ministry of Natural Resources and maintained in research archives by the Ontario Forest Research Institute.

armandii x albicaulis
ayacahuite x strobus
cembra x armandii
cembra x albicaulis
cembra x strobus
flexilis x wallichiana
koraiensis x albicaulis
koraiensis x lambertiana
lambertiana x koraiensis
monticola x ayacahuite
*monticola x (wallichiana** x strobus (P. Schwerinii))*
monticola x parviflora
(monticola x parviflora) x strobus
(monticola x parviflora) x (wallichiana x strobus)
(monticola x parviflora) x pentaphylla
monticola x pentaphylla
monticola x peuce
(monticola x peuce) x (wallichiana x strobus)
monticola x strobus
parviflora (glaucous)*
parviflora (glaucous) x (strobus x parviflora)*
parviflora glauca
parviflora glauca x strobus
parviflora x albicaulis
parviflora x wallichiana
parviflora x strobus
parviflora x (strobus x parviflora)
(parviflora x strobus) x strobus
(parviflora x strobus) x (strobus x parviflora)
pentaphylla x peuce
pentaphylla x (strobus x parviflora)
peuce x (flexilis x wallichiana)
peuce x (wallichiana x strobus)
peuce x flexilis
peuce x wallichiana
peuce x (monticola x parviflora)
peuce x parviflora
peuce x pentaphylla
peuce x (peuce x strobus)
peuce x strobus
(peuce x strobus (wind) x (peuce x strobus)*
(peuce x strobus (wind) x strobus*
peuce x (strobus x wallichiana)
(peuce x strobus) x (flexilis x wallichiana)
peuce x (strobus x peuce)
(peuce x strobus) x (peuce x strobus)
pumila x strobus
pumila x (wallichiana x strobus)
strobus x albicaulis
strobus x (flexilis x wallichiana)
(strobus x wallichiana) x (wallichiana x strobus)
(strobus x wallichiana) x (wallichiana x strobus) (P. Schwerinii)
strobus x (wallichiana x strobus)
strobus x wallichiana
strobus x monticola
strobus x parviflora
(strobus x parviflora) x peuce
(strobus x parviflora) x strobus
(strobus x parviflora) x (strobus x parviflora)
strobus x pentaphylla
strobus x peuce
(strobus x peuce) x wallichiana
(strobus x peuce) x peuce
(strobus x peuce) x monticola
(strobus x peuce) x (peuce x strobus)
strobus x (peuce x strobus)
strobus x (strobus x parviflora)
wallichiana x albicaulis
wallichiana x ayacahuite (P. Holfordiana) x parviflora
wallichiana x (wallichiana x parviflora)
wallichiana x lambertiana
wallichiana x (wallichiana x strobus)
wallichiana x (wallichiana x strobus) (P. Schwerinii)
wallichiana x strobus
wallichiana x koraiensis
wallichiana x parviflora
(wallichiana x parviflora) x (wallichiana x parviflora)
wallichiana x pentaphylla
wallichiana x peuce
wallichiana x strobus (P. Schwerinii) x wallichiana
wallichiana x strobus (P. Schwerinii) x (wallichiana x strobus)
wallichiana x strobus (P. Schwerinii) x peuce
(wallichiana x strobus) x (wallichiana x parviflora)
(wallichiana x strobus) x (wallichiana x strobus)
(wallichiana x strobus) x (wallichiana x strobus) (P. Schwerinii)
(wallichiana x strobus) x pentaphylla
(wallichiana x strobus) x strobus
wallichiana x strobus (P. Schwerinii)

* Recognition of this name uncertain.
** Syn. *P. griffithii McClelland*
Source: Personal communication from B. Sinclair, Ontario Forest Research Institute, 2001.

on the results of this program were never made and included in operational seed orchards (Cherry and others 2000). In a study using eight *P. wallichiana* x *P. strobus* clones, Zsuffa (1975) found moderate broad sense heritability values for tree heights (0.62) and diameters (0.45) and moderately high broad sense heritability values for branch lengths (0.76) and branch angles (0.71).

Although significant efforts were also made to develop varieties resistant to the white pine weevil, they were mainly unsuccessful, particularly the hybrids of such promising species as *Pinus peuce* Griseb. and *Pinus monticola* Doug. The *P. monticola* hybrids were poorly adapted to climatic conditions in Ontario while the resistance of several *P. peuce* hybrids broke down (Zsuffa 1985). In the mid-1980s, the financial resources allocated to the production and the selection of interspecific hybrids and to the development of blister rust resistant eastern white pine were reduced and finally the program ended. The most promising material (pure or hybrid species) produced in this program was established on six different sites in Ontario and comprised, at the beginning, more than 4500 trees. The Ontario Forest Research Institute (OFRI) is conserving these research archives. The status of these collections is however presently unknown and there is a high probability that most of the trees are dead due to several factors including lack of cold hardiness (pers. comm. B. Sinclair 2001).

In the late 1970s, an intensive plus-tree selection program was launched by the Ontario Ministry of Natural Resources (OMNR) in all the major white pine regions to meet the needs of an expanding reforestation program. By the late 1980s, the province had developed eight breeding populations and an extensive network of seed orchards, comprising 18 orchards covering over 130 hectares. However, few seeds were collected from the selections and, therefore, no progeny tests were carried out (Cherry and others 2000). Despite warnings by Zsuffa (1985) that only continued efforts would allow full benefits to be derived from all the work done to date, significant budget cuts in the mid-1990s stripped breeding programs to their bare bones. The white pine breeding program and the development of seed orchards were put on hold. Unfortunately, to all intents and purposes, no improved seed has been obtained from the orchards for reforestation. All the seeds being used in the current reforestation program were collected in natural stands during logging operations (pers. comm. D. Joyce 2001).

In the mid-1990s, a genecology study of eastern white pine that sampled the current Ontario natural range of eastern white pine east of Lake Superior was initiated by the OMNR. Genetic tests were set up and preliminary results were used to establish a breeding zone for the 'North Bay' tree improvement program. Growth variation among populations was significant and showed a clinal pattern along environment gradients; southern populations generally grew faster than the northern ones (pers. comm. P. Lu 2001). Now there is a renewed interest in Ontario for research on eastern white pine resistance to blister rust and progeny testing of the genotypes present in the seed orchards (Cherry and others 2000). P. Lu has recently proposed a study of genetic resistance to blister rust (pers. comm. D. Joyce 2001). To meet some forest management objectives, the forest company Tembec Inc. has also recently reactivated a genetic improvement program for eastern white pine in the North Bay area. Open-pollinated families collected on 265 clones present in the regional seed orchard were recently sown. The company plans to carry out progeny tests in 2002.

History and Present Status in Quebec

In the late 1970s, an eastern white pine breeding program was initiated for Quebec by Corriveau and Lamontagne (1977), under which genetically improved varieties would be created by selecting and hybridizing superior genotypes for growth, shape and resistance to white pine blister rust and white pine weevil. Although a 1995 program review recommended this area of activity be transferred to the provincial government, the program has remained headed by the Canadian Forest Service (CFS). The ministère des Ressources naturelles du Québec (MRNQ) has not been able to take it over due to limited human and financial resources. Progress and accomplishments of the breeding program were reported by Daoust and Beaulieu (1999).

From 1976 to 1986, over 150 selections were made to create the first-generation breeding population in Quebec. Selections were propagated by grafting and grown in a breeding orchard at the Cap-Tourmente National Wildlife Area east of Quebec City (fig. 2). Beginning in 1992, large crops of seed and pollen cones allowed the production of full-sib families; in addition, a 6 x 6 diallel was created for a study on genetic variation in the capacity of somatic embryogenesis initiation. Several experimental designs to evaluate general and specific combining ability were developed in the last few years and are now in production at the Valcartier Forest Station or are in their first post-planting year. Seeds produced in breeding orchards that are not needed for the breeding program are collected by the MRNQ for its reforestation program. In the 1980s, plus-tree selections made in natural stands by the MRNQ as well as selections formerly made for the breeding program were used to establish a network of six seed orchards for producing more than 3 million seedlings yearly. Significant seed production has begun in two out of the six orchards. However, cones were heavily damaged by a white pine cone beetle (*Conophthorus coniperda* (Schwartz). Insect populations will have to be monitored and controlled. Use of pheromones to control this insect seems to be promising. A research project is in progress at the Institut National de Recherche Scientifique (INRS) - Institut Armand-Frappier (pers. comm. R. Trudel 2001).

Since the inception of the breeding program, seeds were collected in more than 100 eastern white pine natural populations in Quebec for *ex situ* conservation and genecological studies. Seed lots from neighbouring provinces and states have also been obtained from a number of collaborators. In 1986, the first phase of a genecology study involving 300 progenies derived from 160 populations was established on three different sites in Quebec (Fort-Coulonge, Notre-Dame-du-Rosaire, Saint-Elzéar-de-Bonaventure; fig. 2). These tests were set up in cut strips under a partial canopy of tolerant hardwoods, a plantation management technique recommended to reduce risks of infestation by the white pine weevil. Despite an initially high survival rate (+ 80 percent after 6 years) and intensive stand tending, it became clear that little valuable information about genetic variation in juvenile growth could be obtained from these tests because blister rust was ravaging the plants, as was significant browsing damage from hares. At Saint-Elzéar-de-Bonaventure, the most eastern site, the survival rate was only 46 percent 11 years after planting and half of the survivors were rust infected; no progeny with more than 50 percent of unaffected seedlings was found. Only 14 percent of the progenies tested showed a proportion between 30-43 percent of unaffected seedlings.

Fortunately for this first phase, growth and phenological traits measured during production in the greenhouse (1 year) and nursery (3 years) made it possible to study the genetic structure and patterns of variation of white pine populations in Quebec (Li and others 1997). Data were also used to delineate preliminary seed zones following a method proposed by Campbell (1986), which estimates relative risks in transferring seed sources. Principal component analysis (PCA) was used to take into account all the traits at the same time. Analysis of variance made it possible to show that provenances were significantly different for each of these traits as well as for the PCA scores. In examining the patterns of variation, it was clear that even though southeastern provenances flushed later, they had superior growth mainly because they set their buds later. Seed source transfer for eastern white pine in southwestern Quebec is now controlled through estimates of relative risks obtained from mathematical models that were developed.

In 1988, another series of three other genecological tests was established (Grand-Mère, Notre-Dame-du-Laus, Notre-Dame-du Rosaire; fig. 2) under a partial canopy of mature pioneer species. These tests included 250 progenies representing 67 provenances. Although intensive stand tending was done from the beginning, the average survival rate for each test 12 years after planting ranged from 53 percent to 69 percent. The main pest affecting tree survival in the tests was blister rust. Genetic variation in juvenile growth was analyzed using these tests and the main conclusions were reported by Beaulieu and others (1996). Provenances and progenies were shown to be phenotypically stable over the three environments. Breeding values were estimated using best linear predictions (BLP) and estimates of genetic gain for height, 10 years after plantation, were obtained for the best 50 progenies. Hence, a 14 percent (9-29 percent) genetic gain is expected for height growth 12 years after planting. For each progeny, three elite trees were chosen and propagated by grafting to create a breeding population. Out of the 50 progenies selected, 22 are from Quebec, 6 from Ontario, 10 from Vermont, 5 from Michigan and 7 from other U.S. states.

During the 1990s, several other provenance-progeny tests comprising a smaller number of progenies were established to improve the distribution of experimental blocks in the province. One of these is located in Béarn in the Témiscamingue region and was carried out in co-operation with Tembec Inc. These tests will supplement the information obtained to date and be used for estimating the number of progenies required to accurately evaluate the value of a provenance.

Up to now, little work has been done to select material resistant to blister rust in eastern white pine. This is mainly because breeders considered that the genetic variability in blister rust resistance in eastern white pine is too low to expect substantial gains through intra-specific selection and breeding. So, most of the work has been directed toward transferring blister rust resistance found in other species to the eastern white pine. It is for this purpose that the most interesting exotic material, identified in Ontario's former program, was included in the breeding program. Thus, 6, 12 and 22 genotypes of *P. wallichiana*, *P. koraiensis* and *P. peuce*, respectively, were obtained from the collection gathered by the OFRI at the Maple Research Station and put together with genotypes making up our eastern white pine breeding population. Seeds obtained from these clones as well as those from interspecific crosses were sown in 2000 and transplanted in the nursery at the Valcartier Forest Station in 2001. Exotics and hybrids will eventually be evaluated for growth, form and resistance.

History and Present Status in the Atlantic Provinces

As mentioned earlier in this document, mortality caused by blister rust infection in natural stands or in plantations is more severe as we move east and closer to the maritime climate. For example, in Newfoundland mortality can reach 30 percent in natural stands and even 100 percent in plantations (pers. comm. J. Bérubé 2001). So, the plus-tree selection program carried out in the Atlantic provinces has always considered blister rust as the main concern and all the trees selected were free of symptoms of the disease at the time of selection.

At present, there is no tree breeding program for eastern white pine in New Brunswick that is headed by the government. However, J.D. Irving. Inc., a forest company, recently initiated a breeding program by establishing a seed orchard including plus-trees selected in New Brunswick and others obtained via collaborators from neighbouring provinces such as Quebec.

Nova Scotia has a breeding program underway for eastern white pine. A clonal seed orchard, made up of 58 locally selected genotypes, was set up in 1981. In 1998, it produced over 26 kg of seed, which exceeds the requirements of the province's reforestation program for the species.

In Prince Edward Island, there is currently no genetic improvement program underway and none is planned in the short term since over 90 percent of forest lands are privately owned. However, first-generation seed orchards were established at the end of the 1980s by the provincial government. Cones have been collected on a regular basis since the mid-1990s (2.7 kg of seeds in 1996).

Although Newfoundland has no active breeding program for the species at this time, interest in eastern white pine is growing. A seed orchard, made up of 200 clones from plus-trees free of symptoms of blister rust selected in natural populations on the island, was established between 1998 and 2000. The orchard will ensure a supply of high-quality seed while allowing the *ex situ* conservation of genetic diversity of the island (English and Linehan 2000).

Progress reports about eastern white pine breeding and improvement are regularly produced and published by the active members of the Canadian Tree Improvement Association in their biennial proceedings. A description of the eastern white pine seed orchards in place in the eastern Canadian provinces is presented in table 2.

Table 2—Description of the eastern white pine seed orchards in the eastern Canadian provinces.

Province	No. of seed orchards	No. of clones	Year of establishment	Breeding generation	Area (ha)	Seed production
Ontario	18	2500	Late 80s	First	130	No
Quebec	7	700	1981-91	First	33.3	Yes
	1	140	1999	Second	1	No
New Brunswick	1	—	1998	First	—	No
Prince Edward Island	2	70	1988-90	First	2.8	Yes
	1	—	1995-97	—	—	No
Nova Scotia	1	58	1981	First	2.1	Yes
Newfoundland	1	200	2002	First	2.0	No

Flower Induction

In the late 1980s, studies on flower induction in eastern white pine were undertaken in Ontario and Quebec. In Ontario, flower induction trials carried out on 3-5-year-old potted grafts revealed that spraying $GA_{4/7}$ at concentrations of 250 and 500 mg /L were effective in promoting pollen- and seed-cone production. Significant increases in pollen-cone production were obtained when applications were made during the period of rapid terminal shoot elongation whereas applications made about one month after completion of the terminal shoot elongation were the best for favouring seed-cone production (Ho and Schnekenburger 1992). Similar results were also obtained at the Valcartier Forest Station, Quebec, between 1990 and 1992, where some trials including root pruning, spray application of $GA_{4/7}$ and heat stress were carried out on 1.5-m to 2-m high potted grafts. Despite high clonal variation, some ramets produced over 200 seed cones and over 500 pollen strobili (Daoust and Beaulieu 1999). For field-grown grafts, Ho and Eng (1995) found that $GA_{4/7}$ injection made during the period of shoot elongation also promoted pollen- and seed-cone production.

Somatic Embryogenesis (SE)

Significant progress has been achieved in this research field at the CFS since the first study was carried out in 1990. Garin and others (1998) investigated the somatic embryogenic process using immature and mature zygotic embryos of eastern white pine originating from 13 open-pollinated seed families sampled in the breeding population maintained at the Cap-Tourmente National Wildlife Area. From the immature zygotic embryos, embryogenic tissues were obtained for 12 out of the 13 families with initiation rates varying from 2.6 percent to 23 percent. Mature somatic embryos were produced for 30 out of 52 cell lines and plants were regenerated. Embryogenic tissues were also obtained from mature zygotic embryos and embryogenic cell lines developed for 5 out of the 13 families tested with a maximum initiation rate of 2.7 percent. Plants were produced from four cell lines. Those produced from immature as well as from mature zygotic embryos are presently under evaluation at the Valcartier Forest Station. After the success obtained in SE, it was decided to make a complete 6 x 6 diallel with maternal trees whose seed families had high, intermediate and low initiation rates. This diallel will make it possible to determine the genetic components and their effects on SE initiation.

Recently, the optimal concentration of plant growth regulator to be put into the culture medium was found. It made it possible to increase the SE initiation rate in eastern white pine from approximately 20 percent to 53 percent (Klimaszewska and others 2001). This study also demonstrated that a low level of plant growth regulator in the medium used for initiation and proliferation consistently produced a high number of somatic embryos. The different concentrations of plant growth regulator tested allowed somatic embryos to convert to plants at an overall frequency of 76 percent. An estimated narrow-sense heritability for somatic embryogenesis initiation (h^2) of 0.25 was found and indicates that selection of responsive families can be done. According to the authors, with these improved protocols, application of eastern white pine somatic embryogenesis in commercial clonal forestry is feasible as an alternative to traditional breeding for reforestation purposes.

Conservation

In eastern Canada, eastern white pine is not considered as a local species at risk or as an endangered species at present. The Ancient Forest Exploration & Research group from Ontario estimated that less than 2 percent of our old-growth white pine forests remain world-wide, making them an endangered ecosystem type (Quinby 2000). After a report prepared by the White Pine Working Group of Newfoundland, describing the deplorable situation of the species in the province, the government issued a moratorium on domestic and commercial cutting licences in 1998. The province's policy is underpinned by a reforestation program and precise guidelines on precommercial thinning operations. A genetic diversity selection and conservation program was launched on the island in 1999 and a 2-ha seed orchard was established.

Presently on crown lands, where harvesting is carried out, forest companies are required to regenerate the species naturally or artifically and must also comply with the sustainable management principles that are enforced in the various provinces. In Ontario, for example, the Ministry of Natural Resources put out a silviculture guide partly designed for white pine stands in 1998 (OMNR 1998) and developed a conservation strategy for old-growth pine forest ecosystems (OMNR 2001). However, it seems that in all the conservation programs in place in eastern Canada, not enough resources are allocated to study the impact of blister rust on the long-term survival of the species.

In situ conservation of the species is also being carried out in existing parks and reserves in eastern Canada (Boyle 1992). This protection may be complete as is the case in national parks or it may be partial, as in some provincial parks. In the latter case, logging activities can take place but they are subject to certain restrictions designed to ensure natural regeneration of harvested species among other things. In Ontario, following the recommendations of the Temagami Comprehensive Planning Council, the government decided in 1996 to protect 11 old-growth red and eastern white pine stands ranging from 100 to 9,000 ha in the Temagami region (Quinby 2000). In Quebec, the most representative ecosystems are protected by a network of ecological reserves. Other stands, identified as exceptional forest ecosystems, will be protected in the near future by a recently adopted law (pers. comm. N. Villeneuve 2001).

In Mauricie National Park, Quebec, a master plan has been drawn up for the ecological rehabilitation of white pine (Quenneville and others 1998). Tests involving prescribed burning were conducted but proved to be inconclusive since they did not coincide with a good cone crop year. In eastern Quebec, work is also in progress to establish a white pine restoration program. In spring 2001, for instance, the Lower St. Lawrence Model Forest collaborated on the establishment, within its territory, of two open-pollinated progeny tests that will be used to estimate the breeding values of the trees making up the first-generation breeding population built up by the CFS. In Ontario, the OMNR has begun to develop restoration goals for white pine ecosystems along the northern edge of its range and a task force has been mandated to develop principles, criteria and management guidelines for ensuring the long-term persistence of old-growth forest in unprotected areas. The task force will use white pine as a test case (pers. comm. D. Joyce 2001).

Ex situ conservation activities include all the experimental plots used for breeding programs, such as provenance tests, progeny tests, breeding orchards and clonal archives. Similarly, the regional seed orchards put in place by provincial governments and forestry companies are excellent tools for conserving genetic resources. These orchards, which are made up of locally selected genotypes, represent a broad sample of the genetic diversity that is present in eastern white pine in eastern Canada. The different provincial reforestation programs, which are carried out using local seed sources most of the time, also help to conserve genetic diversity at the regional level.

Another valuable *ex situ* conservation approach is the maintenance of seed banks such as the one that the CFS maintains at its National Tree Seed Centre in Fredericton (Simpson and Daigle 1998) and at the Laurentian Forestry Centre in Quebec City. The seedlots in these collections provide a very good sampling of the genetic diversity of eastern white pine populations in northeastern North America.

Acknowledgments

The authors thank the following persons who graciously provided pertinent information allowing us to present an accurate overview of the eastern white pine status in eastern Canada: D. Joyce, B. Sinclair, P. Lu, from the Ontario Ministry of Natural Resources; B. English, from the Newfoundland Department of Forest Resources and Agrifoods; K. Tosh, from the New Brunswick Department of Natural Resources and Energy; R. Lavallée, J. Bérubé, R. Hamelin and D. Simpson, from Natural Resources Canada, Canadian Forest Service; A. Deshaies, N. Villeneuve, from the ministère des Ressources naturelles du Québec; and R. Trudel, from the INRS, Institut Armand Frappier. They are also grateful to Pamela Cheers, from Natural Resources Canada, Canadian Forest Service, for editing the paper.

References

Abubaker, H.I. and Zsuffa, L. 1991. Provenance variation in eastern white pine (*Pinus strobus* L.): 28th- year results from two southern Ontario plantations. *In* Proc. of a Symposium on White Pine Provenances and Breeding, USDA For. Serv., Tech. Rep. NE-155.

Beaulieu, J., Plourde, A., Daoust, G. and Lamontagne, L. 1996. Genetic variation in juvenile growth of *Pinus strobus* in replicated Quebec provenance-progeny tests. For. Genet. 3:103-112.

Beaulieu, J. and Simon, J.-P. 1994. Genetic structure and variability in *Pinus strobus* in Quebec. Can. J. For. Res. 24:1726-1733.

Beaulieu, J. and Simon, J.-P. 1995. Mating system in natural populations of eastern white pine in Quebec. Can. J. For. Res. 25:1697-1703.

Bouillon, D. 1998. Pin blanc et rouge en Outaouais. *In* Recueil des conférences du colloque “Du pin blanc pour l’avenir c’est possible”, 3-4 juin 1998, Mont-Laurier, QC, Canada. Compiled by Conseil de la recherche forestière du Québec, pp. 91-99.

Boulet, B. 1998. Un pin blanc vulnérable. *In* Recueil des conférences du colloque “Du pin blanc pour l’avenir c’est possible”, 3-4 juin 1998, Mont-Laurier, QC, Canada. Compiled by Conseil de la recherche forestière du Québec, pp. 13-23.

Boyle, T.J.B. 1992. Forest tree genetic conservation activities in Canada. For. Can., Science and Sustainable Development Directorate, Ottawa, Inf. Rep. ST-X-4.

Campbell, R.K. 1986. Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. Silvae Genet. 35:85-96.

Cherry, M., Lu, P. and Sinclair, B. 2000. Ontario Forest Research Institute. *In* J.D. Simpson, ed. Proc. 27th Meeting Can. Tree Improvement Assoc., Part 1, Sault Ste. Marie, ON, August 15-17, 2000, Nat. Resour. Can., pp. 81-82.

Corriveau, A.G. and Lamontagne, Y. 1977. L’amélioration génétique du pin blanc au Québec. Fisheries and Environment Canada, Laurentian For. Res. Centre, Inf. Rep. LAU-X-31.

Daoust, G. and Beaulieu, J. 1999. Bilan des réalisations du programme d’amélioration génétique du pin blanc. *In* Actes du colloque “L’amélioration génétique en foresterie : où en sommes-nous?” Ressour. nat. Can., Serv. can. for., pp. 87-94.

English, B. and Linehan, B. 2000. The status of tree improvement in Newfoundland and Labrador. *In* J. D. Simpson, ed. Proc. 27th Meeting Can. Tree Improvement Assoc., Part 1, Sault Ste. Marie, ON, August 15-17, 2000, Nat. Resour. Can., pp. 18-19.

Et-touil, K., Bernier, L., Beaulieu, J., Bérubé, J.A., Hopkin, A. and Hamelin, R.C. 1999. Genetic structure of *Cronartium ribicola* populations in eastern Canada. Phytopathology 89:915-919.

Farrar, J.L. 1995. Trees in Canada. Fitzhenry & Whiteside Limited and Canadian Forest Service.

Fowler, D.P. and Heimburger, C. 1969. Geographic variation in eastern white pine, 7-year results in Ontario. Silvae Genet. 18:123-129.

Garin, E., Isabel, N. and Plourde, A. 1998. Screening of large numbers of seed families of *Pinus strobus* L. for somatic embryogenesis from immature and mature zygotic embryos. Plant Cell Rep. 18:37-43.

Hamelin, R.C., Hunt, R.S., Geils, B.W., Jensen, G.D., Jacobi, V. and Lecours, N. 2000. Barrier to gene flow between eastern and western populations of *Cronartium ribicola* in North America. Phytopathology 90:1073-1078.

Heimburger, C.C. 1972. Breeding of white pine for resistance to blister rust at the interspecies level. *In* R.T. Bingham, R.J. Hoff and G.I. McDonald, eds. Biology of rust resistance in forest trees. USDA For. Serv. Misc. Publ. 1221, pp. 541-549

Ho, R.H. and Eng, K. 1995. Promotion of cone production on field-grown eastern white pine grafts by gibberellin $A_{4/7}$ application. For. Ecol. Manag. 75:11-16.

Ho, R.H. and Schnekenburger, F. 1992. Gibberellin $A_{4/7}$ promotes cone production on potted grafts of eastern white pine. Tree Physiol. 11:197-203.

Isabel, N., Beaulieu, J., Thériault, P. and Bousquet, J. 1999. Direct evidence for biased gene diversity estimates from dominant random amplified polymorphic DNA (RAPD) fingerprints. Mol. Ecol. 8:477-483.

Klimaszewska, K., Park, Y.-S., Overton, C., MacEacheron, I., and Bonga, J.M. 2001. Optimized somatic embryogenesis in *Pinus strobus* L. In Vitro Cell. Dev. Biol.-Plant 37:392-399.

Lavallée, A. 1986. Zones de vulnérabilité du pin blanc à la rouille vésiculeuse au Québec. For. Chron. 62:24-28.

Lavallée, A. 1991. La protection du pin blanc. L'Aubelle 86, Formation continue, cours n° 21, 12 pages.

Lavallée, A. 1992. Observations on the evolution of damage by *Pissodes strobi* Peck and characterization of young white pine plantations affected by this weevil. For. Can., Quebec Region. Inf. Rep. LAU-X-98E.

Lavallée, R., Archambault, L. and Morissette, J. 1996. Influence of drainage and edge vegetation on levels of attack and biological performance of the white pine weevil. For. Ecol. Manag. 82:133-144.

Ledig, F.T. and Smith, D.M. 1981. The influence of silvicultural practices on genetic improvement: height growth and weevil resistance in eastern white pine. Silvae Genet. 30:30-36.

Li, P., Beaulieu, J., Daoust, G. and Plourde, A. 1997. Patterns of adaptive genetic variation in eastern white pine (*Pinus strobus*) from Quebec. Can. J. For. Res. 27:199-206.

Li, P., Beaulieu, J., Magnussen, S., Daoust, G. and Plourde, A. 1996. Application of spatial statistics in two genetic tests of eastern white pine. Nat. Resour. Can., Can. For. Serv., Laurentian For. Centre, Sainte-Foy, Qc. Inf. Rep. LAU-X-120.

OMNR. 1998. A silvicultural guide for the Great Lakes-St. Lawrence conifer forest in Ontario. Version 1.1. Ontario Minist. Nat. Resour., Sault Ste. Marie, ON.

OMNR. 2001. A Conservation Strategy for Old Growth Red and White Pine Forest Ecosytems for Ontario. http/www.mnr.gov.on.ca/MNR/forests/forestdoc/oldgrwstr.html.

Quenneville, R., Thériault, M. and Foisy, L. 1998. La restauration des écosystèmes de pin blanc (*Pinus strobus*) au parc national de la Mauricie. *In* Recueil des conférences du colloque "Du pin blanc pour l'avenir c'est possible", 3-4 juin 1998, Mont-Laurier, QC, Canada. Compiled by Conseil de la recherche forestière du Québec, pp. 33-40.

Quinby, P.A. 2000. An overview of the Conservation of Old-Growth Red and Eastern White Pine Forest in Ontario. Ancient Forest Research Report No. 23. http/www.ancientforest.org/rr23.html.

Rajora, O.P., DeVerno, L., Mosseler, A. and Innes, D. J. 1998. Genetic diversity and population stucture of disjunct Newfoundland and central Ontario populations of eastern white pine (*Pinus strobus*). Can. J. Bot. 76:500-508.

Simpson, J.D. and Daigle, B.I. 1998. A list of seed at the National Tree Seed Centre. Nat. Resour. Can., Can. For. Serv., Atlantic For. Centre, Inf. Rep. M-X-203E/F.

Stiell, W.M. and Berry, A.B. 1985. Limiting white pine weevil attacks by side shade. For. Chron. 61:5-9.

Wendel, G.W. and Smith, H.C. 1990. Eastern White Pine. http://www.na.fs.fed.us/spfo/pubs/silvics_manual/Volume_1/pinus/strobus.htm

Whitney, G.G. 1986. Relation of Michigan's presettlement pine forests to substrate and disturbance history. Ecology 67:1548-1559.

Wright, J.W., Amiel, R.J., Cech, F.C., Kriebel, H.B., Jokela, J.J., Lemmien, W.A., Matheson, A.C., Merrit, C., Read, R.A., Roth, P., Thor, E. and Thulin, I.J. 1979. Performance of eastern white pine from the southern Appalachians in eastern United States, New Zealand and Australia. *In* Proc. 26th Northeastern Forest Tree Improvement Conference, July 25-26, 1978, University Park, PA. pp. 203-217.

Zsuffa, L. 1975. Broad sense heritability values and possible genetic gains in clonal selection of *Pinus griffithii* McClelland x *P. strobus* L. Silvae Genet. 24:85-88.

Zsuffa, L. 1976. Poplar and pine *Populus*, *Pinus* breeding in 1973 and 1974. *In* Proc. 15th Meeting Can. Tree Improvement Assoc., Part 1. Edited by C.W. Yeatman and K. Illingworth. Can. For. Serv., Ottawa. pp. 89-95

Zsuffa, L. 1981. Experiences in breeding *Pinus strobus* L. for resistance to white blister rust. *In* Proc. XVII IUFRO World Congress, Division 2, Ibaraki, Japan. pp. 181-183.

Zsuffa, L. 1985. The genetic improvement of eastern white pine in Ontario. *In* Proc. Entomol. Soc. Ont., White pine symposium, Petawawa Natl. For. Inst., September 14, 1984. 116:91-94

Zufa, L. 1971. Poplar and pine breeding at the Southern Research Station, Maple, Ontario in 1970. *In* Proc. 13th Meeting Can. Tree Improvement Assoc., Part 1. Edited by D.P. Fowler and C.W. Yeatman. Can. For. Serv., Ottawa. pp. 85-93.

Five Needle Pines in British Columbia, Canada: Past, Present and Future

John N. King
Richard S. Hunt

Abstract—In British Columbia (BC), Canada, we have been involved with white pine and blister rust since the rust's discovery on imported infected pines through the port of Vancouver in 1910. Just after the rust's introduction, the USDA Forest Service established monitoring plots and species trials in BC, but these were abandoned when the rust became well established in the USA. Resistance research began again in 1946 with a collection of western white pine (*Pinus monticola* Dougl. ex D.Don) seed that was sent to Ontario for testing. In about 1950 grafted plus trees were inoculated in a disease garden, but this work was also abandoned in 1960 when it was demonstrated that seedlings from such selections could be susceptible. Parent tree selection and seedling inoculation of open-pollinated families of western white pine began again in earnest in 1987. From this material we have the basis of a breeding and seed orchard program based on partial resistance mechanisms. An F_1 generation is being produced for future research. Additionally, we are considering single gene resistance traits, such as MGR, which can be pyramided onto the partial resistance of our breeding population. Efforts, particularly for conservation interests, are also being started for whitebark pine (*P. albicaulis* Engel.).

Key words: genetic resistance, western white pine, whitebark pine, limber pine

Five Needle Pines in British Columbia

Both western white pine (*Pinus monticola* Dougl. ex D.Don) and whitebark pine (*P. albicaulis* Engel.) achieve the northern extent of their distributions in British Columbia (BC), Canada, while limber pine (*P. flexilis* James) achieves the limit of its distribution in the Canadian Rockies in both Alberta and BC. The two alpine species, whitebark and limber pine, provide valuable tree cover for wildlife in exposed alpine country, food for birds and small mammals, stabilizing elements for snow packs and soils in these steep and fragile environments, and are an important feature of the aesthetics of the high mountains. Western white pine, besides providing many of these features, is also a fast growing and highly valuable component of BC's timber industry. Although these species may suffer from the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), and *P. albicaulis* relies on Clark's nutcracker (*Nucifraga columbiana* Wilson) for regeneration, their most serious threat has been the introduction of blister rust (*Cronartium ribicola* J.C. Fischer) in the early part of the 1900s. So great was the damage due to blister rust, that it was felt that these species might be lost completely. The major research effort with the white pines has therefore been an intensive search for resistance to blister rust. To this end, the USDA Forest Service established rust resistance programs in Idaho, Oregon, and California. The Canadian and BC Forest Services also established rust screening programs in BC. The prognosis for western white pine is now considerably better – although some of the other species such as whitebark pine are still at a risk. We outline below the events and the progress made to date and strategies we are hoping to develop to protect this valuable natural resource.

Past—Introduction of *Cronartium ribicola*

In 1911 British Columbia (BC) was experiencing a boom with a population of 392,500, more than double the previous 10-year census. The population was largely farmers, loggers, and coal miners with little education. Quite likely Tom Newman planned to take advantage of the boom when he imported 1,000 exotic *P. strobus* L. seedlings from France for resale in 1910. However, most of the recent immigrants were from Britain, and in 1914 many of the men returned to Europe to fight in World War I. Before the war, Newman and others sold some imported pines, but sales crashed with the outbreak of the war, and the remaining plants seem to have been abandoned (Gussow 1923). Perhaps Newman never returned from the European conflict; certainly many did not, and the war had effectively put an end to the boom. Under these circumstances it is amazing that blister rust was first identified in BC only 3 years after the war, in the fall of 1921 (Gussow 1923). Although the potential value of the white pines was understood, in those years in BC there was a stronger commercial interest in currants (*Ribes* spp), the alternate host of blister rust (Eastham 1922; 1922/3). However, the U.S. Forest Service was worried about the threat to western white pines, particularly *P. monticola*, and they dispatched field personnel to BC as early as 1922.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

John N. King is Research Scientist, Research Branch, British Columbia Ministry of Forests, P.O. Box 9519 Stn Prov Govt, Victoria, B.C. V8W 9C2, Canada. Phone: 250 387-6476. Fax: 250 387-0046. Email: John.King@gems7.gov.bc.ca. Richard S. Hunt is Research Pathologist, Pacific Forestry Centre, Canadian Forest Service, 506 West Burnside Road, Victoria, B.C. V8Z 1M5, Canada. Email: R.Hunt@PFC.forestry.ca.

From the Portland, OR, office researchers came to Vancouver, BC, then north via train to near present day Whistler, BC. Here they established a species susceptibility trial (Childs and Bedwell 1948) and various research plots (Lachmund 1934; Childs and Kimmey 1938). Once it was clear that the rust was well established in the United States, no new plots were established in BC. About that time (1927) the lone collaborating Canadian scientist was killed in a car crash (Estey 1994), and Canadian rust research stopped until 1946.

In 1946 the provincial chief forester had *P. monticola* seed collected from the BC interior and sent to Heimburger in Ontario for resistance testing. Both Heimburger and Riker in Wisconsin were doing resistance testing in *P. strobus*. In 1948 the Canadian government hired Porter to do resistance testing in BC. Porter followed Riker's protocol of grafting plus-trees and placing them in a ribes (disease) garden (fig. 1). He obtained scions from survivors in the old U.S. Forest Service plots near Whistler, trees in similar plots that he had established, and a few trees recommended by the BC Forest Service. He rated clones by percentage of ramets cankered after 5 or 7 years in a disease garden. These were also placed in three forest sites and subjected to natural infection. The most promising *P. strobus* and *P. monticola* from Heimburger and *P. strobus* from Riker were also placed at these sites. When it was discovered that grafts from old trees can produce susceptible offspring (Patton 1967), the program ended, and Porter left to become a school teacher. All the material from one field site was transferred to the University of BC experimental forest, and the other sites were abandoned. These sites were revisited in the 1980s, and the cankering of the clones tended to follow Porter's (1960) original ranks (Hunt and Meagher 1989).

The success of Bingham's resistance work in Idaho (Bingham 1983) and the need for *P. monticola* for reforesting laminated-root-rot (*Phellinus weirii* (Murr.) Gilb.) sites was the catalyst for the BC Forest Service and Canadian Forest Service (CFS) to sign a cooperative memorandum of understanding on blister rust resistance in 1983. Disease free plus-trees were selected for both Interior and Coastal populations. Open-pollinated (OP) cones were collected from the selected trees, and the resulting progeny seedlings were exposed to blister rust in inoculation chambers. The first successful inoculation occurred in 1987 and was repeated annually to 1995. This material is now the basis for white pine seed orchards and resistance breeding programs in BC.

Present

The resistance program continues in BC primarily through the continuing cooperative relationship between the provincial government, the CFS, which provides pathology research and screening, and increasingly the Forest Industries, which provide technical support through their seed orchard programs. The efforts of the past have allowed us, at this stage, to assess the resistance found to date, not just in the populations screened in BC, but also in other jurisdictions in Western North America. We can also assess the transferability of seed sources of western white pine and are looking at the most appropriate strategy of seed deployment from our seed orchards in order to use the best available resistance with well-adapted seed sources. Not all of these questions can be answered at present, but current research should answer them in the near future.

Figure 1—Porter's screening for blister rust resistance by growing grafted *Pinus monticola* ramets from blister rust resistant candidates in a disease (ribes) garden at Duncan BC in 1955.

Resistance Story to Date

Most of the resistance programs in Western North America to date have concentrated on selections and screening of open-pollinated families from surviving canker-free parent trees. The strong selection pressure, first in the natural stands and then in inoculation chambers, almost assures that these are not mere "escapes" but that there is a genetic basis to this resistance. However, as with the original Riker method of screening grafts, it has been difficult to determine the basis of resistance and how the resistance is inherited. The exception is the case of the hypersensitive response (HR), a major gene resistance (MGR) found in sugar pine (*P. lambertiana* Dougl.) and some populations of *P. monticola* (for example, Champion Mine) (Kinloch and others 1970, Kinloch and others 2003). Although there are some complexities to MGR (Kinloch and Dupper 1998, Kinloch and others 1999), it is relatively simple and easily understood because it is a classical vertical resistance controlled by a single dominant gene. More complex resistances, falling under the headings of "partial resistance" and "tolerance", are more difficult to characterize, and we have a much poorer understanding of their genetic basis.

In BC we have now made a series of nearly 600 selections from the CFS screening program. This included a fairly intensive parent tree selection from both the Interior and Coastal BC (about 300 from each population) and rust screening of the OP progeny for what may be considered two "partial resistance" mechanisms: "slow-canker growth" (Hunt 1997) and "difficult-to-infect seedlings" (Hunt and others 1998). We have also selected a set of trees from established plantation trials and from Texada Island where a stand was characterized with "tolerance" and trees were selected for their marked "bark reaction" response.

Although the first orchard selections were based on forward selection of the progeny from the screening trials, lately, where it is feasible, we have switched to collecting scion from the original selected parents. Selection of parent material, rather than progeny, has allowed us to proceed much faster with our breeding program, as seed cones can be produced on ramets in as little as 2 years. Also the hypothesis presented that some of the resistance found in the Idaho populations may be controlled by recessive genes (McDonald and Hoff 1971; Hoff 1988) has encouraged us to use parents rather than OP progeny and concentrate future screening on a F_1 population constructed from the best parents. Crossing for this breeding program consists of crosses between parents of similar putative mechanisms, crosses with susceptible parents and selfs. Selfs, where they can be made, will be particularly valuable if recessive genes are involved. The construction of this F_1 breeding population is now well under way (fig. 2), making use of structured mating designs that will help in future genetic interpretations.

Transferability and Adaptability of White Pine Seed Sources

Seed transfer guidelines have been, and continue to be, developed from three series of trials that test most of the

Figure 2—Pollination bags for breeding program crosses on top-pruned young grafts of western white pine at CanFor Seed Orchard, Sechelt BC (photo courtesy R. Sniezko).

range of western white pine on 24 sites throughout BC (fig. 3). The first series contrasted the R.T. Bingham (Moscow, ID) arboretum seed source with a local BC source. Trials were established within and north of the species range. The second was established in nine root disease sites and included 14 provenances covering the range limits of the species (Hunt 1987). The third had 12 provenances with family structure on six sites. These trials have been described in detail (Hunt 1987, Hunt 1994, Hunt and Meagher 1989, Meagher and Hunt 1998, Meagher and Hunt 1999), and these results are summarized below. We also report results from recent assessments from two of the family/provenance trials.

Results from these series show that western white pine does not show a strong clinal response to growth or disease resistance, but rather there are abrupt changes. Most striking are the southern populations from the Sierras, Klamath, and Warner Mountains that grow poorly and are generally highly susceptible to rust, even more so than the northern populations (perhaps as a result of physiological opportunities that the rust can exploit) (Hunt 1994; Meagher and Hunt 1998). Rehfeldt and others (1984) also showed this absence of a strong geographic pattern of variation except in these southern populations. Sources north of these (especially north of the Columbia River) and extending east as far as Montana are less dramatic in their differences. Interior sources, including Idaho, grow well at the coast (Bower 1987; Meagher and Hunt 1998). Coastal sources tended to be slightly inferior for growth (Meagher and Hunt 1998) and less hardy (Thomas and Lester 1992) than interior sources on the interior sites. Some of our more northerly populations do well for juvenile vigour on our interior sites (Meagher and Hunt 1998). The Idaho material was more winter-damaged than BC sources in the trials north of the species range. The resistance of the Moscow, ID, arboretum material held up well in BC's interior but was lower on coastal sites (Hunt and Meagher 1989; Meagher and Hunt 1999). Thus, the Idaho material is not recommended for the northern part of the range nor at the coast but is recommended for the southern Interior. At 5 years, the Champion Mine (MGR) source from southern Oregon showed poor vigour as did the more northern Oregon sources (Mt. Hood and Willamette; fig. 3, sources 29 and 30). Based on this it was recommended that the Columbia River should be a southern transfer boundary and that sources from Oregon should not be used in BC (Meagher and Hunt 1998).

Recent, 2001, Reassessment of Coastal Family/Provenance Trials

In 2001 12-year assessments were made on the two coastal family/provenance trial sites (Ladysmith and Sechelt, fig. 3, locations close to sources 24 and 26) for growth and survival. We present here some preliminary results that allow a reflection on the above recommendations after rust has greatly affected these two coastal sites. Details of the Provenance Plantations experiments are provided in Meagher and Hunt (1998). But briefly this included 12 provenances with four to five cone-parents per provenance with an additional five more bulked provenances. Figure 3 shows the

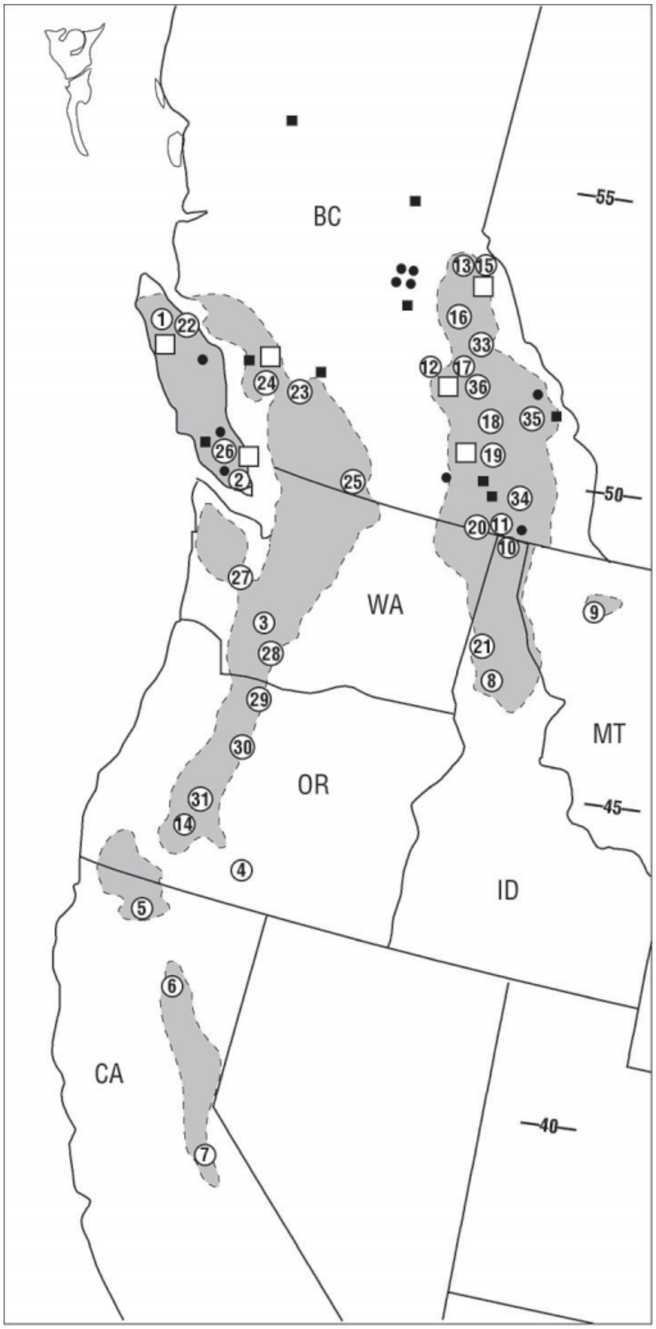


Figure 3—Distribution of western white pine (shaded area), provenance origins (circled numbers) and plantations. Solid circles indicate plantations using R.T. Bingham (Moscow) arboretum seed source contrasted to local sources. Solid squares indicate plantations in the root-rot disease experiment sites with provenances 1 through 14. Open squares indicate plantations that have some or all of provenances 15 through 35. The Ladysmith and Sechelt site are these latter open squares near provenaces 26 and 24, respectively.

provenance collections site (numbers 15 through 31) and the trial locations. Six plantations (3 Coastal and 3 Interior) were established with 25 replicates per plantation. The measurements reported here include height, rust and overall survival on two of the Coastal sites (fig. 3, trial sites close to origin sources, 24 (Sechelt) and 26 (Ladysmith)). In terms of survival, rust has not been the only mortality agent although it is by far the most causative agent. Both sites have been damaged by bough pickers (white pine is highly desirable for Christmas decorations), but this was not deemed to hinder our results and interpretations.

Anova models were run on each of these sites for the following effects: replicates, geographic origin and families within geographic origin. Means analysis – Student-Newman-Keuls (SNK) were also conducted on the geographic origin groupings (Steel and Torrie 1980). Geographic origin groups included: Northern Interior BC (Valemont, Raft River, Barriere and Mt. Revelstoke, fig. 3, sources 15, 16, 17, and 18); Southern Interior BC (Arrow and Trail, fig. 3, sources 19 and 20); Idaho (bulk unselected collections not F_2, fig. 3, source 21); Vancouver Island (includes low elevation Sunshine Coast) (fig. 3, sources 22, 24 and 26); Lower Mainland High Elevation (Cascade) BC (Whistler and Manning Park, fig. 3, sources 23 and 25); Washington Olympic Peninsula (fig. 3, source 27); Northern Oregon Cascade (Mt Hood and Willamette, fig. 3, sources 29 and 30); Southern Washington Cascade (White River, fig. 3, source 28) and Dorena Oregon – "Champion Mine" (fig. 3, source 31). Also included were some selected seedlots. These include the Westar selections – Southern Interior BC but selected as clean parent trees; the Dorena "Champion Mine" MGR selections; and the Porter selections as described above; and at the Ladysmith site, only exotics (mainly *P. strobus* but also *P. koraiensis* Sieb. and Zucc.).

Results are presented for: the percentage canker-free stems in 1995 assessment (CF95); the percentage canker-free stems in 2001 (CF01); mean height and standard errors (in cm) and finally - percent likely crop tree survivors (CT) - those trees, both tall and healthy either canker-free or just minor infections (table 1). Although survival in this last measure reflects other factors such as vigour (height growth), frost survival, and other factors, blister rust escape was by far the major factor.

Ladysmith, although showing the results of blister rust ahead of Sechelt, has now grown beyond the worst of the infection, and 30 percent of the original healthy trees are alive and likely to remain so (some as tall as 12m at age 12). On this site three sources were significantly less infected than the rest. One of the exotic species, *P. koraiensis*, showed markedly less infections in the 1995 assessment (only 9 percent), but this species quickly fell behind for growth rate and had faded from the planting by 2001. Although *P. strobus* has continued to show good survival, it has also shown signs of frost damage and poor overall vigour (table 1; fig. 4). The Dorena seedlot did the best for rust survival (as expected) but was lower ranked for growth (reflecting the earlier assessment) (table 1; fig. 4). One of the more impressive lots was the Porter families, which were second only to the Dorena source for both clean trees in 2001 (CF01) and potential crop trees (CT). The Porter families were also the tallest and were significantly different from the Dorena lot for vigour (height growth) (table 1; fig. 4). This selected lot screened for early survival, using Riker's *P. strobus* protocols, appears to have been effective on a site such as Ladysmith.

The Vancouver Island, Washington, and northern Oregon sources performed quite similarly. The Idaho lots as a whole, as in the earlier analysis, were poor on these coastal sites for survival (mainly blister rust) but were good for growth; these, however, were nonselected Idaho material. The Northern Interior BC source and the high elevation Lower Mainland BC were poor for both growth and survival. Although geographic origins were significant in our model (P<0.0001) so were families within origin. The Southern Interior BC origin, which had the largest number of families, showed up

Table 1—Results showing : % clean trees 1995 – CF95, % clean trees 2001 – CF01, mean height and standard error, and % crop trees (CT), which are defined as those trees that are alive and healthy (either canker free, branch canker, or tolerant stem reaction) and greater than 3 m at Ladysmith or greater than 2 m at Sechelt.

	Trial Sites							
	Ladysmith				Sechelt			
Seedlots	CF95	CF01	HT01 ± se	CT	CF95	CF01	HT01 ± se	CT
Overall plantation	56	30	610 ± 4	27	65	20	461 ± 4	18
Northern Interior BC	46	15	576 ± 15	26	62	14	399 ± 12	12
Southern Interior BC	53	25	639 ± 24	36	73	20	467 ± 24	21
Westar S. Interior BC	52	27	627 ± 5	35	66	23	484 ± 7	21
Idaho	51	22	603 ± 12	29	56	9	460 ± 11	9
Coastal BC, High Elevation	44	10	551 ± 22	15	59	10	455 ± 17	8
Vancouver Island BC	46	20	614 ± 15	28	60	16	470 ± 14	17
Porter families BC	68	52	679 ± 13	55	78	25	510 ± 20	27
Olympic Peninsula WA	51	29	619 ± 31	37	70	22	452 ± 24	21
Southern Cascade WA	62	40	605 ± 25	42	72	33	426 ± 23	23
Northern Cascade OR	54	29	585 ± 20	35	64	17	457 ± 17	15
Dorena OR Champion Mine	74	65	573 ± 20	65	84	49	494 ± 17	40
Exotics (*P strobus*)	73	48	534 ± 15	46				

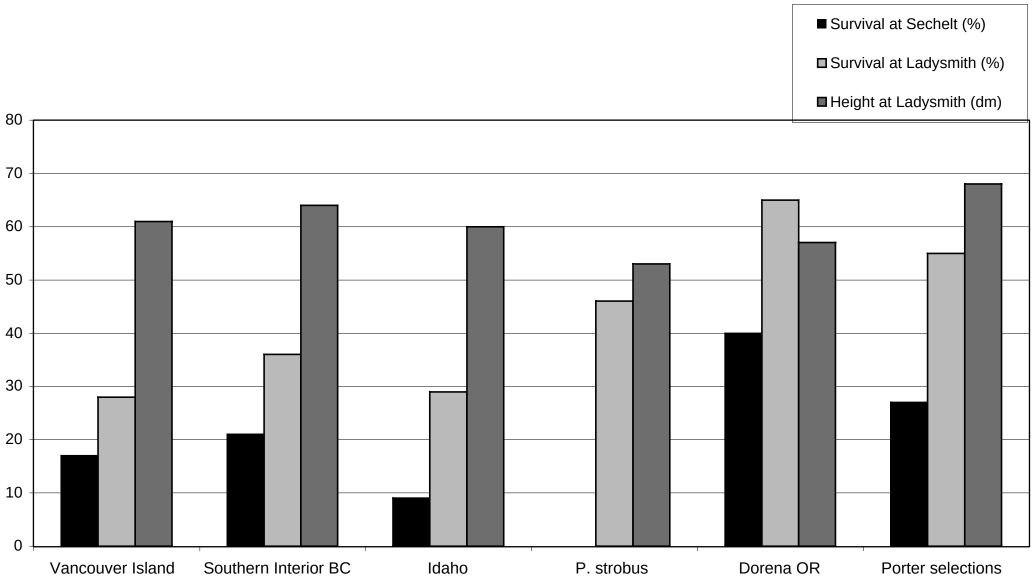


Figure 4—Height (dm) at Ladysmith plantation and Survival (%) at Sechelt and Ladysmith plantations for five *P. monticola* sources and the *P. strobus* source in 2001 at age 12. (Survival refers to crop tree survival as discussed in the text.)

well for growth but not survival; however; several families showed consistently better survival over both sites.

Although the Porter families, screened for phenotypic survival in ribes gardens (fig. 1), did well on such sites as Ladysmith, on higher rust hazard sites such as Sechelt heavy mortality continues (overall plantation infections going from 35 percent to 80 percent in 5 years; table 1). The phenotypic survival selection as conducted by Porter is likely equivalent to the partial resistance screening of the current programs. This indicates to us that on severe rust sites, MGR, such as in the Dorena "Champion Mine" source, will need to be combined with the partial resistances in order to have any trees survive.

Future

The future prospect for western white pine against blister rust is hopeful. Certainly compared to other exotic pathosystems, such as chestnut blight (1904 introduction) caused by *Cryphonectria parasitic* (Murrill) Barr. on American chestnut (*Castenea dentata* Marsh. Borkh.) or to Dutch elm disease caused by *Ophiostoma ulmi* (Buism.) Nannf. (1920's introduction) and *O. novo-ulmi* Brasier (1940's introduction) on elm species (*Ulmus* spp.), there does appear to be a reasonable degree of native resistance. Confirmation of this resistance from the inoculations to field trials is under way through a series of excess stock trials (Hunt 2002). Investigation of these trials together with continued measurements of the provenance trials will help us to establish deployment guidelines for the orchard seed which will soon be available.

Deployment Potential for Western White Pine in BC

Orchards in the interior BC have a predominant element of material from the Idaho program. The emphasis here will be to incorporate our own selections and compare them to Idaho material.

On the coast, three seed orchards will soon be producing seed. Earlier use of seedling progeny for orchard establishment has now given way to the use of selected parents based on results of the inoculation of their progeny. New material, primarily selections from heavily infected trials, is also being added. All of these selections fall under the general categories of "partial resistance" or "tolerance". In addition to this, we have been encouraged to use "total resistance" pollen based on the performance of "Champion Mine" and "Champion Mine" pollinated seedlots in the "root disease" trials (Hunt 1987, Hunt these proceedings) and shown here (table 1; fig. 4). These seedlots (Dorena in table 1) have the Cr2 gene which conditions a hypersensitive response (HR) in western white pine. The strategy of pyramiding HR can be implemented in seed orchards by either supplemental mass pollination or mass control pollination. Both of these methods have found practical use in BC (Webber 1995).

While Cr2 is a powerful form of resistance (the Dorena seedlot, table 1), a pathotype of rust that overcomes it does exist (vcr2), and Cr2 cannot be seen as an ultimate solution (Kinloch and others 2003). Although there are few examples of "total" or "vertical resistance" pathosystems being durable (Leach and others 2001), a completely durable resistance may not be required. Because most cankering occurs close to the ground in BC (Hunt 1991), resistance may therefore only be needed during the plantation's early years. How fast and far vcr2 will spread and its durability are the more relevant questions. If vcr2 becomes widely distributed, it would negate any further planting of single gene resistance solely based on Cr2. Investigations of Cr2 material in the BC root disease trials have failed to show any virulent pathotypes up to 15 years (Hunt and others these proceedings), and in a Bear Pass, OR, plantation some resistant Cr2 trees are still canker-free after more than 60 years (Sniezko, pers. comm). However, the observation of vcr2, the virulent strain, in a relatively small population (hence small selection pressure) of *P. monticola* with Cr2 at the Happy Camp field station in northern California (Sniezko and others these proceedings) is most certainly disturbing. Some encouragement for using the strategy of pyramiding HR has come from observations made in the long-term deployment and monitoring of sugar pine with the Cr1 hypersensitive response gene. As in western white pine, a pathotype of blister rust virulent to Cr1 exists (Kinloch and Comstock 1980). Data indicate that the virulent strain of the rust (vcr1) does not always arise quickly or spread rapidly (Kinloch and Dupper 1998). This has encouraged us to develop a deployment strategy that attempts to manage the Cr2/vcr2 pathosystem by integrating it into a silvicultural option that would incorporate hazard assessment area to be planted and distance from other plantations.

Future Research Directions

Further investigation of the Cr2 gene and its potential durability is needed. This will include: careful investigation of all plots in which it has been deployed in BC, follow up of the material that the Dorena program has deployed, and continued interaction with the Region 5 sugar pine program which has provided a model for this deployment. Besides Cr2, other "total resistant" genes may exist and be made available. The Dorena Genetic Resource Center is investigating other potential dominant gene resistances in western white pine (Sniezko pers. comm). Although *P. monticola* and *P. lambertiana* do not naturally hybridize (Bingham 1972), there are now *in vitro* fertilization methods (Fernando and others 1997) which may permit such a cross, and thus add Cr1 as a resistance gene in *P. monticola*. The multiplicity of these "total resistance" genes should add to their durability and strategies to use multiple "total resistance" genes need developing.

The pyramiding of several race-specific resistances into a single plant genotype theoretically has the ability to greatly reduce the probability of a mutation to multiple virulence (Wheeler and Diachun 1983). However, this assumes that the mutations to virulence are independent of each other. Empirical evidence from crop literature, however, points to the fact that there is no clear association between the number of resistance genes in cultivars and their durability (Mundt 1990). Some single resistances have proven highly durable while others have been highly ephemeral, and combinations are not necessarily more durable unless specific resistances are included (Johnson 2000). It has been hypothesized that the quality and durability of a plant resistance gene is a function of the fitness penalty of virulence. Even where genes fail, they may be beneficial through a residual effect because they may add a cost to the pathogen of not having the avirulence (Leach and others 2001). The advent of molecular genetics technology to investigate gene function has offered some insights into the potential relationships between viurlence/avirulence and durability. Although avirulence can confer a high degree of fitness in some cases (resulting in durability of HR), in others this does not appear to be so, and these relationships can be complex (Leach and others 2001). Bacterial blight resistance in rice has shown such a positive functional relationship between the avirulence gene in the pathogen and fitness through its aggressiveness (rate a virulent isolate produces an amount of disease) (Vera Cruz and others 2000). The study of gene function and the protein – ligand relationships between resistance, virulence/avirulence in HR pathosystems in the white pines are being investigated by the CFS (Ekramodddoullah and Tan 1998, Yu and others 2002) and may lead to some insights and potential indicators of durability.

HR total resistance is only one component of our resistant breeding program. We will continue to rely on partial resistances and tolerances for the major part of our effort, and the breeding program is directed to families and individuals selected for this type of resistance. By using a structured mating design and cloning of individuals we can begin to construct pedigreed lines to more carefully observe the partial resistances and be in a position to start to understand some of the underlying genetics.

Another part of the investigation of resistance will be the observation of blister rust as an endemic pathosystem with Asian white pines. Some early work with Asian hybrids was conducted by Heimberger in Ontario, and some of this material may still be available (G. Daoust pers. comm). Unlike the other two exotic pathosystems mentioned earlier (chestnut blight and Dutch elm disease), we are not obliged to use species hybrids and backcrossing to save our native gene pool as there does appear to be ample resistance in our native populations. However, the observation of the endemic pathosystem in Asian species and their hybrids with North American white pines should help us greatly in understanding resistance and identifying which resistances are likely to be the most durable.

Biotechnology can help in our efforts. This will include in-vitro fertilization (Fernando and others 1997) to help in hybrid crosses; embryogenesis to clone lines for the pedigreed breeding program (some successful lines have already been produced); molecular biology to detect the protein precursors of HR; and molecular genetic techniques to help in understanding the genetic basis of resistance. A lot of classical breeding and pathological research will need to be continued to realize this effort.

Although a lot of effort has been spent on western white pine to the point where we can start to see the results and envision its return as an important species to our landscape (Fins and others 2001), other species are still in danger. Whitebark pine, *P. albicaulus*, is considered an endangered species in BC and the U.S. Pacific Northwest (Krakowski 2001, Mahalovich, these proceedings). To this end we have initiated a large-scale seed collection. This is both to preserve important gene pools that are under threat and to start some initial screening in this species. The successes we have had to date should encourage us to keep up the effort in reestablishing these species and continue the co-operative atmosphere of this effort throughout the regions where the white pines grow.

References

Bingham, R.T. 1972. Taxonmy, crossability, and relative blister rust resistance of 5-needled white pine. *In* R.T. Bingham (Ed). Biology of rust resistance in forest trees: proceedings of a NATO-IUFRO advanced study institute, August 17-24, 1969. USDA Misc. Publ. No. 1221, pp 271-280.

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: USDA For. Serv. Gen. Tech. Rept. INT-146.

Bower, R.C. 1987. Early comparison of an Idaho and a coastal source of western white pine on Vancouver Island. West. J. Appl. For. 2: 20-21.

Childs, T.W. and J.L. Bedwell. 1948. Susceptibility of some white pine species to *Cronartium ribicola* in the Pacific Northwest. J. For. 46: 595-599.

Childs, T.W. and J.W. Kimmey. 1938. Studies on probable damage by blister rust in some representative stands of young western white pine. J. Agric. Res. 57:557-568.

Eastham, J.W. 1922. Report of provincial plant pathologist, Vancouver, in Report of the BC Dept. Agric. For 1921.

Eastham, J.W. 1922/3. White-pine blister rust in B.C. The Agric. Journ. (Canada) 7:29, 41,57,64.

Ekramodddoullah, A.K.M and Y. Tan. 1998. Differential accumulation of proteins in resistant and susceptible sugar pine inoculated with blister rust fungus, *Cronartium ribicola*. Can. J. Plant Pathol. 20:308-318.

Estey, R.H. 1994. Essays on the Early History of Plant Pathology and Mycology in Canada. McGill-Queens University Press, Montreal, 384 pp.

Fernado, D.D., J.N. Owens, P. Von Aderkas, and T. Takaso. 1997. *In vitro* pollen tube growth and penetration of female gametophyte in Douglas-fir (*Pseudotsuga menziesii*). Sexual-Plant-Reproduction 10:209-216.

Fins, L., J. Byler, D. Ferguson, A. Harvey, M. F. Mahalovich, G. McDonald, D. Miller, J. Schwandt, and A. Zack. 2001. Return of the giants. University of Idaho, Station Bull. 72, 20pp.

Gussow, H.T. 1923. Report of the dominion botanist for the year 1922. Dept. Agric. (Canada) pp 7-9.

Hoff, R.J. 1988. Blister rust resistance in western white pine for eastern Washington, Idaho, and western Montana. In R.S. Hunt (complier) Proc. of a western white pine management symposium, Nakusp BC, May 2-5, 1988, pp12-20.

Hunt, R.S. 1987. Is there a biological risk of western white pine provenances to root diseases, In G.A. DeNitto (compiler) Proc. West. For. Dis. Wk. Conf. 37:29-24.

Hunt, R.S. 1991. Operational control of white pine blister rust by removal of lower branches. For. Chron. 67:284-287.

Hunt, R.S. 1994. The transferability of western white pine to and within British Columbia – blister rust resistance. Can. J. Plant Pathol. 16:273-278.

Hunt, R.S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. Can. J. Plant Pathol. 19:352-357.

Hunt, R.S. 2002. Relationship between early family-selection traits and natural blister rust cankering in western white pine families. Can. J. Plant Pathol. 24:200-204.

Hunt, R.S. and M.D. Meagher.1989. Incidence of blister rust on “resistant” white pine (*Pinus monticola* and *P. strobus*) in coastal British Columbia plantations. Can. J. Plant Pathol. 11: 419-423.

Hunt, R.S., G.D. Jensen, A.K. Ekramoddoullah, and E.E. White. 1998. Western white pine improvement program for British Columbia. Can. Tree Improv. Assoc. 26(1): 157-159.

Johnson, R. 2000. Classical plant breeding for durable resistance to diseases. Jour. Plant Path. 82: 3-7.

Kinloch , B.B. Jr., G.K. Parks and C.W. Fowler. 1970. White pine blister rust: Simply inherited resistance in sugar pine. Science 167:193-195.

Kinloch, B.B. Jr. and M. Comstock. 1980. Cotyledon test for major gene resistance. Can. J. Bot 58:1912-1914.

Kinloch, B.B. and G.E. Dupper. 1998. Evidence of cytoplasmic inheritance of virulence in *Cronartium ribicola* to major gene resistance in sugar pine. Phytopathology 89:192-196.

Kinloch, B.B., R.A. Sniezko, G.D. Barnes, and T.E. Greathouse. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. Phytopathology 89: 861-867.

Kinloch, B.B., R.A. Sniezko, and G.E. Dupper. 2003. Origin and distribution of Cr2, a gene for resistance to white pine blister rust in natural populations of western white pine. Phytopathology 93: 691-694.

Krakowski, J. 2001. Conservation genetics of whitebark pine (*Pinus albicaulus* Engelm.) in British Columbia. MSc Thesis, University of British Columbia, Vancouver BC.

Lachmund, H.G. 1934. Growth and injurious effects of *Cronartium ribicola* cankers on *Pinus monticola*. J. Agric. Res. 48:475-503.

Leach, J.E., C.M. Vera Cruz, J. Bai and H. Leung. 2001. Pathogen fitness penalty as a predictor of durability of disease resistance genes. Annu. Rev. Pathol. 39:187-224.

McDonald, G.I. and R.J. Hoff. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: genetic control of needle-spots-only resistance factors. Can. J. For. Res. 1: 197-202.

Meagher, M.D. and R.S. Hunt. 1998. Early height growth of western white pine provenances in British Columbia Plantations. West. J. Appl. For. 13: 47-53.

Meagher, M.D. and R.S. Hunt. 1999. The Transferability of western white pine to and within British Columbia based on early survival, environmental damage, and juvenile height. West. J. Appl. For. 14: 41-47.

Mundt, C.C. 1990. Probability of mutation to multiple virulence and durability of resistance gene pyramids. American Phytopathology Soc. 80: 221-223.

Patton, R.F. 1967. Factors in white pine blister rust resistance. IURFO XIV Congress (Section 22), Munich, Proc. pp. 876-890.

Porter, W.A. 1960. Testing for resistance to the blister rust disease of western white pine in British Columbia. For. Biol. Lab. Dept. Agric. Victoria BC mineo 19pp.

Rehfeldt, G.E., R.J. Hoff and R.J. Steinhoff.1984. Geographic patterns of genetic variation in *Pinus monticola*. Bot. Gaz. 145: 229-239.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Company, New York.

Thomas, B.R. and D.T. Lester.1992. An examination of regional, provenance and family variation in cold hardiness of *Pinus monticola*. Can. J. For. Res. 22: 1917-1921.

Vera Cruz, C.M., J. Bai, I. Ona, H. Leung, R. Nelson, T-W Mew and J.E. Leach. 2000. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. PNAS 97: 13500-13505.

Webber, J.E. 1995. Pollen management for intensive seed orchard production. Tree Physiology 15:507-514.

Wheeler, H. and S. Diachun. 1983. Mechanisms of pathogenesis. Pages 324-333 In: T. Kommedahl and P.H. Williams (eds.) Challenging Problems in Plant Health. American Phytopath. Soc. St. Paul, MN, USA.

Yu, X., A.K.M Ekramoddoullah, D.W.Taylor, and N. Piggott. 2001. Cloning and characterization of a cDNA of *cro r I* from the white pine blister rust fungus, *Cronartium ribicola*. Fungal Genetics and Biology. 35: 53-66.

Genetics and Breeding of Five-Needle Pines in the Eastern United States

Howard B. Kriebel

Abstract—Research and breeding of five-needle pines in the eastern USA has been concerned mainly with eastern white pine (*Pinus strobus* L.), which has been found to be a highly variable species. Principal attention has been given to the inheritance of growth traits within and among stands and among provenances. Growth of trees of Tennessee, North Carolina and Georgia provenances exceeds that of other origins in many areas of the US and even in other countries. Research basic to breeding indicates that the genetic barrier to species crossability in the soft pines is embryo failure, whereas in hard pines it is pollen tube incompatibility. Patterns of premature cone drop in relation to genetic affinity have also been determined; cone retention is not closely related to capacity to produce viable seed. The expression of inbreeding after selfing in young plantations of *P. strobus* varies with the parents and the site, but the species is more tolerant of inbreeding than most other species of pines. Eastern white pine population studies show that genetic variability may be maintained even in small isolated stands and in the next generation after heavy thinning, although gene pool deterioration may follow cutting if silviculture disregards gene pool conservation. A genetic gain in volume of about 22 percent has been obtained in *P. strobus* from age 13 family selections in first-generation progeny tests. Potentially useful species crosses are eastern white pine x Himalayan pine and eastern white pine x western white pine. The best hybrid families of the eastern white pine x Himalayan cross have been found to exceed *P. strobus* in volume by 22 to 44 percent at ages 17-22 in progeny tests. These two hybrids also exceed *P. strobus* in wood specific gravity. Selection and breeding of *P. strobus* for blister rust resistance has been difficult and has not yet yielded commercially useful resistant genotypes, nor has selection and breeding for white pine weevil resistance been successful. However, new approaches may overcome these problems. Air pollution tolerance varies widely in *P. strobus*; natural selection in native stands has yielded highly tolerant progenies while eliminating the most sensitive genotypes from the gene pool. Future research directions are suggested.

Key words: Five-needle pines, eastern white pine, breeding, species incompatibility, genetic variability, growth rate, species hybrids, resistance, white pine weevil, white pine blister rust, air pollution tolerance.

Introduction

Research on the genetics and breeding of five-needle pines in the eastern USA began about 1950 with species hybridization experiments and early tests for resistance to the white pine weevil (*Pissodes strobi* Peck) and the white pine blister rust (*Cronartium ribicola* J.C. Fischer). During the subsequent 30-year period, eastern white pine (*Pinus strobus* L.) was probably the subject of more extensive cooperative tree improvement research over the eastern half of the USA and eastern Canada than was any other forest tree species, with the exception of the southern pines. The greatest effort was applied to the study of geographic variation over the entire species distribution by replicated provenance tests, first using seed collections made at the range-wide level, then concentrating on the southern Appalachian part of the species range. The results of this work by numerous state university research stations, the USDA Forest Service and Canadian research centers were combined in joint publications issued at various ages. They provide a very useful knowledge base, especially on adaptability and growth traits as related to seed origin interacting with plantation location. These results have now been put into practice in the commercial planting of eastern white pine.

Studies also provided some measure of stand-to-stand and within-stand variation. Controlled breeding research, although not as extensive as the common-garden testing of open–pollinated seed, has provided estimates of the inheritance of growth rate and the potential for genetic gain from half-sib and full-sib families. Efforts to develop weevil and blister rust resistant eastern white pine have, on the other hand, not yet been successful, although work continues. Research on other species of five-needle pines has been primarily limited to investigations of the potential usefulness of some of the more adaptable species and their hybrids as a source of resistance to the weevil and blister rust. A later research effort was the study of the genetic aspects of air pollution tolerance in *P. strobus*, which was, prior to natural selection for tolerance, highly sensitive to sulfur dioxide and ozone, both major pollutants over much of the natural range of the species.

Applied genetic research directed toward improved planting stock of five-needle pines has declined in the eastern USA during the last 20 years as part of the general curtailment of public funding for tree improvement research and low level of support from other sources. Thus available new genetic information on five-needle pines published during the last two decades is mainly concerned with 1) biochemical and molecular analysis of variability in natural stands, and 2) continuing efforts to develop rust-resistant strains of eastern white pine and to apply molecular technology to the weevil resistance problem. This paper covers principal results of fivc-needle pine breeding and improvement

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The author is with Ohio State University (Emeritus), 625 Medford Leas, Medford, NJ 08055 USA. Tel.: 1-609-654-3625 or Fax: 1-609-654-3625 or E-mail: kriebel@medleas.com.

research in the USA; results of Canadian studies of stand and provenance variation in field trials and the long-term Canadian efforts to develop blister-rust resistant eastern white pine are reported in the paper by Daoust and Beaulieu in these proceedings.

Variability in Eastern White Pine

Geographic Variation in Growth Rate in Test Plantations

Extensive range-wide provenance testing has shown that *P. strobus* is a highly variable species, as compared, for example, with the more range-restricted species *Pinus resinosa* Ait. (Fowler 1965). Of particular importance for tree improvement, eastern white pine includes a wide range of genotypes with respect to rate of growth in height, diameter and stem volume, while at the same time retaining the capacity for adaptation to a broad range of environmental conditions. These conclusions are based on statistical analysis of the performance of trees in widely replicated range-wide seed source trials (Wright 1970). In trials at mid-latitudes within the species distribution (Pennsylvania, Maryland, Tennessee, Indiana, Ohio, southern Michigan, Illinois, West Virginia, Nebraska), genotypes of western North Carolina, Tennessee and northern Georgia origin outgrew those of all other provenances by 70 to 80 percent in tree height growth rate by the end of the first decade. The same growth pattern was found in trials in Australia and New Zealand. After the first decade, absolute differences between fast-growing southern and slow-growing northern seedlots increased but relative differences decreased. Diameter growth differences persisted, sustaining large differences in volume during the second decade (Funk 1979, Kriebel 1982). Later trials concentrating on southern Appalachian seed origins confirmed the growth superiority, in all tests, of seed sources in North Carolina, Tennessee and Georgia over those from Virginia, West Virginia and Maryland. The fastest-growing seedlots commonly grew 40 percent faster than the slowest ones, translating into a 2 to 1 superiority in rate of volume growth (Wright and others 1979).

Population Structure

Progeny tests have shown that family differences in height growth rate are commonly large within stands of eastern white pine, the variance component for general combining ability on a family mean basis averaging over several trials about 25 percent of the mean variance over all plots (De Vecchi Pellati 1967, Wright 1970, Kriebel 1978, Thor and Gall 1978). This evidence of high genetic variability is supported by more recent studies of gene diversity in natural stands before and after thinning, facilitated by new technology, using isozyme and DNA analysis. In Wisconsin, using simple sequence repeated satellite DNA markers, Echt (2000) found that genetic diversity in eastern white pine was the same in both 160-year-old trees and their natural regeneration after a shelterwood thinning that removed most trees. Likewise, in Newfoundland, Rajora and others (1998) were unable to show that genetic diversity had declined in *P. strobus* populations, even after a century of decline in population size. Allelic diversity in heavily thinned stands was as high as it was in unthinned stands. In fact, the Newfoundland populations were as genetically variable as those from Ontario, near the center of the species range. On the other hand, isozyme and microsatellite DNA analyses of diversity in two old-growth Ontario stands showed that harvesting caused a loss in genetic diversity (Buchert and others 1997, Rajora and others 2000). Differences in silvicultural treatment of the old-growth stands may explain the differences between the Wisconsin and Ontario results; the forestry program on the Menominee Indian Reservation in Wisconsin area is considered by many to be the finest example of sustainable management of eastern white pine on the continent (Echt 2000).

Genetics of Outbreeding and Inbreeding

Genetic Control over Embryo Formation and Cone Set

High yields of viable seed are usually obtained in eastern white pine from both open and controlled crossing with other individuals of the species. Hybrids can be obtained from crosses with several other species of five-needle pines with varying success in terms of seed yield (Wright 1953). Reported histogenetic and serological studies of fertilization and embryogenesis in “soft” pines (Subgenus *Strobus,* Section *Strobus*) have shown that fertilization and early embryogenesis can occur normally in ovules of non-crossable species combinations, i.e. those crosses never reported to yield viable seed, with subsequent post-fertilization breakdown from embryo inviability. In fact, early development of the true embryo has been observed in some of these crosses that do not yield viable seed (Ueda and others 1961, Hagman and Mikkola 1963, Hagman 1967, Kriebel 1972, 1981). In contrast, incomplete pollen tube penetration and failure of ovule fertilization have been the pattern in similar studies of hard pines (McWilliam 1959, Hashizume and Kondo 1962a, 1962b, Chira and Berta 1965). The evidence from these painstaking studies, therefore, usually based on daily or-near daily ovule collections and sectioning over a period of several weeks and some including more than one year’s data, suggests a fundamental difference in species isolation mechanisms between the soft and hard pines, the soft pines being characterized by embryo inviability and the hard pines by pollen tube incompatibility.

Soft pines and hard pines also differ in the way in which interspecific hybridization affects cone retention. In soft pines, non-crossable combinations consistently retain first-year cones, whereas in hard pines they often do not. It is possible, for example, to cross *P. strobus* with *P. koraiensis* pollen without first-year cone abscission. Table 1 shows the decrease in maternal control over cone abscission as genetic divergence increases between parent species (left column), for both pine subgenera. Subgenus *Strobus* seed maturation is characteristically blocked by embryo inviability and subgenus *Pinus* by pollen incompatibility. In comparisons of the effect of female parent, pollen species and pollination year on premature cone drop in *P. strobus*,

Table 1—Species isolation mechanism and extent of maternal control over cone abscission in relation to degree of outcrossing in soft and hard pine subgenera (Kriebel 1976b).

	Subgenus *Strobus*	**Subgenus *Pinus***
Isolation mechanism ⇒	**Embryo inviability**	**Pollen incompatibility**
Type of cross	**Degree of maternal control[b]**	
Intraspecific, outcross	Strong	Strong
Interspecific, viable seed	Strong	Strong
Interspecific, no viable seed	Strong	Usually weak
Intraspecific, limited pollen [c]	Threshhold	Threshhold
Unpollinated	Almost nil	Almost nil
The other subgenus	Nil	Nil
Another conifer genus	Nil	Nil

[a] Summary constructed from research papers cited in Kriebel (1981), including chronological histogenetic studies of fertilization and embryogenesis, and breeding experiments.

[b] Strong = high level of cone retention.

[c] Different species seem to have different threshold values for the number of collapsed (unpollinated) ovules per cone above which abscission occurs.

extensive histological analysis of developing ovules showed that female parent exercised overriding genetic control in crosses within and between species,. Pollen species had no effect on cone abscission, even in the case of a totally non-crossable species combination, nor did year of pollination. The year effect could be significant if, for example, prolonged heavy rain seriously reduced the pollen supply (Kriebel 1981).

Practical Significance of Inbreeding in *P. strobus*

P. strobus is more tolerant to inbreeding than most other species of pines (Fowler 1965). Inbreeding is found in both unmanaged and managed populations, and appears to be a natural characteristic of the species (Echt 2000). Among randomly-selected trees crossed in a small isolated Ohio stand, six of 8 trees manually self-pollinated had no reduction in seed yield; yield of the other two averaged 22 percent of outcross families. Reduction in growth rate in 5-10-year old offspring resulting from controlled self-pollination was variable compared to outcross progenies, averaging 18 percent on a good site and 38 percent on a poor site. Both open- and control-pollinated progenies from this stand were as vigorous as those from larger stands of comparable structure. (Kriebel 1975, 1982).

Growth Improvement in Eastern White Pine

Heritability of Growth Rate

Estimates of narrow sense heritability of height growth have been calculated from several progeny tests of eastern white pine, and from these estimates, genetic gain has been estimated using fixed assumptions. The heritability estimates have been fairly consistent when based either on individual tree means or on family means. Table 2 summarizes early estimates for height growth from open-pollinated ("half-sib") and control-pollinated (full-sib) progeny tests, and a later estimate of genetic gain in volume at age 13 in Ohio. Height estimates based on individual trees are in general agreement for both half-sibs and full-sibs, as are those with a family mean basis (Adams and Joly 1977, Kriebel and others 1972, Kriebel and others 1974, Thor and Gall 1978).

Keathley (1977) estimated the heritability of volume growth in an Ohio white pine half sib progeny test at age 13 as h^2 (plot mean basis) = 0.45 ± 0.02. From this estimate, using a selection differential of 1.3s and assuming family means to be normally distributed around the plantation mean, estimated genetic gain in volume was about 22 percent (Table 3).

Table 2—Estimates of heritability of tree height and stem volume in eastern white pine (Keathley 1977).

Parameters for estimates	TN[a]	OH[b]	NH[c]
Height, half-sib, individual tree	0.27	0.28	0.33
Height, half-sib, family mean	—	0.59	0.59
Height, full-sib, individual tree	—	0.32	—
Height, full-sib, family mean	—	0.54	—
Volume, half-sib, family mean	—	0.45	—

[a] Age 7, Tennessee, Thor and Gall 1978

[b] Age 13, Ohio, Kriebel 1978

[c] Age 3. New Hampshire, Adams and Joly 1977

Table 3—Estimated genetic gain in tree volume after thinning in a half-sib progeny test of eastern white pine (Kriebel 1978).

Parameter	Statistic
Initial spacing	1 m (within rows) x 2 m
Trees per linear plot	4
Plots per family	12
50% thinning to 2 best trees/plot	Age 13 (to 2500 trees/ha)
h^2 for volume (plot mean basis)	0.45 ± 0.02
Mean 2-tree plot volume	0.021 m^3
Standard deviation (s)	0.008 m^3
Selection intensity	6 families out of 48
Final stocking	313 trees/ha
Selection differential	1.3s
Genetic gain (ΔG)	0.223
Realized gain, thinning at age 18	0.40

Actually, thinning was not made until age 18, since selection was a 2-stage process, combining a low-level individual tree selection within family plots with a subsequent high-level family selection. The realized gain in volume at age 18, based on plot means, was about 40 percent (Kriebel 1983).

Species Trials and Species Hybridization

In addition to arboretum plantings of many of the five-needle pines, a number of species have been tested in the eastern United States for their possible economic value as commercial plantation trees. The two species of most interest have been the Himalayan pine (*P. wallichiana* A. B. Jacks., syn. *P. griffithii* McClel.*)*, and western white pine (*P. monticola* Dougl. ex D. Don). Himalayan pine, or blue pine, as it is known in its native region, has been tested on the provenance level, including some families within provenances. It has fast growth and variable cold hardiness, depending on the seed source and planting region. Although it is cold-hardy in parts of the mid-Atlantic coastal plain, in Ohio it suffers from late spring frost damage to new shoots, resulting in deformity with multiple branching. Seed sources in the eastern Himalayas (Nepal) do not survive winters in this region (Kriebel 1976a, Kriebel and Dogra 1986). Tests were made in Tennessee of some of the same provenances tested in Ohio. Survival and height growth were probably affected by a severe drought rather than cold winter temperatures. Families that had a high percentage of survival and good growth at each plantation came from a wide geographic spectrum (Schlarbaum and Cox 1990). Himalayan pine is generally susceptible to the white pine weevil (Heimburger and Sullivan 1972) and variable in resistance to white pine blister rust (Bingham 1972).

Western white pine has the necessary cold hardiness in the eastern US and Canada, where it has a variable growth rate and good form. It appears to suffer less weevil damage than does eastern white pine, and this is probably its principal value for planting in the these regions (Heimburger and Sullivan 1972). It does not seems to be well adapted to the warmer, drier interior part of the eastern USA within the native range of *P. strobus* and is not recommended for Ohio (Kriebel 1982).

Macedonian white pine (*P. peuce* Griseb.) has been reported to have some resistance to the white pine weevil. It has good form but it has a slower growth rate in the eastern US than eastern white pine (Wright and Gabriel 1959).

Species Hybrids

At least seven F_1 hybrids and some of their reciprocals frequently outgrow either parent during the first decade (Wright 1959). The two of these hybrids that may have value for forest planting are eastern white pine x Himalayan pine and the reciprocal cross, and eastern white pine x western white pine and its reciprocal (Kriebel 1972). The first of these hybrids has had excellent growth and survival in Ohio (Kriebel 1982). In a 43-family full-sib progeny test, the two eastern white x Himalayan pine families were in the top 10 in volume growth at age 13, and one of these families outranked all others in the plantation. Hybrids with western white pine were more variable in volume growth; among the 43 families, two of the five eastern x western white pine families ranked third and sixth. When wood specific gravity of the hybrids *P. strobus* x *wallichiana* and *P. strobus* x *monticola* was measured in the same test and compared with that of eastern white pine, both hybrids clearly outranked *P. strobus* (Table 4). For the 152 trees tested for specific gravity, the results were:

P. strobus x strobus, 120 trees, mean wood specific gravity = 0.266

P. strobus x wallichiana, 15 trees, mean wood specific gravity = 0.295

P. strobus x monticola, 17 trees, mean wood specific gravity = 0.312

Since eastern white pine wood fiber is now in use at some paper mills, there could be a two-way gain from the use of these two hybrids in plantations, both from wood volume and wood specific gravity (Whitmore, F.W. and Kriebel, H.B., unpublished data).

Weevil Resistance Breeding

Repeated destruction of terminal shoots by the weevil larvae does not affect the health of white pines but it

Table 4—Relative stem volume and wood specific gravity of the hybrids *P. strobus* x *P. wallichiana* and *P. strobus* x *P. monticola ,* compared with *P. strobus*; top 10 of 43 families in a full-sib progeny test at age 13 ranked by volume (Kriebel and Whitmore, unpublished, Kriebel 1982).

Hybrid family	Cross[a]	♀ x ♂	Relative stem vol.[b]	Rank[c]	Relative specif. gr.	Rank, sp. gr.[d]	Rank, sp. gr. x vol.[e]
1430	st x wa	1278 x 1213	202	1	110	4	1
1375	st x st	1130 x 1279	171	2	100	4	4
1428	st x mo	1278 x 635	166	3	115	2	2
1408	st x st	1276 x 1277	155	4	93	8	6
1420	st x st	1278 x 1275	148	5	98	5	7
1429	st x mo	1287 x 645	143	6	124	1	3
1424	st x st	1278 x 1280	139	7	97	6	9
1393	st x wa	1275 x 1213	139	8	112	3	5
1448	st x st	1280 x 1279	139	9	94	710	
1373	st x st	1130 x 1277	138	10	98	5	8

[a] st = *strobus,* wa = *wallichiana*, mo = *monticola*.

[b] Mean stem volume in m^3 at age 13 from seed, as a percentage of the mean of all 43 family means in the progeny test (0.0337 m^3 per tree). The experiment included 33 full-sib *P.strobus* families*, 5 P. strobus* x *monticola* families, 2 *P.strobus x wallichiana* families, and 2 *P. strobus x peuce* families.

[c] Rank in volume among the 43 families in the progeny test.

[d] Mean specific gravity of the stem wood at age 13 from seed, expressed as a percentage of the mean of all 43 familiy means (266 kg/m^3).

[e] Rank in family mean specific gravity x mean volume.

destroys their commercial value by causing permanent deformities in the main stem. Although significant progress has been made in breeding for superior growth rate in eastern white pine and hybrids, genetic improvement in resistance to the white pine weevil and white pine blister rust has so far been unattainable in eastern North America. In geographic seed source trials, weevil damage was usually heavy in trees from all sources. Although seed sources varied in the degree of weevil injury, there was no total resistance, i.e. complete absence of weevil attack, in trees of any origin (Garrett 1972). However, since a few geographic provenances have been identified that have low susceptibility to repeated weevil attack, selection within stands based on relative degree of susceptibility in provenance tests may be useful as a first step in a selection program (Genys 1981, Wilkinson 1981). Noncrystallization of cortical oleoresins, earlier thought to be a resistance factor (Santamour 1965, Van Buijtenen and Santamour 1972) was subsequently found to be unrelated to weevil susceptibility (Bridgen and others 1979, Wilkinson 1979a, 1979b). Later work indicated that the concentrations of two monoterpenes may be useful as selection criteria for reducing susceptibility to weevil attack (Wilkinson 1980).

Two other species of five-needle pines have been considered in breeding for resistance to the white pine weevil. One is *P. peuce,* which has variable resistance (Zsuffa 1979). Although it has a moderate growth rate, its weevil resistance and crossability with *P. strobus* suggests its use as the male parent in hybrids for the introduction of resistance into eastern white pine. The hybrids could then be backcrossed to *P. strobus* to improve the growth rate. The long-term nature of this option, with low yields of hybrid seed, makes it impractical. The second species is *P. monticola*, and in this case instead of hybridization the species would be used directly, since *P. monticola* appears to be more weevil-resistant than *P. strobus* (Heimburger 1972). But western white pine varies in vigor and adaptability in the eastern United States, necessitating local progeny testing of trees screened for weevil resistance. Since 1980, no studies have been undertaken in the eastern USA to explore the feasibility of using hybrids or alternate species for weevil resistance. For a more detailed historical review of weevil resistance breeding, see Kriebel (1982).

Using Silviculture as an Alternative to Breeding

An alternative strategy to minimize weevil damage is particularly suitable for the northeastern and northern Lake States (Michigan, Wisconsin, Minnesota). It integrates genetics with silviculture by the planting of fast-growing selections of eastern white pine in a mixture or under an associated pioneer species such as aspen or birch, to provide partial shade for weevil protection. There are two possibilities. One is to allow the pine to overtop the short-lived aspen or birch, commonly in 20 years or less. The other is to remove the aspen or birch, if economically feasible, after the pine has developed one clear log that is free of weevil damage or nearly so. This is based on the weevil preference for trees growing in full sun over trees under shade or partial shade (MacAloney 1952, Ledig and Smith 1981).

Breeding For Rust Resistance

In the eastern USA, the white pine blister rust is a serious problem primarily in cool, humid regions, especially in the northern Lake States and the northeastern states. The rust is ubiquitous under these conditions and there are no reported unique populations that are threatened. The rust is not a problem in warm and dry localities within these regions and in low-elevation stands in the southern and southwestern parts of the range of *P. strobus.* Resistance appears to be polygenic in nature, thus requiring several generations of breeding to build up a practical level of resistance in offspring (Heimburger 1972). Artificial inoculation of eastern white pine alone does not accurately identify breeding stock suitable for field planting, since progenies succumbing to high concentrations of inoculum may be tolerant to the levels to which they are actually exposed in the field. Blister rust hazard is not high in all regions of the northeastern US and Canada. In warm zones and in cool zones with large openings to the sky, resistant strains are not essential (Van Arsdel 1972, Zsuffa 1979). Interspecific hybridization with other more rust-resistant species of five-needle pines has so far not been an effective breeding strategy (Zsuffa 1981).

Given these constraints on breeding eastern white pine for resistance to the white pine blister rust, new technology is now being applied to the problem by the US Forest Service in forest genetics and forest pathology projects in the North Central Region. Objectives are: (1) to develop a better understanding of the genetic structure of tree and pathogen populations, using molecular markers; (2) to identify genes for important quantitative traits; (3) to develop micropropagation techniques for production of elite trees; and (4) to develop gene transfer technologies for introduction of novel genetic constructs. Plantations of self- and reciprocal-crosses of eastern white pine progenies are being established for studying the genetics of blister rust resistance. The blister rust program has the objectives of determining rust variation in the Lake States and comparing it with rust variation in the rest of North America, determining whether resistance can be reliably identified in inoculated seedlings at an early age and whether there is a race-specific component in seedling resistance. Although current work involves inoculation of seedlings from clonal parents, diallel crosses and selfs among selected parents have been made for future studies. Sugar pines (*Pinus lambertiana* Dougl.) with and without the MGR gene (Kinloch 2001) have also been included in each inoculation for detection of one form of race-specific virulence. Michler and Pijut have developed improved micropropagation technologies for eastern white pine, and Michler and Davis have isolated eastern white pine chitinase genes with the goal of developing efficient genetic engineering protocols for pines using constructs with pine promoters. Eastern white pine seedlings are currently being tested in greenhouse studies to determine stability of transgene insertion (Michler and Zambino, personal communications).

Integrated Management for Rust and Weevil Control

The feasibility of applying a silvicultural regime integrating management for growth, blister rust and weevil resistance is currently being studied in northern Wisconsin (Ostry 2000). Variables in the study include survival, height, rust infection and weeviling. Treatments include clearcut vs shelterwood, nonselected stock vs selected stock, and no pruning vs pruning. Age 10 analysis, although preliminary, showed that:

1. Height growth was greater in clearcut than in shelterwood plots.
2. Weevil attack was greater in clearcut than in shelterwood plots.
3. Blister rust infection was higher in shelterwood than in clearcut plots.
4. Selected planting stock outgrew nonselected stock.
5. Blister rust infection was higher in nonselected than selected stock.
6. Blister rust infection was higher in unpruned than in pruned trees.

Pruning of lower branches had double benefits, both in correcting stem form for weevil damage, and also by removal of the branches most susceptible to rust infection.

Genetics of Air Pollution Tolerance

Because of inherent sensitivity and geographical distribution in relation to industry, population centers and prevailing winds, eastern white pine has probably been more impacted by air pollution than any other tree species in eastern North America (Gerhold 1977). Karnosky and Houston (1979) reviewed the genetics of air pollution tolerance of eastern white pine in the northeastern US. White pines are sensitive to both SO_2 and O_3, but the interaction of these pollutants has more serious effects than either pollutant alone (Houston 1974). There is significant tree-to-tree variation that has a strong genetic component (Houston and Stairs 1973). The effect of this large genetic component has been natural selection against sensitive trees in native stands over a wide region of eastern North America, with losses to the gene pool that may have included linked genes of unknown biological or commercial value. It was estimated that in northern Ohio stands of *P. strobus*, more than 40 percent of the potential seed-bearing trees had dropped out of the breeding population. Only pollution-tolerant trees survive to produce seed, and progenies from stands in polluted regions were found to be almost totally free of air pollution injury while retaining the vigor of the parent stands (Kriebel and Leben 1981). Similar evidence of the inheritance of pollution tolerance was obtained from the progenies of healthy trees growing in Tennessee for many years under polluted conditions. The progenies had darker green needles than progenies from other southern Appalachian stands and were consistently faster-growing than others in three polluted areas in Tennessee and in Ohio (Thor and Gall 1978, Kriebel 1982).

As a result of this natural elimination of sensitive genotypes throughout the regions where eastern white pine is native and planted, SO_2 is not currently a serious problem. Ozone injury tends to be more localized and is primarily a problem where white pines are planted close to major highways with high emission levels from motor vehicles. However, the future effect of air pollution on eastern white pine is uncertain. Industrial emissions of SO_2 from coal-burning power plants in the midwestern states continue to have an impact on northeastern forest land and waters. Unless the currently inadequate emission controls in the midwestern region of the US are strengthened, increases in coal-burning power plant outputs may further affect the survival and growth of eastern white pine in the northeastern states.

Directions for the Future

The primary goal for future research on the genetics and improvement of five-needle pines in the eastern United

States should be the restoration of the superior position of eastern white pine as a high-quality timber tree. The most promising methods of achieving this goal through genetic research and its applications are the following:

1. Ecology, management and resistance screening can be integrated to achieve the objective of planting pest-free eastern white pine planting stock in regions where white pine blister rust and the white pine weevil are major deterrents to the commercial planting of this valuable native tree.
2. Existing older eastern white pine progeny tests can be converted into seed orchards for rapid gain from the use of open-pollinated seed of known parentage and proven potential. Seedlings from elite trees should be planted in rust-free areas at close initial spacing to minimize weevil damage. This procedure has already been applied in some progeny tests.
3. Weevil resistance should be incorporated into eastern white pine through genetic engineering to incorporate genes for insect resistance into the species. A program has already been initiated to develop the molecular technology that may make possible the introduction of weevil resistance into white pine.

References

Adams, W.T. and Joly, R.J. 1977. Analysis of genetic variation for height growth and survival in open-pollinated progenies of eastern white pine. *In*: Proc. 25th Northeastern For. Tree Impr. Conf., Orono, ME, pp. 117-131.

Bingham, R.T. 1972. Taxonomy, crossability and relative blister rust resistance of 5-needle white pines. *In* Biology of Rust Resistance in Forest Trees. Proc. NATO-IUFRO Advanced Study Institute, 17-24 August 1969, Moscow, ID. USDA Forest Serv. Misc. Pub. 1221, pp. 271-280.

Bridgen, M.R., Hanover, J.W. and Wilkinson, R.C. 1979. Oleoresin xharacteristics of eastern white pine seed sources and relationship to weevil resistance. Forest Sci. 25:175-183.

Buchert, G.P., Rajora, O.P., Hood, J.V. and Dancik, B.P. 1997. Effects of harvesting on genetic diversity in old-growth eastern white pine in Ontario, Canada. Conservation Biol. 11:747-758.

Chira, E. and Berta, F. 1965. Eine der Ursachen der Nichtkreuzungsfähigkeit von Arten aus der Gattung *Pinus* (Slovak, German summary). Biologia, Bratislava, Czechoslovakia 20:600-609.

De Vecchi Pellati. 1967. Prove comparative fra progenie di diverse provenienze e diverse piante singolo di pino strobo. 1. Primi risultati delle osservazioni in vivaio. Nota Tec. 4, Istituto Nazionale Per Piante da Legno Giacomo Piccarolo, Torino, Italy, 4 pp.

Echt, S.C. 2000. Use of microsatellite markers in management of conifer forest species. *In* Strategies for Improvement of Forest Tree Species, *Ed.* G.C. Douglas. National Council for Research and Development, Dublin, Ireland, pp. 75-82.

Fowler, D.P. 1965. Effects of inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northeastern *Pinus* species. Silvae Genet. 14:76-81.

Funk, D.T. 1979. Genetic variation in volume growth of eastern white pine. For. Sci. 25:2-6.

Garrett, P.W. 1972. Resistance of eastern white pine (*Pinus strobus* L.) provenances to the white pine weevil (*Pissodes strobi* Peck). Silvae Genet. 21:119-121.

Genys, J.B. 1981. Preferences of *Pissodes strobi* in attacking different geographic strains of *Pinus strobus*. *In:* Proc. 17th IUFRO World Congress, 6-12 September 1981, Kyoto, Japan, pp. 191-194.

Gerhold, H.D. 1977. Effect of air pollution on *Pinus strobus* L. and genetic resistance-a literature review. Ecol. Res. Ser. Rep. EPA-600/3-77-002. US Environmental Protection Agency, Corvallis OR, 45 pp.

Hagman, M. 1967. Genetic mechanisms affecting inbreeding and outbreeding in forest trees; their significance for microevolution of forest tree species. *In* Proc. XIV IUFRO World Cong., Munich, Germany, Section 22:347-365.

Hagman, M. and Mikkola, L. 1963. Observations on cross-, self-, and interspecific pollinations in *Pinus peuce* Griseb. Silvae Genet. 12:73-79.

Hashizume, H. and Kondo Y. 1962a. Studies on the mechanism of fertilization in forest trees. I. Inhibitors to the growth of pollen tubes in the reproductive organs of *Pinus densiflora*. Jour. Jap. For. Soc. 44:43-48..

Hashizume, H. and Kondo, Y. 1962b. Studies on the mechanism of fertilization in forest trees. II. Inhibitors to the growth of pollen tubes in the reproductive organs of reproductive organs of *Pinus thunbergii*. Trans. Tottori Soc. Agr. Sci. 14:93-97.

Heimburger, C. 1972. Relative blister rust resistance of native and introduced white pines in eastern North America. *In* Biology of Rust Resistance in Forest Trees. Proc. NATO-IUFRO Advanced Study Institute, 17-24 August 1969, Moscow, ID. USDA Forest Serv. Misc. Pub. 1221, pp. 257-269.

Heimburger, C. and Sullivan, C.R. 1972. Screening of Haploxylon pines for resistance to white pine weevil. II. *Pinus strobus* and other species and hybrids grafted on white pine. Silvae Genet. 21:210-215.

Houston, D.B. 1974. Response of selected *Pinus strobus* L. clones to fumigations with sulfur dioxide and ozone. Can. J. For. Res. 4:65-68.

Houston, D.B. and Stairs, G.R. 1973. Genetic control of sulfur dioxide and ozone tolerance in eastern white pine. For. Sci. 19:267-271.

Karnosky, D.F. and Houston, D.B. 1979. Genetics of air pollution tolerance of trees in the northeastern United States. *In*: Proc. 26th Northeastern Forest Tree Improvement Conference, 25-26 July 1978, University Park, PA, pp. 161-178.

Keathley, D.E. 1977. An analysis of the genetic variance in height, diameter, and volume for two experimental plantations of eastern white pine. M.S. Thesis, School of Natural Resources, Ohio State University, Columbus, OH, 37 pp.

Kinloch, B.B., Jr. 2001. Genetic interactions in the white pine/blister rust pathosystem. *In:* Program and Abstracts, Western For. Gen. Association, July 30-Augsut 2, 2001, Davis, California (unpaginated).

Kriebel, H.B. 1972. Embryo development and hybridity barriers in the white pines (Section Strobus). Silvae Genet. 21:39-44.

Kriebel, H.B. 1975. Inbreeding depression in eastern white pine. *In* Proc. 9th Central States For. Tree Impr. Conf., Ames, IA, pp. 48-55.

Kriebel, H.B. 1976a. Variation in cold-hardiness in blue pine. *In* Proc. 10th Central States For. Tree Impr. Conf, West Lafayette, IN, pp. 166-170.

Kriebel, H.B. 1976b. Maternal control over cone abscission in pines. *In:* Proc. XVI IUFRO World Congress, Oslo, Norway, pp. 239-245.

Kriebel, H.B. 1978. Genetic selection for growth rate improvement in *Pinus strobus*. Genetika (Belgrade, Yugoslavia) 10(3):269-276.

Kriebel, H.B. 1981. Maternal control over premature cone abscission of pines. Genetika (Belgrade, Yugoslavia) 13(3): 215-222.

Kriebel, H.B. 1982. Recommended genetic selections of some forest trees for Ohio. Ohio State Univ., Ohio Agr. Res. and Devel. Center, Res. Bul. 1148, 19 pp.

Kriebel, H.B. 1983. Breeding eastern white pine: a world-wide perspective. For. Ecol. And Management 6:263-279.

Kriebel, H.B. and Leben, C. 1981. The impact of SO_2 air pollution on the gene pool of eastern white pine. *In* Proc. XVII IUFRO World Cong., Kyoto, Japan, Div. 2, pp. 185-189.

Kriebel, H.B. and Dogra, P.D. 1986. Adaptability and growth of 35 provenance samples of blue pine in Ohio. *In* Proc. XVIII IUFRO World Cong., Ljubljana, Yugoslavia, pp. 30-38.

Kriebel, H.B., Namkoong, G., and Usanis, R.A. 1972. Analysis of genetic variation in 1-, 2-, and 3-year-old eastern white pine in incomplete diallel cross experiments. Silvae Genet. 21:44-48.

Kriebel, H.B., Roberds, J.H. and Cox, R.V. 1974. Genetic variation in vigor in a white pine incomplete diallel cross experiment at age 6. *In* Proc. 8th Central States For. Tree Impr. Conf., Columbia, MO, pp.40-42.

Ledig, F.T. and Smith, D.M. 1981. The influence of silvicultural practices on genetic improvement: height growth and weevil resistance in eastern white pine. Silvae Genet. 30:30-36.

MacAloney, H.J. 1952. White pine weevil. *In* Important Tree Pests of the Northeast. New England Sect., Soc. Am For. Evans Printing Co., Concord, NH, pp. 82-84.

McWilliam, J.R. 1959. Interspecific incompatibility in *Pinus*. Am. J. Bot. 46:425-433.

Ostry, M.E. 2000. Restoration of white pine in Minnesota, Wisconsin, and Michigan. Hortechnology 10(3):542-543.

Rajora, O.P., DeVerno, L., Mosseler, A. and Innes, D.J. 1998. Genetic diversity and population structure of disjunct Newfoundland and central Ontario populations of eastern white pine (*Pinus strobus*). Can J. For. Res. 76:500-508.

Rajora, O.P., Rahman, M.H., Buchert, G.P. and Dancik, B.P. 2000. Microsatellite DNA analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. Molec. Ecol. 9:339-348.

Santamour, F.S., Jr. 1965. Insect-induced crystallization of white pine resins. I. White pine weevil. USDA Forest Serv. Res. Note NE-98, 8 pp.

Schlarbaum, S.E. and Cox, R.A. 1990. Survival and growth of *Pinus griffithii* genetic tests in Tennessee. *In* Proc. XIX IUFRO World Cong., Montreal, Canada, Div.2, pp. 130-134.

Thor, E. and Gall, W.R. 1978. Variation in air pollution tolerance and growth rate among progenies of southern Appalachian white pine. *In* Proc. 1st Conf. Of the Metropolitan Tree Improvement Alliance (METRIA), pp. 80-86.

Ueda, K., Yoshikawa, K. and Inamori, Y. 1961. On the fertilization of interspecies crossing in pine. Bul. Kyoto Univ. For. 33:137-155.

Van Arsdel, E.P. 1972. Environment in relation to white pine blister rust infection. *In* Biology of Rust Resistance in Forest Trees. Proc. NATO-IUFRO Advanced Study Institute, 17-24 August 1969, Moscow, ID. USDA Forest Serv. Misc. Publ. 1221, pp. 479-493.

Van Buijtenen, J.P. and Santamour, F.S., Jr. 1972. Resin crystallization related to weevil resistance in white pine (*Pinus strobus)*. Can. Entomol. 104:215-219.

Wilkinson, R.C. 1979a. Cortical strobic acid concentrations in eastern white pine resistant and susceptible to the white pine weevil. *In*: Proc. 26th Northeastern Forest Tree Improvement Conf., 25-26 July 1978, University park, PA, pp. 121-132.

Wilkinson, R.C. 1979b. Oleoresin crystallization in eastern white pine: relationships with chemical components of cortical oleoresin and resistance to the white pine weevil. USDA Forest Serv. Res. Pap. NE-438, 10 pp.

Wilkinson, R.C. 1980. Relationship between cortical monoterpenes and susceptibility of eastern white pine to white pine weevil attack. For. Sci. 26:581-589.

Wilkinson, R.C. 1981. Analyses of susceptibility of eastern white and other Haploxylon pines to white pine weevil attack in the northeastern United States. *In:* Proc. 17th IUFRO World Cong., 6-12 September 1981, Kyoto, Japan, pp. 199-203.

Wright, J.W. 1953. Summary of tree-breeding experiments by the Northeastern Forest Experiment Station 1947-1950. USDA Forest Serv., NE For. Exp. Sta., Sta. Pap. 56, 47 pp.

Wright, J.W. 1959. Species hybridization in the white pines. For. Sci. 5:210-222.

Wright, J.W. 1970. Genetics of eastern white pine. USDA Forest Serv., Res. Pap. WO-9, 16 pp.

Wright, J.W. and Gabriel, W.J. 1959. Possibilities of breeding weevil-resistant white pine strains. USDA Forest Serv., Northeastern For. Exp. Sta. Paper 115, 35 pp.

Wright, J.W., Amiel, R.J., Cech, F.C., Kriebel, H.B., Jokela, J.J., Lemmien, W.A., Matheson, A.C., Merritt, C., Read, R.A., Roth, P., Thor, E. and Thulin, I.J. 1979. Performance of eastern white pine from the southern Appalachians in eastern United States, New Zealand and Australia. *In* Proc. 26th Northeastern For. Tree. Impr. Conf, University Park, PA, pp. 203-217.

Zsuffa, L. 1979. The genetic improvement of eastern white pine in Ontario. *In* Proc. Tree Improvement Symp., *Ed.* J.B. Scarrat,. COJFRC, Toronto, ON. Can. For. Serv. Pub. O-P-7, pp. 153-160.

Zsuffa, L. 1981. Experience in breeding *Pinus strobus* L. for resistance to blister rust. *In* Proc. XVII IUFRO World Cong., Kyoto, Japan, pp. 181-183.

Breeding Rust-Resistant Five-Needle Pines in the Western United States: Lessons from the Past and a Look to the Future

Geral McDonald
Paul Zambino
Richard Sniezko

Abstract—Introduction of *Cronartium ribicola* into the Western United States created major disruptions in forests where five-needle pines were important components. In response, various control measures were implemented on two commercially important species—western white pine (*Pinus monticola*) (WWP) and sugar pine (*P. lambertiana*) (SP). The USDA Forest Service developed three programs to breed for resistance: one directed at northern Rocky Mountain WWP; a second for WWP and SP in Oregon and Washington; and a third for SP in California. The Rocky Mountain program developed a resistant population composed of durable or multigenic resistance that shows no evidence to date of any R genes (i.e., genes for "Major Gene Resistance"). The Cascade WWP program utilizes a mixture of an R gene and multigenes. Washington populations correspond to the Rocky Mountain model, but resistance of the Oregon populations seems to be partly due to an R gene. An R gene is the threshold basis for selection of SP for the California program. Progeny that carry R genes are screened for additional resistance mechanisms to develop populations with multiple sources of resistance. These breeding programs have created large seed banks, several seed orchards, and numerous additional plantings of pedigreed material. However, new concepts for examining genotype by environment (G x E) interactions, recognition of phenotypic plasticity of hosts and pathogens and of induced defenses, and evidence of disease attenuation in the blister rust pathosystem may have important consequences for future breeding, integrated management, and ecosystem restoration efforts in five-needle pine ecosystems. Reanalysis of some old results and update of data from some long-term plantings were coupled with current knowledge of additional pathosystems to suggest an altered paradigm for white pine blister rust in which complex interactions among the pine, rust, and environmental components are the norm.

Key words: Induced defenses, ontogenic resistance, reaction norms, phenotypic plasticity, disease genotype x environment interaction, ecologic restoration

Introduction

Introduction and spread of the causal agent of white pine blister rust (WPBR), *Cronartium ribicola* J. C. Fischer ex Rabh., in western North America was recently reviewed (McDonald and Hoff 2001). WPBR introduction precipitated a major reduction in populations of western white pine (WP) (*Pinus monticola* Douglas ex. D. Don.) in the northern Rocky Mountains (Neuenschwander and others 1999). In this region, WWP was a modifier keystone species whose presence influenced many ecosystem processes (McDonald and others 2000). Removal of WWP has also altered patterns of mortality from Armillaria root rot on replacement species and thus, of fire regimes that could ultimately produce shortened fire return intervals and more intense stand replacement fires (McDonald and others 2003). Ecosystems where whitebark pine (WBP) (*P. albicaulis* Engelmann) is a keystone species are also at risk of experiencing major perturbations (Tomback and others 2001).

The ecologic and economic importance of North American species of five-needle pines and their susceptibility to WPBR have prompted most genetics work on western five-needle pines. Breeding work has centered on the two important commercial species of WWP and sugar pine (SP) (*Pinus lambertiana* Douglas). Programs were delineated by geographic areas. The first, initiated in 1946, was designed to produce WPBR resistant selections of WWP for use in the "Inland Empire" (northeastern Washington, northern Idaho, and western Montana) and utilized controlled crosses to obtain full-sib progeny for selection within and among crosses (Bingham 1983). A program to develop resistance in WWP and SP for Washington and Oregon was started in 1956. This program switched to screening open pollinated progeny of candidate trees in 1971, after initially using full-sib progeny (Sniezko 1996). A breeding program designed to develop resistance in SP for California was initiated in 1957 (Kitzmiller 1982).

Conditions influencing development of WPBR and types and distributions of resistance mechanisms each vary considerably among these western geographic regions. Differences in approaches among the breeding programs are reflected in differences in kinds of seed orchards and other breeding resources. Our intent in this paper is to briefly describe each program, examine the development, deployment, and historical evidence of effectiveness of resistance to WPBR, and provide insight into the development of management strategies for restoration of ecosystems. Some recent literature will be reviewed. Results in existing

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Geral McDonald and Paul Zambino are with the USDA Forest Service, Rocky Mountain Research Station. Email: gimcdonald@fs.fed.us or pzambino@fs.fed.us. Richard Sniezko is with the USDA Forest Service, Dorena Genetic Resource Center. Email: rsniezko@fs.fed.us

literature will be examined and some previously unpublished results will be presented using new paradigms for interpreting adaptation and epidemiological processes and understanding the physiology of resistance. Finally, we will discuss relevance of lessons learned from breeding WWP and SP to new efforts directed at whitebark pine (WBP), limber pine (LP) (*P. flexilis* James), southwestern white pine (SWWP) (*P. strobiformis* Engleman), bristlecone pine (BP) (*P. aristata* Engelmann), foxtail pine (FP) (*P. balfouriana* Greville & Balfor.), and Great Basin bristlecone pine (GBBP) (*P. longaeva* D. K. Bailey).

The Breeding Programs

Northern Rocky Mountain Western White Pine—This breeding program has had two phases. The first phase was based on about 400 phenotypically resistant (canker-free to five cankers/tree) selections obtained from areas of high rust severity (cankers/tree). This phase was initiated in 1946 when R. T. Bingham selected a full-crowned 60-year-old tree almost 30 meters tall as the only rust-free tree within a northern Idaho stand of 380 trees (Bingham 1983). By June of 1950, 58 trees had been selected from similar areas of high rust severity. Counts of cankers from neighboring susceptible trees at the time of selection (data on file at the Rocky Mountain Research Station, Moscow, Idaho) allowed infection rates and probability of escape to be calculated. Under the levels of rust incidence and severity observed for stands containing the 58 selections, only one uninfected tree out of 143 million would be expected to be an escape (McDonald and Hoff 1982). The full contingent of 400 candidate trees had been located by 1970 and the ratio of resistant (rust-free to five cankers per tree) to susceptible phenotypes in natural populations was established at 0.00001 (McDonald and Hoff 1982).

Beginning in 1950, selections were crossed to produce full-sib families whose seedlings could be artificially inoculated to measure general combining ability (GCA) and specific combining ability (SCA) (Bingham 1983). Wild inoculum from two to three collection sites was used for each inoculation. Inoculations were conducted under a double-layered tent (Bingham 1983). One fourth of the 400 parent trees selected as phenotypically resistant proved to have GCA for enhanced rust resistance. These 100 parents were ascribed to three populations corresponding to elevation, with the "low elevation" class originating below 1065 m, the "mid-elevation" class from 1066-1250 m, and the "high elevation" class from over 1251 m. Since a balanced design of crossing was desired and the smallest elevation class contained 24 GCA parent trees, 24 GCA trees were selected to represent each elevation in subsequent controlled crosses. Foundation stock for each of the three F_2 seed orchards representing the three elevation classes was of 12 unrelated GCA x GCA families. Early artificial inoculation tests indicated that about 65 percent of the seedlings of GCA x GCA F_2 families remained free of infection after intense rust exposure (Hoff and others 1973).

The Phase I program was noted for selection of candidate trees from stands under intense rust pressure that were then used in various crossing schemes (Bingham and others 1969). Full-sib progeny were subjected to artificial inoculation in tests containing many thousands of seedlings (Bingham 1972). Phase I generated many peer-reviewed papers and left a legacy of nine well-documented plantations and four seed orchards (Mahalovich and Eramian 1995). Several are composed of well-marked pedigreed materials suitable for new genetic research (records on file USFS Region 1). A recently accepted paper uses these historical records and materials to analyze the influence of blister rust resistance breeding on the genetic structure of WWP as revealed by AFLP DNA markers (Kim and others 2003). Several plantings of Phase I materials have received repeated rust examinations and new data obtained from these plantations will be discussed later in this report. The Phase I program was also the source of descriptions of most of the resistance mechanisms (see Hoff and McDonald 1980 for descriptions) that have since been applied to the ongoing breeding programs in the northern Rocky Mountains (Mahalovich and Eramian 1995) and Oregon and Washington (Sniezko 1996).

Northern Rocky Mountain WWP—Phase II Program—Region 1 of the USDA Forest Service and the Inland Empire Tree Improvement Cooperative (see Fins and others 2002) administers the Phase II breeding program that was initiated in 1967 (Mahalovich and Eramian 1995). This program is based on open pollinated (OP) selections screened for inheritance of a set of resistance mechanisms (Hoff and McDonald 1980, Mahalovich and Eramian 1995). The objective is to select for resistant progeny among seedlings of 3100 candidate WWP that have been chosen from stands at least 25 years in age. Maximum severity (cankers/tree) acceptable for select trees is set relative to severity in the surrounding stand as follows: 0 if severity was 10 to 20, 1 if severity was 21 to 40, 2 if severity was 41-75, 3 if severity was 76-150 and 4 to 5 if severity was 150+. Severity on most selected trees was less than three cankers. OP cones have been collected from about 200 candidates each year for screening at the USFS Region 1 Tree Nursery, Coeur d'Alene, Idaho. Seedlings are grown under standard nursery regimes for inoculation at the end of their second growing season.

Inoculum is generated in a disease garden located at the Lone Mountain Tree Improvement Site, Spirit Lake, Idaho. Aeciospores are collected each year at 10 sites covering a target-breeding zone that includes the Idaho panhandle and western Montana (Mahalovich and Eramian 1995). Collected spores are used to initiate a controlled epidemic on *R. nigrum* L. and *R. hudsonianum* var. *petiolare* (Dougl.) Jancz. Telia-bearing leaves are collected and transported to the Coeur d'Alene nursery for use in controlled inoculations as had been done for the Phase I program. Following September inoculations, seedlings are planted in beds outdoors and inspected the following June for numbers of needle lesions and the following September for presence/absence of needle lesions, bark reactions, and cankers. The second and third September after inoculation, seedlings are inspected for presence/absence of bark reactions. Data are used to select both families and individuals within families in an attempt to accumulate resistance mechanisms (Mahalovich and Eramian 1995). Families selected from the Phase II program as having high proportions of rust-free seedlings at the fourth inspection are rigorously outplanted to evaluate long-term rust behavior and growth under operational conditions and natural levels of inoculum (Mahalovich and Eramian 1995). Over 20 plantings have been established, using sound statistical designs, and plans are in place to conduct regular inspections (Mahalovich and Eramian 1995). Survival, damage to terminal stems, presence/absence of bole and branch cankers, number of stem cankers, number of branch cankers, and total tree height will each be tracked. The planned assessment schedule is 5, 7, and 10 years after planting, followed by 5-year intervals until one half of the rotation age has been reached (Mahalovich and Eramian 1995).

Three additional testing regimes have been implemented in the Phase II program. A crossing study with full-sibs and selfs was initiated in 1993 to verify the genetic mechanisms and inheritance for needle shed, short shoot, and bark reaction traits, with crossing taking place at two field sites and the Coeur d'Alene nursery. Secondly, realized gain trials have been designed to test resistance of seed orchard populations from both Phases under operational conditions. For this study, F_1, F_2, B_1 and several control lots will be outplanted in 2004 at sites where infection levels have been increasing in plantations of resistant material. Lastly, four tests, each replicated at two locations per year beginning in 2001, are being planted to evaluate genotype by environment interactions and the idea that a single breeding zone is sufficient for the northern Rockies. Results of the new testing regimes will facilitate validation or revision of the breeding strategy.

Additional breeding was initiated in 1995 using elite tree selections from the Phase I and Phase II programs to generate a second-generation program and obtain further potential gains in resistance. Up to 360 selections will be divided into 18 sublines that will ensure that seed from the 2^{nd} generation program will minimize the potential for inbreeding. Also, a WWP clone bank was established in 1999 at Dry Creek Tree Improvement Area, Clark Fork, Idaho to protect unique trees, elite trees, and progeny of candidate trees in the field that have been lost through timber removal, road construction, and catastrophic fire. Entries are added annually.

Oregon and Washington Sugar Pine and Western White Pine—The Washington and Oregon breeding program is operated out of the Dorena Genetic Resource Center, USFS, Region 6 and is located near Cottage Grove, Oregon (Sniezko 1996). The USDI Bureau of Land Management, Oregon Office, has been a major cooperator, particularly for sugar pine, in the phenotypic selection of trees and development of seed orchards for many years. Other cooperators represent a wide array of public agencies, Indian nations, and other organizations.

Objectives of the resistance-breeding program for Oregon and Washington include identifying the amount and type of genetic resistance present in natural populations of WWP and SP, selecting families and individuals within families for resistance, and developing durable resistance to WPBR while retaining broad genetic diversity and local adaptation within both species. The program utilizes conventional breeding techniques to increase the durability of the partial resistance currently available, while maintaining diverse genetic populations. Patterns of genetic adaptation in SP and WWP identified through growth responses in common garden studies (Campbell and Sugano 1987, 1989) are the primary basis for delineation of breeding zones.

The Dorena facility has screened seedlings of WWP or SP from more than 9500 parent trees. The program has the capability to inoculate more than 600 families per year and evaluate over 100,000 seedlings annually with consistently high seedling infection rates, as evidenced by development of needle lesions and stem symptoms. Inoculations are performed in a building that has been fitted with humidifiers and associated controls to act as a large, self-contained incubation chamber (McDonald and others 1984). Seedlings are inoculated after their second growing season and are evaluated for five years thereafter to discern different resistance mechanisms. Historically, this program has used the same set of definitions of resistance as the northern Rocky Mountain Phase I and Phase II programs (see Sniezko 1996), although some re-evaluation of mechanisms is underway. In general, 80 to 240 (often 120) families are inoculated per 'run', with each family represented by 60 seedlings divided among six replications. Inoculum comes from two sources: collections of telia produced in the wild from natural inoculum under ambient conditions vs. in a disease garden under more controlled conditions.

Until recently, conditions and techniques used in inoculation and evaluation of most forms of resistance had changed only slightly since the 1960s. However, Dorena has recently begun to use a modified screening technique to differentiate families expressing an R gene (*Cr2*) specific to WWP (Kinloch and others 1999) that develops a hypersensitive reaction as a barrier to colonization in infected needles from other families that have a high incidence of canker-free seedlings. Previously, disease-garden telia were generated after *Ribes* were inoculated with bulked collections of aeciospores obtained from across Oregon and Washington. Currently, *Ribes* in the disease garden become naturally infected by aeciospores from WWP in the general vicinity. Teliospore-bearing leaves from the disease garden are used to represent a locally prevalent, virulent strain of rust (the "Champion Mine Strain") that can overcome *Cr2* resistance (McDonald and others 1984, Kinloch and others 1999). Since wild-collected (wild-type race) and disease garden (virulent race) *Ribes* leaves are each used in half of the inoculation replications, differences in frequency of resistance between inoculum sources identify resistance of families conferred by the R gene, and differentiate them from forms of resistance (partial resistance) that are not known to be overcome by differences in rust race. Results from such inoculations indicate that in Region 6, the frequency of the WWP R gene is very low and that existing levels of partial resistance are low, although a few outstanding parents exist (Kinloch and others 1999, Sniezko 1996, Kegley and Sniezko this proceedings).

Recent re-sowing of some previously tested families has confirmed their resistance. Demonstration plantings at Dorena that include representatives of such resistant families alongside susceptible families that develop extensive cankering and mortality provide a dramatic visual demonstration of the effectiveness of the program to visitors. Tentative discovery of a new resistant mechanism, termed "mechanism X", may cause further changes to the program. One of us (RS) has observed that "X" is not *Cr2* because of resistance to the *vcr2* race of the pathogen and that "X" families do better than *Cr2* families in field tests; one of us (GM) has also observed that needle shed morphology of "X" is reminiscent of Idaho WWP needle shed. Only Washington populations of WWP have been shown to express "X" but further investigations of Oregon populations are underway (Sniezko unpublished data).

Much of the early activity in the Oregon-Washington white pine blister rust (WPBR) resistance program involved selecting trees in natural stands in each of eight breeding zones, inoculating and evaluating offspring of these trees for resistance, and establishing seed orchards from the most rust-resistant parents or progeny. Seed orchards have been established for both WWP and SP in most breeding zones and resistant seed is available for several zones. Orchards include a diverse set of parents and an array of putative resistance mechanisms.

Recently, an important, but long neglected, component has received additional attention. Establishment of field plantings is now under way to monitor resistance, validate screening results, and demonstrate the potential for resistant seed in ecosystem restoration throughout Oregon and Washington. Since 1996, families of WWP and SP have been established at 20 and 10 sites, respectively, in conjunction with cooperators that include USDA Forest Service Region 5, BLM, Confederated Tribes of Warm Springs, OR, and Josephine County, OR. Additional trials, using control crosses or open-pollinated seedlots from Dorena, have been established in British Columbia and on WA Department of Natural Resources lands (Rich Hunt, personal communication 2001). A planting in the Oregon coast range is planned by Plum Creek Timber Company (Jim Smith personal communication). Full sib or OP families are utilized and have been established at sites that differ in representation of the presumed races of blister rust. These plantings will help establish whether there are changes in rust virulence over time, and will potentially give information on inheritance of some of the resistance mechanisms (for example results, see Sniezko and others 2000, Sniezko and others, these proceedings). Information from a few older plantations has also recently been collected, summarized, and presented at technical meetings (Sniezko and others #2, these proceedings). In these older trials, infection levels after 15 or more years in the field are high (>75 percent of trees infected). One goal of these plantings is to determine the relative field performance of WWP and SP lots that differed in levels of rust resistance during screening, to calibrate by species and resistance level the WPBR extension (McDonald and others in preparation) of the Forest Vegetation Simulation Model (Teck and others 1997) for use in Region 6.

California Sugar Pine—A program designed to identify and utilize WPBR resistance in California sugar pine was initiated in 1957 (Kitzmiller 1982) and is conducted out of facilities located near Placerville, CA. The goal of this portion of the USDA Forest Service Region 5's genetics program is to maintain locally adapted SP having a mixture of resistance mechanisms throughout sugar pine's natural range (Samman and Kitzmiller 1996). This is being achieved through two approaches. First, resistant trees are identified that can be used as seed sources for each of 27 California seed zones (Kitzmiller 1976) where sugar pine occurs, with trees additionally grouped according to 500 ft altitudinal bands. Secondly, a subset of these and additional selected resistant materials are being used to establish seed orchards and breeding populations for each of seven breeding zones that have been established based on physiographic and environmental parameters (Samman and Kitzmiller 1996).

Criteria for tree selection vary according to geographic location and the frequencies at which resistance mechanisms occur within local populations. The first-level criterion for seed tree selection is the presence of the sugar pine R gene (*Cr1*; Kinloch and Davis 1996). This was the first dominant gene for resistance identified in a forest tree species (Kinloch and Littlefield 1977), and like *Cr2* described above, restricts needle colonization and stem infection by *C. ribicola* by development of a hypersensitive response (Kinloch and Littlefield 1977). Assessment is based on symptom development in inoculated progeny seedlings. In regions where frequency of the R gene, and consequently, the occurrence of disease free sugar pine are sufficiently high, timber and growth traits are also considered. Conversely, in regions such as the northern part of the range of sugar pine where the R gene for sugar pine is low in frequency (Kinloch 1992), initial tree selection is based solely on freedom of trees from disease in stands with high incidence of blister rust. Selections are protected from cutting until screened.

Open pollinated seed is collected and progeny seedlings screened for the R gene at the Placerville facility using artificial inoculations. Control lots are included that contain several representative genotypes homozygous or heterozygous for *Cr1* or homozygous for lack of the R gene. Telia used as screening inoculum are produced by inoculating greenhouse-grown plants of susceptible cultivars of *Ribes nigrum* L. with aeciospores of rust that lacks virulence to *Cr1* that have been collected from sugar pine in different areas, or with urediniospores produced after previous inoculations. Inoculated plants are bagged for 24 hours until infection has occurred, and then maintained in a cool, partially shaded greenhouse for four to five weeks until dense telia develop on leaves. Basidiospores are cast from telia on trays of detached leaves onto 8-month-old seedlings in three-layered, cloth-lined chambers in which temperature and humidity are kept at optimum levels for infection by water flowing over the outer walls. Seedlings are each evaluated three times approximately three weeks apart after susceptible yellow or yellow-green needle spots can be distinguished from necrotic spots or yellow spots with necrotic margins that signify resistance due to the sugar pine R gene. Open pollinated families with approximately 50 and 100 percent resistant seedlings indicate heterozygous and homozygous parent trees, respectively, whereas lower proportions indicate a non-*Cr1* tree that has received pollen from one or more *Cr1* trees.

Those seedlings with the R gene are further screened for slow rusting under natural conditions at a location (Happy Camp, California) where a strain naturally occurs that can overcome the sugar pine R gene (Kinloch and Davis 1996; Samman and Kitzmiller 1996). *Ribes sanguinium* Prush. is planted among the R-gene-resistant seedlings to produce natural inoculum having a high proportion of *vcr1* virulence to the sugar pine R gene resistance. Seedlings are exposed to a minimum of two, and usually three "wave" years of heavy rust infection, with final slow rust determinations made for seedlings and families a minimum of ten years after planting. Detection of slow-rusting resistance at the Happy Camp site is based on observable low rates of increase in rust incidence and severity, slow or abortive colonization, and reduced persistence of infections. Normal cankers and bark reactions (corking out of branch and bole infections) in SP appear similar to those in WWP in the other resistance programs, although the Region 5 sugar pine program also differentiates a "blight" reaction in which necrotic tissue extends beyond the canker to the end of the branch proximally to the next branch whorl and distally to the end of the branch (Kinloch and Davis 1996). Materials selected at Happy Camp for having slow-rusting resistance in addition to R gene resistance are then grafted and planted into seed orchards and clone banks.

To date, 28654 candidate trees have been selected; 1395 living MGR trees identified (John Gleason, R5 Genetics, pers. comm.); and approximately 2900 pounds of MGR seed is available for restoration efforts. The three seed orchards closest to completion represent the Sierra Nevada (i.e., Breeding zones 4, 5,and 3, in order of completeness). Each of these orchards has separate but adjacent breeding blocks for R gene only versus R gene plus slow-rusting resistance mechanisms (durable resistance), and additional divisions based on geographic and elevation differences. Duplicate plantings of some of this material are being developed as seed orchards by cooperators in industry, providing a buffer against loss by fire or other causes. Although the numbers of R-gene parent trees needed for the Sierra have been met and exceeded (currently over 700 represented in the orchard design), adequate numbers of selections have been difficult for some extremes of elevation and for the northern range of sugar pine. To increase the number of R-gene phenotypes from such locations, the program is also identifying resistant MGR progeny of phenotypically resistant seed trees in the northern range that lack the R gene. Lack of the R gene in these phenotypically resistant trees may indicate high levels of non-MGR resistance mechanisms. Fertilization by R gene pollen may thus result in offspring that have several resistance mechanisms. Pollen receptor, R-gene-carrying progeny that are being used in the program are geographically distant from known R-gene trees, to maximize genetic diversity. Alternative methods are being investigated that may enable north zone candidate trees with potentially high slow-rusting resistance to be identified during the initial progeny screening for R gene resistance.

A network of widely dispersed plantations has been established to evaluate silvicultural growth characteristics and resistance of SP to both the wild type and the *Cr1*-virulent races of WPBR,(Kitzmiller and Stover 1996). Eight plantations established in 1983 utilized the same families at each site, and included families that were homo- and heterozygous for *Cr1* and susceptible families. Four plantations established in 2000-2001 include homo- and heterozygous *Cr1* and susceptible families, as well as R gene plus slow-rusting families. Within each category, some families have demonstrably wide geographic adaptation, while other families are of local origin (P. Stover, personal communication). Plots are monitored primarily for incidence of disease and presence and numbers of cankers on branches and stems. Thus far, monitoring after wave years has failed to detect the race of WPBR virulent to the sugar pine R gene outside of the immediate vicinities of its two known sites of occurrence at Happy Camp and Mountain Home, California.

Other Five Needle Pine of the Western United States—Some breeding work for other five needle pines is just getting underway (Mahalovich and Dickerson these proceedings). A common garden study designed to delineate seed zones for northern Rocky Mountain WBP was planted at the Priest River Experimental Forest in 2000 (D. Ferguson, personal communication). Open pollinated seed, collected from 10 trees in each of 45 stands, was planted in 2000. Growth, development, and periodicity of shoot elongation will be monitored using data collection methods patterned after a previous WWP study (Rehfeldt and others 1984). A second planting of most of the same sources was installed in 2001 on a dry site at the Priest River Forest to study drought tolerance of WBP (Ferguson personal communication). Seedlots from a number of national forests in OR and WA were also included in a recent common garden study initiated at the University of British Columbia (Andy Bower, personal communication). These studies should provide insights into adaptive variation in WBP.

Evaluations of a small number of California WBP seedlots for R genes (Kinloch and Dupper 2002) and OR/WA WBP (unpublished data) have failed to find them. However, R genes were only recently confirmed in LP and SWWP (Kinloch and Dupper 2002). A recent sowing of WBP at Dorena will increase the number of parents evaluated in Region 6 for R genes and other resistance responses. Protocols will be adapted from the Region 6 WWP and SP resistance program and other efforts to screen for resistance in WBP (Hoff and others 2001). Spore load for evaluation and comparison of WBP sources will be a critical factor. An inoculation trial in 2001 at Dorena has identified the spore load needed for infection of this species under local conditions (Kegley and others in press). A recent preliminary screening of phenotypically resistant WBP growing in the northern Rocky Mountains demonstrated a resistance signature (low frequency and proportion of spotting among progeny seedlings) very similar to that of northern Rockies WWP (Hoff and others 2001). Early in the development of WPBR in western North America, a study quantitatively compared WPBR incidence and severity on WWP and WBP at six sites in Washington, Oregon, and Idaho (Bedwell and Childs 1943). Their data were used to compute infection rates using the "monomolecular" or "monocyclic epidemic" equation (see below) in WWP and WBP (McDonald and Hoff 2001). Comparison of these rates indicated WBP growing in the northern Cascade Mountains of Washington and in the northern Rocky Mountains in northern Idaho were about 70x more susceptible than contemporary WWP, while WBP populations in the vicinity of Mount Hood, OR were only 4x more susceptible (McDonald and Hoff 2001). Genetic differentiation of populations was revealed as an explanation for differences in relative susceptibility by a recent study of genetic structure of WBP (Richardson and others 2002). This study demonstrated distinct southern and northern populations of WBP that exhibited a distinct zone of hybridization between Bedwell and Childs' (1943) Mount Hood and northern Washington Cascade populations of WBP.

New Looks at Historic Issues

Separating Fact from Fiction—The WPBR pathosystem has been in North America for almost a century; intense efforts to control the disease by minimizing the alternate host had been applied; and we have 50 years of experience in developing resistance. Yet, detection of multiple forms of resistance, virulence that overcomes one form of resistance in several hosts, observations on incidence and severity of infection, and behavior of local epidemics all lead us to ask critical questions of the breadth of our knowledge of this system. Have we examined the blister rust pathosystem in sufficient detail to predict whether disease in stands genetically improved for resistance will perform as predicted by our past experience with the epidemic on susceptible plants, by artificial screening results, or by models that predict disease incidence over time and space? Have important assumptions about WPBR behavior remained unchallenged? Can new tools be employed to critically measure WPBR pathosystem dynamics in time and space? What is the relative importance of classic genetic trait-oriented selection vis-à-vis other sources of genetic expression and change?

R genes and Resistance-Virulence Interaction—The classic gene-for-gene system seems to play an important role in WPBR. A series of papers has demonstrated that SP, WWP, SWWP, and LP have R genes (major genes for resistance) at some frequency in their populations (Kinloch and Comstock 1980, Kinloch and others 1999, Kinloch and Dupper 2002). Two virulence genes (*vcr1* and *vcr2*) are known that negate the resistance conferred by R genes in SP and WWP, respectively. Virulence genes have not been found that negate R genes in SWWP and LP, indicating that the three genes are distinct (Kinloch and Dupper 2002). At least one variant of *C. ribicola* is virulent on both SP and WWP R genes (Kinloch and Dupper 2002). Some evidence indicates that the *C. ribicola* gene for virulence to the sugar pine R gene may be inherited by way of the cytoplasm (Kinloch and Dupper 1999). The WWP R gene has not been found in Northern Rocky Mountain populations but is found in the Oregon Cascade Mountains (Kinloch and others 1999), with additional occurrence in the northern Sierra (Kinloch and others, unpublished). In some cases, expression of the WWP R gene seems to be subject to maternal influences, which may complicate its recognition (Kinloch and others 1999). Distribution of the virulence gene that overcomes the WWP R gene was tested using teliospore samples collected for seedling inoculations, and was identified as prevalent in recent years at Champion Mine (CM) and Grass Creek (GC) (Kinloch and others 1999, Kinloch and Dupper 2002) in the Oregon Cascade Mountains. Presence of the virulence gene was signified by the development of typical susceptible interactions in a group of full-sib families from Champion Mine that carry the WWP R gene (Kinloch and others 1999).

Inoculations of some of these same full-sib WWP families with rust collected in different ways at both CM and GC in 1984 and 2000 demonstrated an effect of *Ribes* source (or environmental aspects correlated with occurrence of *Ribes* species) and disease development. In their analysis, McDonald and others (1984) used three different sources of CM inoculum: telia collected directly at CM from *R. sanquineum* or *R. bracteosum* (Dougl.), and telia produced on *R. bracteosum* at Dorena, Oregon from aeciospores collected from WWP at CM (McDonald and others 1984 table 3). The most striking difference among these sources was the 4X higher rates of rust incidence (needle lesions) on WWP per equivalent basidiospore density when telia had been produced *in situ* at CM on *R. bracteosum* vs. the nearby *R. sanquineum*. Telia produced at Dorena from CM aeciospores behaved like the telia developed at CM on *R. bracteosum* except that it took significantly longer to kill seedlings so that significantly more stunted leaders of living plants were noted at the time of assessment (McDonald and others 1984). These results strongly suggest that growth on different *Ribes* species at this location provides an important G x E effect on ability of *C. ribicola* inoculum to subsequently infect and develop on pine. These differences may be mediated through differential ability of a virulent race to utilize local *Ribes*, or through more subtle interaction. Even though WPBR from the GC site may now carry *vcr2* virulence (Kinlock and others 1999), in 1984 its apparent lack of virulence to the WWP R gene was similar to WPBR obtained at the Still Creek (SC) site (see McDonald 2000) in its apparent lack of virulence to the WWP R gene (McDonald and others 1984). However, the GC inoculum produced significantly more cankers that were delayed in their development after needle infection than did the SC inoculum (McDonald and others 1984).

Ontogenic Resistance—An increase in ability of plants or plant parts to resist a pathogen as they age and mature and the physiological mechanisms by which this originates are well documented in agricultural pathosystems (Ficke and others 2002). However, in agricultural systems, plant development is generally restricted to a single season. The situation is much less clear for pathosystems involving long-lived hosts that nonetheless have yearly production of foliage and stems. Two types of ontogenic resistance have been described for WPBR: resistance related to types and maturity of needle tissues, and resistance related to physiological aging or other phenomena correlated with tree age.

Seedlings of the five-needle pines pass through four distinct maturation phases. Seeds germinate and produce cotyledons, primary (simple) needles, and occasionally secondary or fascicled needles during their first growing season. In most seasons in subsequent years, only secondary needles are produced, and these may be retained for multiple seasons. However, under certain environmental conditions, even older seedlings may produce primary needles in varying numbers, as part of a variant shoot morphology known as “lammas growth”.

Cotyledons and primary needles are extremely susceptible to infection and colonization and have been used for early screening of R gene resistance in SP where resistant and susceptible spots can be visually differentiated (Kinloch and Comstock 1980). These juvenile needles have also been used experimentally to assess rates of seedling colonization and subsequent mortality in families of eastern white pine (EWP) (*P. strobus* L.), since infection of both primary needles and stems of such immature plants is uniformly high (Zambino unpublished data). Conversely, the Phase I WWP resistance-breeding program relied on the differential between needle lesions and stem canker incidence to signify resistant families. An attempt to cut costs by inoculating seedlings at the end of their first growing season nearly eliminated the differentiation between resistant and susceptible seedling families (Bingham and others 1969). A large-scale inoculation of WWP subsequently confirmed that significant difference in needle lesion and canker incidence was associated with inoculation age and family

(Bingham 1972). The same pattern was observed in an interspecific cross of EWP with an Asian pine presumed to have a well-evolved resistance to WPBR. Patton and Riker (1966) inoculated two families of EWP x Himalayan white pine (*P.griffithii* McClelland) hybrids at 4.5 months and 48 months, and obtained canker incidences of 0.997 and 0.4 respectively.

Ontogenesis and maturity of individual needles also has a strong influence on resistance; lack of appreciation for this fact may explain variability in screening success and in ability to model the response of selected materials under natural conditions that have repeated opportunities for infection, versus controlled inoculations that are generally exposed at a single time. In inoculation experiments, “year old” secondary needles of EWP produced 3.7 times more infection per linear distance of needle than “fully mature current year” needles (Hirt 1938). In similarly inoculated WWP, the ratio between “year-old” and “current year” needles was 14.5 to 1 based on needle area (Pierson and Buchanan 1938). Most screening includes infection data on needles within or at the end of the first year of development, even though no studies have shown how this practice might influence field performance of selected materials or the expression of canker resistance mechanisms. Timing for inoculation within the current year needle development may also be critical as inoculation of WWP secondary needles before they have completed development circumvents the expression of resistance in needle tissues (Woo 2000). In addition, stomatal density and shape and contact angle of water droplets on needle surfaces showed significant differences in comparisons of resistant and susceptible WWP (Woo and others 2001 and these proceedings).

Finally, branch and stem resistance appears to increase with age: Comparisons among grafts obtained from susceptible and resistant ortets of various ages and susceptible seedlings demonstrated that resistance increases with increasing age of the scion source for both resistant and susceptible grafts of EWP (Patton 1961). In addition, the act of grafting alone reduced incidence from .99 to .81 in 4-year-old seedlings (Patton 1961). In SP exposed to rust in the Happy Camp disease garden, average incidence among grafts of 17 ortets was 0.32, whereas seedling families obtained from the same 17 candidates showed an incidence of 0.92 while susceptible control seedlings had an incidence of .98 (Kinloch and Byler 1981). This apparent increase in a tree’s resistance with age must nonetheless be reconciled with the fact that shortly after the introduction of WPBR, canker incidence in some merchantable WWP stands reached 100 percent and was followed by high levels of mortality (Buchanan 1936), indicating the ineffectiveness of the extant levels of ontogenic resistance at that time.

Ontogenic or Induced Resistance?—Defenses against most enemies in most living organisms may be either constitutive or induced (Fluhr 2001). Most animals have both kinds of defense, while plants may have evolved mostly constitutive defenses in the form of R genes that stand as sentinels on the lookout for virulent pathogens (Fluhr 2001). These R genes have certain molecular signatures found across many plant taxa (Fluhr 2001). Some of these signatures have been observed in both SP (Kinloch and Dupper 2002, Sheppard and others 2000) and north Idaho WWP (Liu and Ekrammoddoulah 2004; M.-S Kim personal communication). Perhaps these signatures are a sign of the widespread occurrence of R genes in the five needle pines as suggested by Kinloch and Dupper (2002).

Other recent reports suggest that induced defenses might also be widespread in plants as well as animals. In the case of the desert locust, *Schistocerca gregaria*, increased population density induced increased resistance to bacteria and fungi (Wilson and others 2002). A large and rapidly developing literature is available for the topic of inducible (epigenetic) defenses in plants and animals that we will not attempt to present here. Induced defenses vary from morphologic plasticity able to ward off predation or infection to induced physiological resistance (Tollrian and Harvell 1999). Epigenetic R-gene resistance that is expressed in subsequent generations was demonstrated in *Arabidopsis thaliana* (L.) Schur (Stokes and others 2002).

Since the topic of inducible defenses impinges on many aspects of WPBR resistance breeding, it should be watched closely. Impacts could range from understanding ontogenic resistance through examination of phenotypically resistant candidates, to improved selection and interpretation of performance of “control” lots used in progeny screening tests and field plantings. Inducible defenses have already been reported in both native (evolutionarily well-established) conifer pathosystems such as fusiform rust-loblolly pine (Enebak and Carey 2000), and Norway spruce (*Picea abies* (L.) Karsten)-blue-stain-(*Ceratocystis polonica*) (Krokene and others 2001, Franceschi and others 2002), as well as recently established interactions such as Monterey pine (*P. radiata* D. Don.) — pitch canker (*Fusarium circinatum*) (Bonello and others 2001). Also, stilbenes — effective conifer phytoalexins — are induced in various plant parts including primary needles of Scots pine by such diverse challenges as fungal attack in the phloem, UV light, and stress (Chiron and others 2000).

Evidence for Induced Resistance to WPBR—In WWP, 36 phenotypically resistant ramets (clonal offspring) that had been obtained by grafting from select trees from the field (ortets) were established in plantations and exposed to levels of field inoculation that produced 0.89 incidence of cankering of control seedlings at the same sites after 13 years of exposure (Bingham 1966). Full-sib offspring were obtained from 34 of the ortets that produced the tested ramets. Controlled crosses were made on the 34 seed parents using a common set of four pollen donors. When 11744 seedlings of these families were subjected to artificial inoculation, canker incidence was 0.84 (calculated from table 4, Bingham 1966). Comparisons between infection of the ortets (plants in the field) and their ramets should indicate presence or absence of induced resistance. Bingham (1966) noted that ramets of all nine of the ortets that were infected when selected did not themselves become infected; however, five of the 25 ortets that were uninfected when selected had ramets that became infected. Three kinds of phenotypically resistant parents (ortets) are suggested. First, those in which a few cankers induced resistance that prevented subsequent infection of their ramets (i.e., the 9 infected ortets). Second, those that may not have been resistant or may have been capable of induced resistance that was not triggered (i.e., the 5 ortets whose ramets became infected). The third class is composed of those trees that possessed putative constitutive resistance (an additional 20 rust-free ortets that produced rust-free ramets). This situation implies induced resistance. Furthermore, comparison of performance of seedling progeny of the three classes may indicate the first evidence of transgenerational induction of resistance in conifers: Log-odds ratios (Sokal and Rohlf 1995) indicated significant differences in rust incidence on seedlings that differentiated the groups from one another. Rust incidence was lowest (0.81) among 6863 seedlings representing the 20 "constitutive resistance" ortets, highest (0.92) among the 1612 seedlings representing the 5 "uninduced" ortets, and intermediate (0.88) among 13269 seedlings representing the 9 "induced" ortets.

G x E in Plantations of Rust Resistant WWP—Over the years, WPBR-resistant stocks developed in the north Idaho Phase I program were planted and/or inoculated in many different environments. Differential responses in results illustrate gaps in our knowledge concerning the relative contributions of environment, rust genes, alternate host diversity, pine diversity, and resistance genetics to the behavior of WPBR. The most interesting of these tests involve long-distance transfer of materials. For example, susceptible controls and several full-sib F_1 and F_2 families from the Phase I program were subjected to natural and artificial inoculation at two sites in Japan where *C. ribicola* basidiospores are produced on a host (*Pedicularis resupinata* L.) in the Scrophulariaceae (Yokota 1983). The overall conclusion was that resistant material developed in north Idaho was not resistant to the rust in Japan. Stock from the Phase I program was also planted at several sites in British Columbia (Bower 1987, Hunt and Meagher 1989). In a direct comparison after 11 years at a low elevation site (Mesachie), disease incidence was higher (.75) for the Phase I F_2 than for local natural unimproved stock (0.52) (Hunt and Meagher 1989). The pattern was reversed at a second site 45 km north of Mesachie at Northwest Bay where a higher elevation site produced incidences after 13 years of 0.12 for the F_2 vs. 0.40 for local stock (Hunt and Meagher 1989). These authors describe further significant G x E interactions for several sources of resistant WWP as well as EWP. Significant G x E constitutes a warning to pay close attention to inoculum sources and geographic distributions of materials selected for resistance. G x E was also observed in field plantings of slash pine (*P. elliottii* Englm var. *elliottii*) resistant to *C. quercuum* (Berk.) Miy. ex Shirai f. sp. *fusiforme* (Schmidt and Allen 1998). Significant G x E in rust behavior signifies that geographic partitions in addition to simple breeding zones may be a required strategy in restoration programs.

New Tools and Concepts

Comparative Epidemiology—Plant disease epidemiologists have focused on modeling and disease management from an endpoint perspective, while medical epidemiologists have focused more on elucidating component parts, enabling them to act as disease detectives who can reconstruct the whole by understanding the interactions of the parts (Waggoner and Aylor 2000). Subsequent to the inception of pine breeding programs in western North America, advances in epidemiological theory and evolutionary biology have added several tools that should enhance our own capability to become good disease detectives and to focus on how the various parts of WPBR interact. Successful breeding and deployment of resistance in a long-lived pathosystem requires significant understanding of how epidemics function across time and space, which can lead to better integrated management of blister rust and restoration of ecosystems dominated by five needle pines.

Rust Progress Curves—Measuring disease is a significant problem often approached by determining incidence (proportion of a population of plants or plant parts infected) and severity (number of lesions or amount of area of plants or plant parts infected) periodically during the progress of the epidemic and estimating curves from the plotted values. Nonlinear disease progress curves and their associated linear incidence rate equations (Madden and Campbell 1990) can be useful for comparing epidemics and predicting outcomes in new times and places. Fracker (1936), the first to apply nonlinear progress curves to plant disease epidemics, was also first to apply them to WPBR. Since then, epidemiological theory has matured into several straightforward concepts (Zadoks and Schein 1979, Madden and Campbell 1990). Almost all epidemics are described by one of two basic forms. For most diseases and environments, epidemics behave as a polycyclic process where diseased plants contribute inoculum to increase local disease within the same growth cycle. Such epidemics are represented as a logistic curve where the inflection point indicates the point at which the acceleration in rate of disease increase due to increased inoculum is offset by the decline in healthy tissues available for colonization. Other epidemics behave as a monocyclic process where local diseased plants do not contribute to additional disease because inoculum is from relatively constant external sources. Because amounts and infection efficiency of inoculum are relatively constant and not affected by local feedback, curves that describe this process have no inflection point. This "monomolecular" disease progress curve and its associated absolute infection rate have been applied to WPBR (Kinloch and Byler 1981, McDonald and Hoff 1982, Goddard and others 1985, McDonald and others 1994, McDonald and Hoff 2001). Presence or absence of an inflection point may provide important clues about fundamental disease processes such as relative importance of local vs. long-distance spread of inoculum and degree of rust multiplication on alternate hosts.

The epidemic asymptote K indicates maximum incidence in an epidemic (Madden and Campbell 1990). The idea that incidence may not reach 1.0 was anticipated by Fracker (1936), who also introduced the concept of the multiple infection transformation, an equation relating incidence to severity. Agricultural epidemiologists have generalized K in nonlinear disease progress curves (Madden and Campbell 1990) and the examination of K has been applied to WPBR epidemics (McDonald and Dekker-Roberson 1998, McDonald and Hoff 2001). Fracker's (1936) ideas were combined with those of Bald (1970) to develop the concept of a factor that measures deviation in K over space for WPBR epidemics (McDonald and others1991, McDonald and others 1994).

If one assumes that total incidence can theoretically approach 1, then the difference between complete incidence and predicted asymptote, 1-K, may be attributed to lack of uniformity in distribution of resistance genes, microclimate, or composition or density of inoculum. Predictions of K in different situations could be vitally important for breeding and deployment programs alike. For a breeding program, clumpy distribution of infection due to clumpy microclimate and/or basidiospore distribution can significantly increase the probability that rust-free candidate trees are susceptible escapes whose processing reduces screening efficiencies. However, asymptotes may also reflect effects of a major or minor gene for resistance, or situations in which even susceptible materials will persist on a site, and changes in K during an epidemic could indicate major changes in rust and/or pine populations (Kinloch and Byler 1981, McDonald and others 1994). Infection rates, especially comparative rates, are useful for assessing durable resistance (McDonald and Dekker-Robertson 1998).

Phenotypic Plasticity, Reaction Norms – Construction of Hypotheses, and Understanding G x E—Since a central tenet of breeding for resistance in any pathosystem is host/pathogen coevolution, it thus seems natural that geneticists and phytopathologists engaged in development, deployment, and maintenance of resistance should examine theoretical interpretations utilized by other disciplines to understand the implications of G x E in plant pathosystems. Indeed, evolutionary biologists have developed very powerful concepts and tools – those of phenotypic plasticity and reaction norms – not yet widely applied in phytopathology and sparingly used in forest genetics. Phenotypic plasticity is the ability of a genotype to express itself as different phenotypes in different environments. A few notable applications of this concept in forest genetics were found (Wu 1998, Wu and Hinckley 2001). As a basis for the following discussion, concepts from the excellent book by Pigliucci (2001) are liberally presented. Central elements in the concept of phenotypic plasticity are the reaction norm and its connection to analysis of variance as applied in quantitative genetics. Reaction norms are functions that relate the range of possible phenotypes a genotype can express across an environmental gradient. They are generally used to visualize genetic, environmental and G x E variance by comparing at least two genotypes, families (any relationship), populations, species, or groups of species across an environmental gradient. Any gradient of interest, e.g., light, temperature, moisture, nutrients, or host organisms, can be displayed. However, if a true gradient is not present, then sites or hosts must be compared in pairs or some kind of artificial gradient needs to be assumed. Reaction norms have a historic association with ANOVA as the preferred method of analysis. Summary statistics are often given in a suitable format such that all ANOVA elements are present, to wit: differences among across-environment means of the genotypes indicate genetic (G) variance; differences among across-genotype means of environments indicate environmental (E) variance; and when G and E interact in ways not predicted by the combined influence of across-environment and across-genotype means, there is plasticity (P) variance. Further note that if means for an individual genotype differ significantly across-environments, that genotype is plastic. This allows computation of the log-odds ratio and its associated standard error for hypothesis testing. A newly published book presents a multivariate approach to the analysis of G x E in a crop-breeding context (Yan and Kang 2003). Although examples of most types of organisms are presented in Pigliucci's (2001) book, fungi and plant pathosystems are notably absent, arguably because phytopathologists and mycologists have been slow to embrace the concepts and methodology of phenotypic plasticity and reaction norms. Yan and Kang (2003) devote two chapters to the analysis of pest resistance G x E in the context of crop resistance breeding rather than in terms used by evolutionary biologists.

To foster a dialogue about these and other questions, we will look at some blister rust pathosystem processes using the concepts and tools just outlined. We will apply the concepts of K, infection rate, inflection points, and reaction norms in a critical assessment of published data as well as updated data obtained from established plantings. Our intent is to advance the idea that becoming better rust detectives will lead to better development, deployment, and management of resistance genes in western North American 5-needle pines.

Materials and Methods

Data

To illustrate the utility of the reaction norm approach and in keeping with the disease detective approach to the study of WPBR epidemiology, reaction norms were constructed for seven cases from previously collected or published data. Case one examines time from inoculation to pustule development for inoculum sources from different geographic areas inoculated onto individual *Ribes* clones; data was obtained from a study (McDonald 2000) that was previously published using average responses over all clones. Reaction norms (fig. 1-3) were selected to provide an elementary lesson in recognizing types of trends that may be encountered. The second case identifies variation in three types of pine response to the Champion Mine race of *C. ribicola* obtained from different alternate hosts through a reexamination (fig. 4-5) of data from McDonald and others (1984). Case three demonstrates the use of reaction norms (fig. 6-7) to dissect effects of developmental stages, and possibly ontogenic resistance, using developmental reaction norms defined by periods of exposure to rust for WWP data published by Bingham (1966 and 1972). Case four uses reaction norms to demonstrate a classic G x E interaction (fig. 8) for WWP stocks grown at two *ex situ* locations in Japan (Yakota 1983). Case five demonstrates the fitting of incidence data from various classes of resistance phenotypes grown at two northern Idaho sites into disease progress curves (fig. 9); further examination of differences in reaction norm parameters among resistance and site categories are presented (fig. 10-12). Case six examines infection and disease progress parameters in three northern Idaho full-sib test plantation sites (Bingham and others 1973, McDonald and others 1994, McDonald and Dekker-Robertson 1998, Fins and others 2002); reaction norms are set up to compare parameters that were obtained for different resistance classes early versus late in the epidemic (fig. 13-16). Case 7 is a detailed examination of infection severity and incidence to reveal clues about the cause of unexpectedly high disease incidence on resistant stocks at one of the three sites.

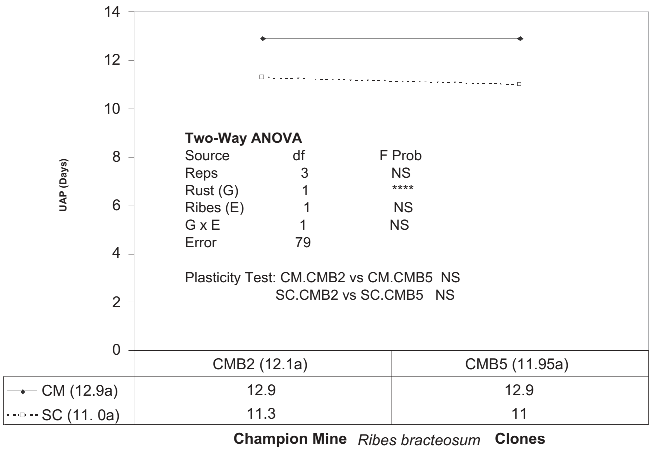


Figure 1—Reaction norm demonstrating significant genetic variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes bracteosum* collected at the Champion Mine site (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination.

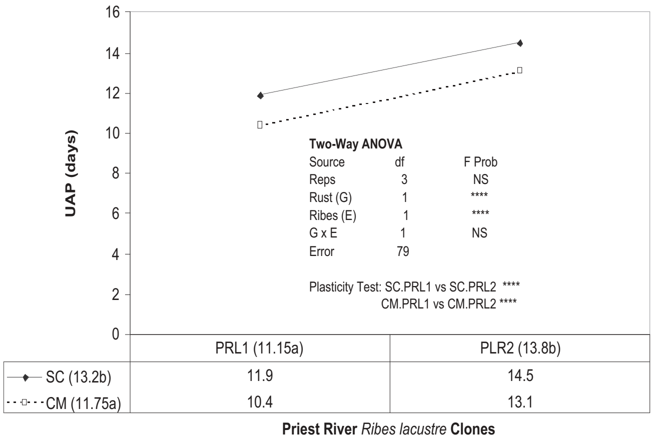


Figure 2—Reaction norm demonstrating significant genetic and environmental variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes lacustre* collected at the Priest River Experimental Forest, Idaho (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination.

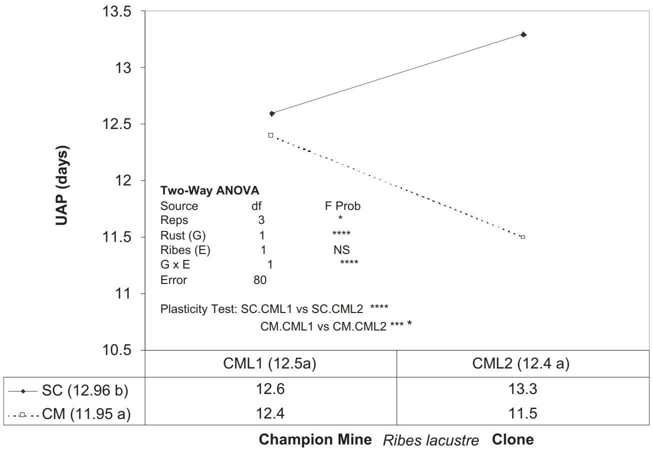


Figure 3—Reaction norm demonstrating significant genetic, environmental and G x E variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes lacustre* collected at the Champion Mine site (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination

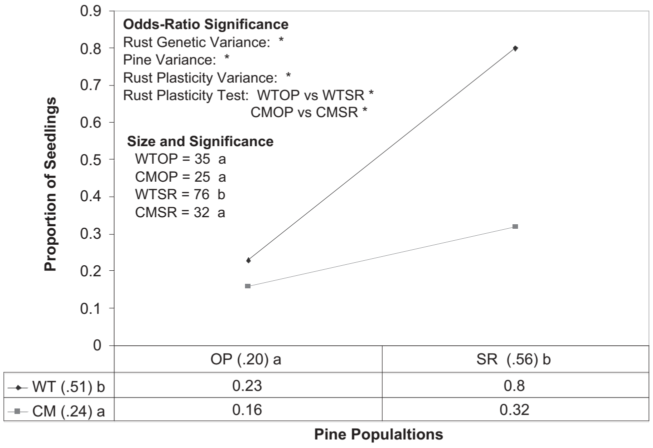


Figure 4—Incidence of premature needle shed resistance in *Pinus monticola* for two sources of *Cronartium ribicola* basidiospores (CM = Champion Mine, OR; WT = pooled Still Creek and Grass Creek, OR) inoculated onto 13 open pollinated pine families obtained from phenotypically resistant parents (OP) vs. seven full sib families obtained from Champion Mine and specifically selected for resistance to WT inoculum (SR) (Case 2 in text; data from McDonald and others 1984).

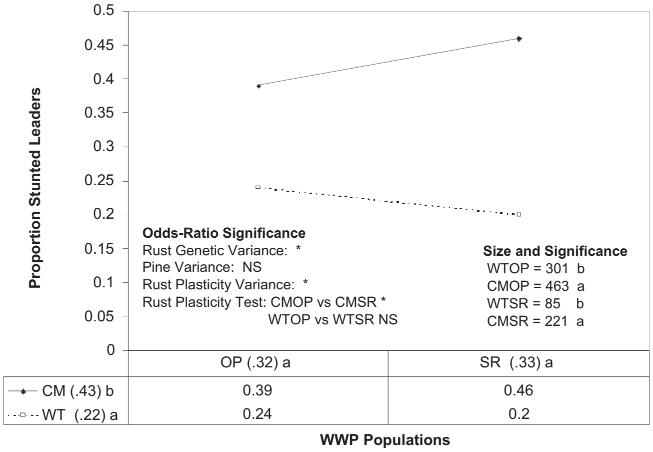


Figure 5—Proportion of stunted leaders in *Pinus monticola* for two sources of *Cronartium ribicola* basidiospores (CM = Champion Mine, OR; WT = pooled Still Creek and Grass Creek, OR) inoculated onto 13 open pollinated pine families obtained from phenotypically resistant parents (OP) vs. seven full sib families obtained from Champion Mine and selected for resistance to WT inoculum (SR) (Case 2 in text; data from McDonald and others 1984).

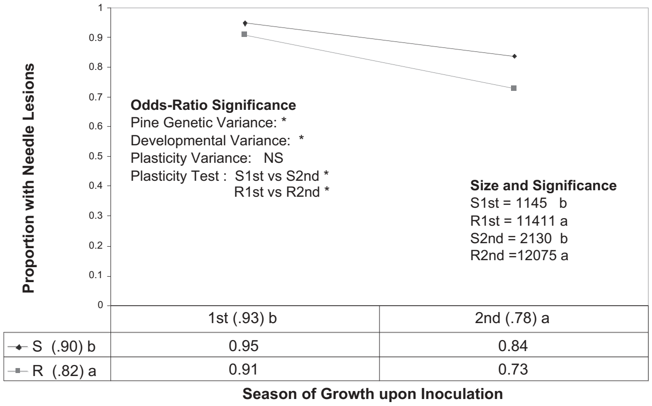


Figure 6—Effect of seedling development stage during rust exposure on needle infection in *Pinus monticola* (Case 3 in text): Incidence of infection for seedlings of phenotypically resistant parents (R; full-sib progeny) vs. susceptible infected parent trees (S; open pollinated progeny from high rust areas in northern Idaho) after exposure to a bulked source of *Cronartium ribicola* basidiospores from north Idaho at the end of first (1st) vs. second (2nd) growing seasons (Data from Bingham 1972).

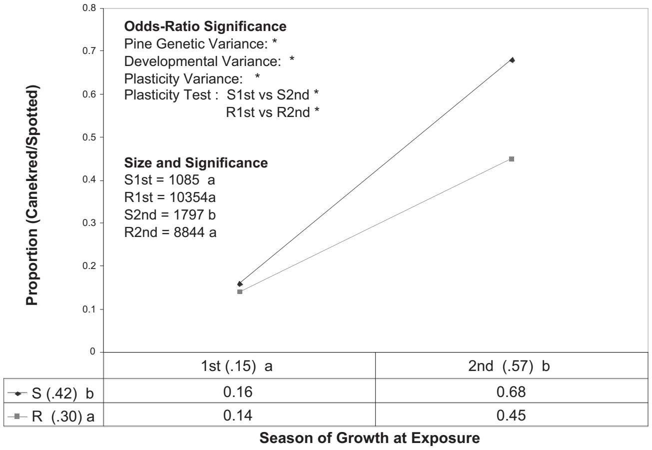


Figure 7—Effect of seedling development stage during rust exposure on stem infection in *Pinus monticola* (Case 3 in text; materials and definitions as in fig. 6).

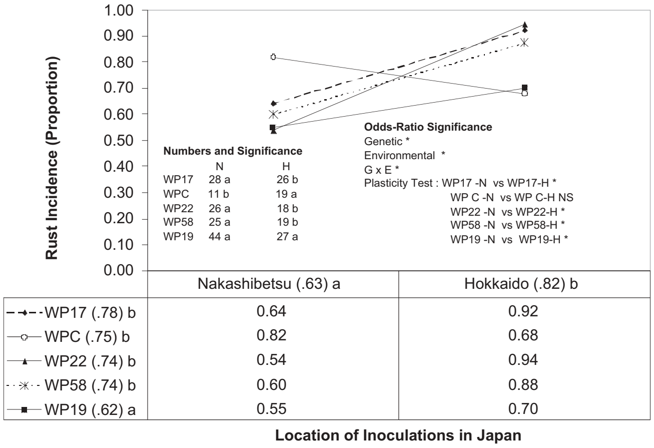


Figure 8—Genetic x Environment interaction affecting resistance in *Pinus monticola* (Case 4 in text): Incidence of canker infections for five half-sib families from crosses of north Idaho seed parents after inoculation with unique sources of *Cronartium ribicola* basidiospores at two localities, i.e. natural inoculations at Nakashibetsu, Japan and artificial inoculation at Hokkaido, Japan. (Data from Yokota 1983).

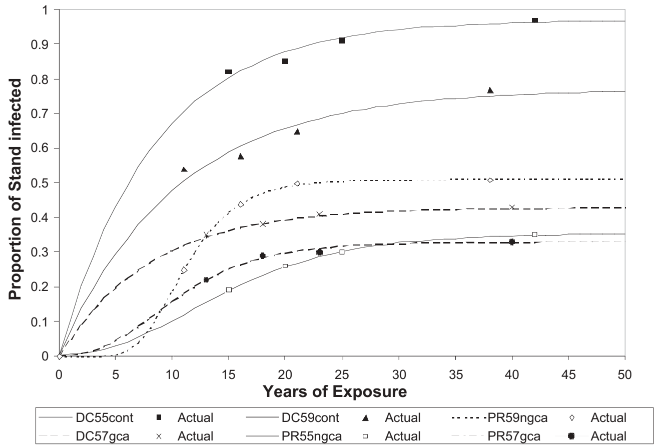


Figure 9—Disease progress curves for epidemics caused by *Cronartium ribicola* showing differences in disease incidence asymptotes (K) from fitting optimal nonlinear equations (Gompertz or monomolecular) to data from groups of *Pinus monticola* families representing three resistance pedigrees (GCA x GCA, NonGCA x GCA, and OP Controls) grown at Priest River (PR) and Deception Creek (DC) Experimental Forests located in northern Idaho, USA (Case 5 in text).

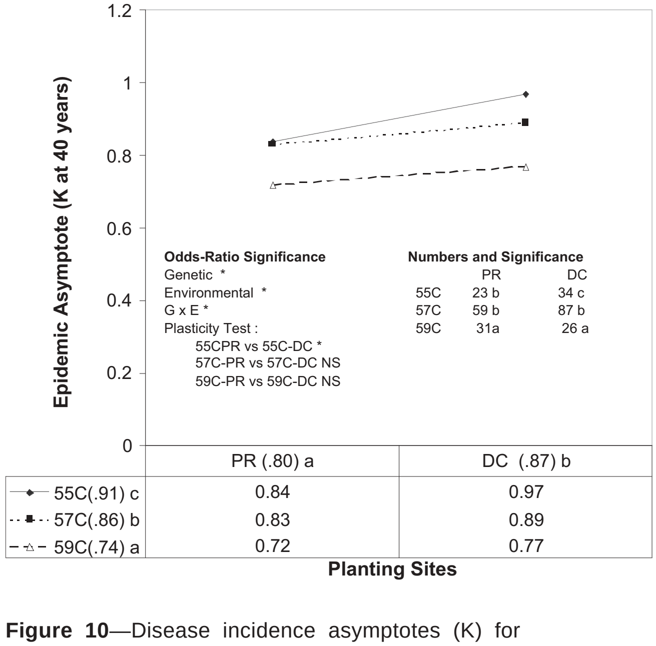


Figure 10—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in *Pinus monticola* open pollinated control lots planted in 1955 and 1956 (55C), 1957 (57C) and 1959 (59C) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

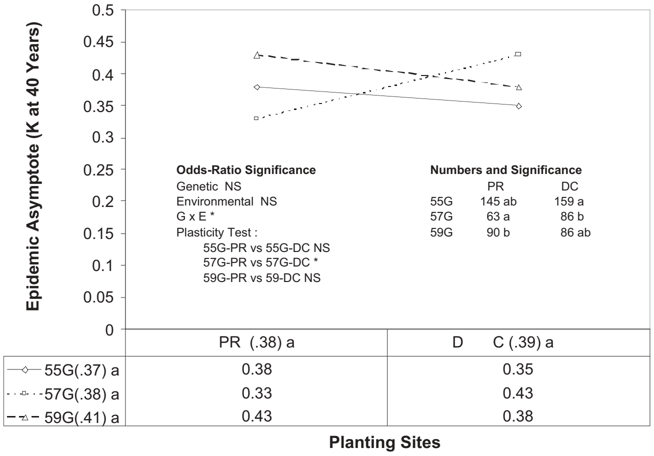


Figure 11—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in groups of *Pinus monticola* full-sib families with a GCA x GCA pedigree planted in 1955 and 1956 (55G), 1957 (57G) and 1959 (59G) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

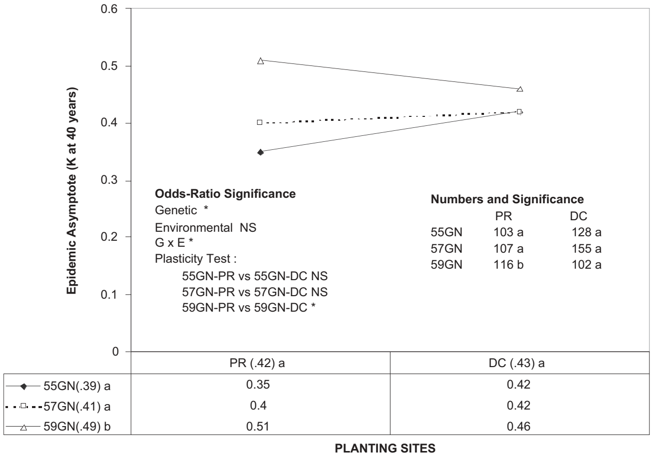


Figure 12—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in groups of *Pinus monticola* full-sib families with a nonGCA x nonGCA pedigree planted in 1955 and 1956 (55GN), 1957 (57GN) and 1959 (59GN) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

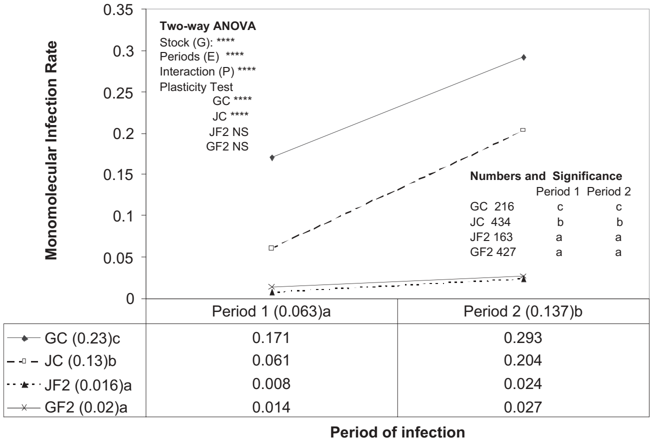


Figure 13—*Cronartium ribicola* infection incidence rate constants computed using the monomolecular equation for the first 11 years (period 1) vs. the last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (Cont) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho (Case 6 in text).

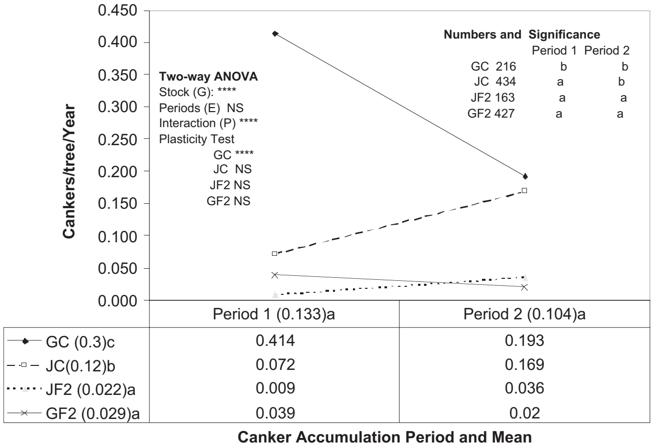


Figure 14—*Cronartium ribicola* average canker accumulation rates for first 11 years (period 1) vs. last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (Cont) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho (Case 6 in text).

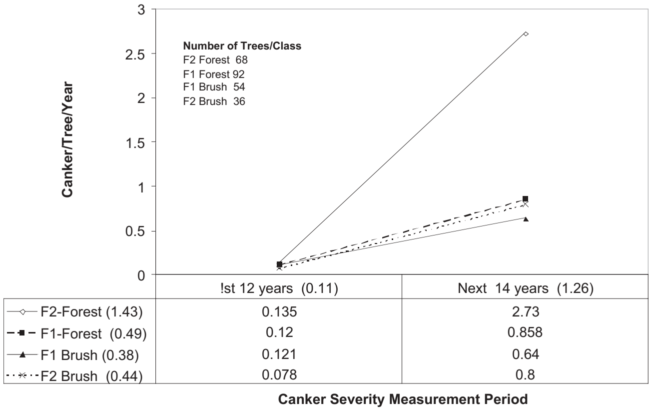


Figure 15—*Cronartium ribicola* average canker accumulation rates for first 11 years (period 1) vs. last 14 years (period 2) during which infection was monitored in *Pinus monticola* first (F_1) vs. second generation resistant selections (F_2) planted at Merry Creek in northern Idaho under forest vs. brush site conditions (Severity data supplemental to study described by McDonald and Dekker-Robertson 1998) (Case 7 in text).

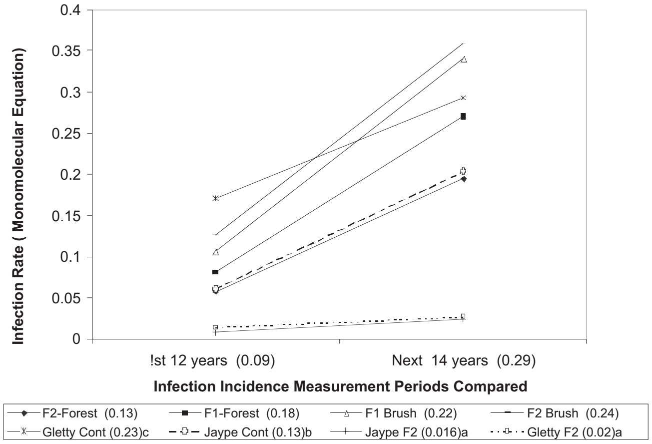


Figure 16—*Cronartium ribicola* infection incidence rate constants computed using the monomolecular equation for the first 11 years (period 1) vs. the last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (C) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho vs. Merry Creek (Case 7 in text; based on data from McDonald and Dekker-Robertson 1998).

Statistical Methods

This paper utilized three main types of statistical tests: ANOVA with multiple means testing, log-odds ratios applied to means of proportions, and nonlinear curve fitting. Data were available for the *Ribes*-rust geographic variation experiment (McDonald 2000) to conduct standard analysis of variance (Systat 9.1 SPSS, Inc) for each set of four comparisons in the reaction norm format. These data meet the normal distribution assumption for ANOVA (see McDonald 2000). This format and details of reaction norm analysis can be found in Pigliucci (2001). Where multiple means were compared in pairwise format the Bonferroni correction was used (Systat 9.1). In the case of nonlinear curve fits, the standard error of the parameter estimate (disease asymptote) was computed by the curve fitting software (Table Curve 3.0 SPSS, Inc).

Analyses based on published data and new analysis of the vigor-quality plantings entailed comparisons of proportions; log-odds ratios were used to determine if any particular pair of proportions was significantly different (Sokal and Rohlf 1995). The standard error of the comparison was determined by taking the square root of the sum of the reciprocals of the number of observations in each cell of the comparison (Sokal and Rohlf 1995). To obtain 95 percent confidence limits, the standard error was doubled and then added and subtracted from the natural log of the odds ratio (Sokal and Rohlf 1995). When this range crossed zero, the proportions under comparison were considered not significantly different (Sokal and Rohlf 1995). Reaction norms require comparison of across-environment means of each family line or genotype, as well as comparison of each family x environment proportion. Mean proportion of all families in an environment must be compared across environments. To accomplish these comparisons, we computed each relevant mean proportion and estimated the standard error by computing the average number of observations per cell of the four cells associated with the proportions being compared.

Results and Discussion

Case 1: Uredinial Sorus Development Time

Data previously presented by McDonald (2000) but pooled over five *Ribes* clones were separated by clone x WPBR source and the period from inoculation to urediniospore appearance. The number of days until urediniospores appeared (urediniospore appearance period; UAP) was then subjected to a new ANOVA. Given that hosts of plant pathogens are a significant component of a pathogen's environment, our first example will include two WPBR populations (Champion Mine and Still Creek) compared on two *R. bracteosum* Douglas clones from the Champion Mine site (fig. 1) and two *R. lacustre* (Pers.) Poir. clones obtained from the Priest River site (fig. 2). We also include two Champion Mine *R. lacustre* clones (fig. 3). The important elements are the mean UAP's obtained from 24 leaf disks — four replications of inoculations of 6 disks each (McDonald 2000).

The across-environment means are important, as are the slopes of the reaction norms. Defining the "genetic" component as differences due to rust source, and "environment" as that due to rust clone in the first comparison (fig. 1), the across-environment means differ significantly, indicating significant genetic variance in the rust populations. There is no environmental variance (means for the two *Ribes* clones across rust populations do not differ), no plasticity variance (no G x E interaction), and no plasticity for either population (the individual environment means for each rust population do not differ). There would be no genetic variance if means for the two rusts were not significantly different. Next, we illustrate sloping parallel reaction norms and associated ANOVA (fig. 2). In this specific combination, the rust populations showed significant genetic variance (across-environment means differ), environmental variance (environmental means differ), and both rust populations show plasticity because means within a rust population differ across environments, but there is no plasticity variance (no G x E). Crossing or divergent reaction norms (fig. 3) also yield specific genetic information about *R. lacustre* clones CML1 and CML2: Genetic variance is present since the across-environment means differ, but environmental variance is not present since the mean performance of the two rust populations on *Ribes* clones, CML1 and CML2 do not differ. Variance for plasticity is present (significant G x E). If we were comparing rust clones derived from single spores, we could make the case that rust clones have heritable plasticity. Since we are comparing rust populations, we can only argue that the two populations are genetically different. Both populations are exhibiting plasticity, since their performances differ by *Ribes* clone.

The real power in such specific G x E comparisons resides in the fact that specific hypotheses can be created and tested. These hypotheses can then form the basis for designing specific experiments, which can include application of molecular techniques (Wu 1998, Pigliucci 2001). For example, figure 3 might suggest that genetic variation in *C. ribicola* is caused by allelic differences within mixed populations, variation in gene expression, or some other factor such as conditionally dispensable chromosomes or transposon activity. Conditionally dispensable chromosomes are small chromosomes that often contain genes coding for pathogencity that can be conditionally removed from the genome (Hatta and others 2002, Covert 1998). Transposons — transportable genetic elements — are known in the basidiomycotina (Fowler and Mitton 2000) and are known to influence the expression of host resistance genes in a rust pathosystem (Luck and others 1998). Another potential role for utilizing reaction norms for understanding pathosystems is to examine quantitative geographic and host differential responses beyond the qualitative virulent – avirulent dichotomy based on R genes, as discussed above.

Case 2: R Gene-Virulence Interaction

We will briefly discuss aspects of the CM (Champion Mine) race in regard to qualitative vs. quantitative differential reactions. The CM race was first described in 1984 (McDonald and others 1984) using a series of quantitative pine host responses relating mostly to reduction in frequency of expression of several resistance traits. Open pollinated families obtained from phenotypically resistant candidate trees growing in Oregon and Idaho were compared to a group of full-sib families obtained from selection and breeding candidates obtained from the CM site (Kinloch and others 1999, McDonald and others 1984). Trees were inoculated with CM and wild-type inoculum and expression of various symptoms was recorded monthly for 24 months. Data were published (tables 4 and 5 in McDonald and others 1984) that now allow reaction norms to be developed for genetic interpretations. Responses of the OP and selected-resistant families with regard to premature needle-shed resistance (McDonald and Hoff 1970) yielded significant genetic and environmental (host genetic) variance when genotype is defined by host and environment is defined by kind of rust inoculum (fig. 4). Plasticity variance was indicated by the combination of nonsignificant (NS) and significant differences in the E means. The reaction norm interpretation is that both sources of blister rust carry genetic variation for plasticity since means from their cross-environment plasticity tests were significantly different. This analysis shows that the OP candidate families carried some premature needle shed resistance that was of equal effectiveness to both populations of the rust; i.e., they did not differentiate the rust populations. However, the resistant population selected by screening and breeding for resistance to inoculum collected at CM dropped from 80 percent expression of resistance to 32 percent. This is still an important level of resistance, which suggests some mechanism other than simple negation of an R gene may underlie susceptibility to rust variants capable of neutralizing R genes. An alternative explanation for the observed pattern in the R-gene-containing/R-gene-lacking couplet is variation in expression of R genes associated with host maternal cytoplasm (Kinloch and others 1999).

Before we leave the CM race, we should investigate the effect of this rust population on frequency of stunted leaders (McDonald and others 1984). Reaction norms were constructed (fig. 5) showing significantly greater incidence of stunted leaders by CM rust across host environments. There was no significant response of this trait to selection for resistance when inoculated with wild-type rust, but significant genetic and plasticity variances in the rust. The pattern of these norms indicates experimental conditions that could facilitate the study of physiological aspects of WPBR. This is especially interesting since the CM inoculum grown at the site of inoculation exhibited a significantly higher incidence of stunted leaders than did the two sources obtained at the CM site. In closing our discussion about CM rust, evidence of many kinds of differential interactions involving CM on both *Ribes* and pine hosts (fig. 1 to 5) sends a message that there is much more to host interactions with WPBR than simple virulence / avirulence alleles in the rust and R genes in the pine hosts. An initial step toward understanding these complexities should be to reanalyze the initial test (McDonald and others 1984) in light of the current knowledge about *Cr2* genes in WWP (Kinloch and others 1999).

Case 3: Ontogenic Resistance

In Bingham's (1972) large-scale screenings of 1966 and 1967, many thousands of seedlings in a number of control lots and full-sib tester families germinated one year late. Thus, seedlings belonging to the same seedlots were simultaneously inoculated at 1 and 2 years of age. Incidence of needle infection and subsequent incidence of cankers were analyzed for each seedling. For incidence of needle lesions, WWP clearly showed genetic variance, environmental (developmental) variance, and plasticity in both resistant and susceptible populations (fig. 6). No variation for plasticity was evident because both populations moved in the same direction. Thus, we can argue that age (primary needles vs. secondary needles) has an equal influence in both resistant and susceptible populations. This leads to the conclusion that screening efficiency could have been enhanced by early inoculation. As pointed out by Bingham (1972) the lower needle lesion incidence in older seedlings is surprising given that target increases about 4x by the end of the second season. Take note that almost 30,000 seedlings were included in this test (fig. 6).

Analysis of canker incidence clearly illustrates why primary needle inoculation did not work (fig. 7). Incidence of cankers does not differentiate populations under first season inoculation (within E1 NS). Meanwhile populations were clearly separable when secondary needles were inoculated. This result also indicates a large amount of resistance (spots but no cankers) in the control populations used in the 1964 and 1965 screenings. Bingham (1972) noted the essential differences from his perspective. So what, if anything, does the reaction norm perspective add? In this case, two obvious advantages are to focus attention on interactions and development of questions from "outside the box" by forcing consideration of a wider viewpoint. For example, comparative incidence of both needle and stem infections brings into question just how much and what kind of resistance was present in materials selected for "susceptible controls" in the 1950s after just 25 years exposure of the WWP to WPBR. During the Phase I screenings at Moscow, Idaho, incidence of combined infection (spots and cankers) varied from 0.5 to 1.0 for individual control lots, and no two inoculations utilized the same control lots (Bingham 1972). It appears that few of the WWP control lots supported cankering incidences > 0.99, as had five of eight control lots of EWP (Patton 1961).

Case 4: Analysis of Pine Rust G x E in Japan

The western breeding programs have been very aware of growth G x E and in some cases have gone to great lengths to capture its potential in selection programs (Kitzmiller and Stover 1996). In our introduction, we discussed indications gleaned from the literature that both rust-pine and rust-*Ribes* G x E may be of great import to understanding WPBR. We have just seen how reaction norms are constructed and interpreted, and from other sources (McDonald and Andrews 1981, McDonald 2000), that WPBR-*Ribes* G x E might play an important role in WPBR dynamics. Now we will take a closer look at WPBR-pine G x E.

Yokota (1983) provided sufficient data and descriptions of his experiments for us to apply a reaction norm analysis. Several full-sib F_1 families created within the north Idaho Phase I breeding program and two lots of susceptible north Idaho WWP were inoculated in Japan at two sites. Potted seedlings were transported from Hokkaido to Nakashibetsu 440 km eastward where they were exposed to basidiospores produced on local *Pedicularis resupinata* L. under natural conditions. The stressful nature of the latter site is indicated by Yokota's statement that in a related test conducted the previous year, many WWP seedlings were killed by winter exposure to cold temperatures. In the experiment we are analyzing, seedlings were exposed from August 22 to October 16, 1978, and then transported back to Hokkaido. Identical families were exposed to artificial inoculation September 18 to 20, 1978, and again September 27 to 30, 1979, at Hokkaido to basidiospores that developed naturally on local *P. resupinata*. Mean spore cast for the two inoculations were 90 and 80/cm^2 (Yokota 1983). Both naturally and artificially inoculated seedlings were placed in the same nursery to allow development of symptoms. Needle lesions were not observed, but incidence of cankering was recorded based on continuous observations for 5 years (Yokota 1983). Reaction norms were constructed from Yokota's canker incidence data.

Our example is a bit light in numbers since the control at one site included only 11 seedlings, but features of the reaction norm facilitate the extraction of useful information. One might say that the differences noted resulted from different rust populations or inoculation procedures or both. At one site the seedlings received a two-month natural exposure, while at the other site they received two short-term artificial exposures that delivered only about 85 spores/cm^2. This is far short of the 2,000 spores/cm^2 or more that are delivered in artificial inoculations from *Ribes* leaves during screening and experimentation (McDonald and others 1984). The fact that one family group (WP22) went from having the lowest to the highest incidence upon change of location, while another went from highest to lowest (this change was not significant due to low numbers) argues that the comparison may have a message, despite the aforementioned shortcomings. For one thing, the families tested generally gave about 30 percent canker-free individuals after artificial inoculation in Idaho and in the Nakashibetsu test so that they still had 30 percent less incidence than controls (fig. 8). However, almost no resistance was expressed at Hokkaido (fig. 8). Significant differences were seen even though the trees spent only two months at Nakashibetsu. There were significant differences associated with family groups and lots of G x E expressed in families WP19 and WP 22. Is this complex picture caused by variation of virulence or aggressiveness on the part of the rust, or by environment acting on gene expression in the pine or the rust? We have seen that planting northern Rockies F_2 WWP at a low elevation (warm) coastal site increases susceptibility while planting the same material nearby at a higher and more northerly site resulted in expected performance (Hunt and Meagher 1989). If the Nakashibetsu site was consistently cold during initial colonization (as mentioned, WP seedlings died of cold exposure the previous winter) and the Hokkaido site was warmer during initial colonization, then cool temperatures during inoculation and early colonization is a common element that may be related to activation of resistance genes. Expression of resistance genes in some pathosystems is influenced by temperature (Pérez-Garcia and others 2001). Another possible source of G x E is needle physiology. In EWP, certain trees showing one-season needle retention also exhibited low WPBR canker incidence (Hirt 1944). Trees retaining one season's needles became more normal in their retention of three seasons of needles after transplantation to a new site, and there exhibited normal rates of infection (Hirt 1944).

Case 5: Vigor-Quality Western White Pine Plantations

Repeated measurements of experimental material [e.g., resistant material having or lacking general combining ability (GCA) as well as susceptible materials] growing in natural environments are a powerful source of information about the dynamics of WPBR epidemics. In 1955, 1957, and 1959, outplantings of early generational materials from the Phase I program (full-sib GCA x GCA and GCA x Non GCA, open pollinated GCA, and Non GCA) were made at three locations in north Idaho to monitor the vigor and quality of the resistant families (Steinhoff 1971, Goddard and others 1985). Control lots arising from seed collected from infected members of the same cohorts as the resistant selections were also planted. In 1953 and 1955, OP seed was collected from infected members of the cohorts of phenotypically resistant trees at the same five locations to serve as control lots for the 1957 and 1959 plantings respectively (tree location data on file). Controls for the 1955/1956 planting were collected, in 1951, from infected trees located outside of resistant-tree selection sites. Plantings were replicated at Priest River, Deception Creek Experimental Forest, and at Emerald Creek on the Saint Joe National Forest, all in northern Idaho, USA. Poor survival at Emerald Creek precluded further consideration of that site. Plantations were inspected for rust incidence in 1970, 1975, 1980, and 1997. The 38 to 42 year-old trees were inspected from the ground for canker incidence in 1997. Records of individual trees were checked for continuity across all dates and records of unknown mortality were removed. Any record of rust infection during the life of a tree placed it in the infected category. A few records lost continuity between the 1980 and 1997 inspections because specific trees could not be relocated. The 1980 data were published (Goddard and others 1985) and data for the other years are on file (Moscow Forestry Sciences Laboratory). Reaction norms were constructed using as data estimates of the asymptote (K) of canker incidence obtained by fitting non-linear functions to rust incidence data recorded at about 13, 18, 23, and 40 years. Equations (Madden and Campbell 1990) fitting expectations of the monomolecular (monocyclic disease assumption), the logistic (polycyclic disease assumption with inflection assumed at 0.5 K), and Gompertz models (polycyclic disease assumption with variable inflection point) were calculated with Table Curve software (SPSS Inc). Each site initially received equal numbers of seedlings from the same families at each planting, although uneven numbers of losses due to planting occurred at each site. Each of the three years contained a unique mix of full-sib families for each resistance category. Pivotal to this discussion is the fact that the same mix of full-sib families was planted at the two sites for each planting time.

Estimated K and rate parameters of the best fitting equation, planting site, family groups, and standard errors of the estimated K and rate values are given in table 1. Out of nine combinations of site, family group, and planting year at Priest River, eight of the best fits (highest R^2 and lowest SE for K and infection rate) were derived using the Gompertz and, out of the nine at Deception Creek, eight were

Table 1—Fit of nonlinear equations to white pine blister rust canker incidence on full-sibs western white pine growing in the vigor-quality plantations located on northern Idaho Experimental Forest.

Full-sib group	Planting year	Planting location	Nonlinear[a] equation	K ± SE	Infection rate ± SE
OP Cont	1955	Priest River	Gomp	0.84 ± 0.025	0.16 ± 0.030
OP Cont	1957	Priest River	Mono	0.83 ± 0.017	0.08 ± 0.005
OP Cont	1959	Priest River	Gomp	0.72 ± 0.027	0.22 ± 0.058
GCA x GCA	1955	Priest River	Gomp	0.38 ± 0.018	0.10 ± 0.017
GCA x GCA	1957	Priest River	Gomp	0.33 ± 0.009	0.20 ± 0.046
GCA x GCA	1959	Priest River	Gomp	0.43 ± 0.011	0.25 ± 0.031
GCA x N	1955	Priest River	Gomp	0.35 ± 0.003	0.14 ± 0.007
GCA x N	1957	Priest River	Gomp	0.40 ± 0.009	0.18 ± 0.028
GCA x N	1959	Priest River	Gomp	0.51 ± 0.003	0.32 ± 0.011
OP Cont	1955	Deception	Mono	0.97 ± 0.024	0.12 ± 0.013
OP Cont	1957	Deception	Mono	0.89 ± 0.032	0.11 ± 0.015
OP Cont	1959	Deception	Mono	0.77 ± 0.046	0.10 ± 0.017
GCA x GCA	1955	Deception	Gomp	0.35 ± 0.008	0.14 ± 0.016
GCA x GCA	1957	Deception	Mono	0.43 ± 0.005	0.12 ± 0.007
GCA x GCA	1959	Deception	Mono	0.38 ± 0.015	0.11 ± 0.015
GCA x N	1955	Deception	Mono	0.42 ± 0.007	0.11 ± 0.008
GCA x N	1957	Deception	Mono	0.42 ± 0.018	0.12 ± 0.022
GCA x N	1959	Deception	Mono	0.46 ± 0.055	0.06 ± 0.016

[a] Gomp = Gompertz and Mono = Monomolecular equations (Madden and Campbell 1990).

derived using the monomolecular model. This indicates the rust was behaving in a fashion expected by a monocylic disease cycle at Deception Creek and in a polycyclic fashion at Priest River (fig. 9). The fit of these standard disease progress curves to the long-term WPBR canker incidence data was generally excellent for 18 combinations of families x site (table 1). Each genetic group seems to have its own unique path (fig. 9). However, some common patterns are evident. All attained their unique asymptote at about 25 years. At these sites and with these materials, once the plateau was reached, it has been stable. Although a stable K value at 25 years may not be obtained for epidemics at all sites, as demonstrated in Kinloch and Byler's (1981) long-term data collected at Happy Camp during the onset of the outbreak of the Happy Camp race in resistant SP, it is nonetheless true that when a stable predicted K value is obtainable, it can be an excellent trait for reaction norm analysis. If our plantings were composed of clones, or even full-sib families, instead of groups of full-sib families, we would have some very robust genetic information for drawing hypotheses about G x E interactions, as has already been demonstrated with poplars (Wu 1998, Wu and Hinckley 2001).

In these plantings, significant differences in K parameter could be due to clumpy distribution of basidiospores, clumpy microclimate, or variation in resistance among trees within a resistance category (McDonald and Hoff 2001). *Ribes* were eradicated from the vicinity of the plantations prior to establishment (Steinhoff 1972), and few bushes can be found in the local vicinity today. If a susceptible control lot can be shown to approach an incidence of one, then we can assume that departures from unity are due to resistance (McDonald and Dekker-Robertson 1998). When K is not constant throughout an epidemic, rust incidence alone is an inadequate measure of disease behavior. Under such cases, considering canker counts may be required to augment the use of incidence data; a multiple infection transformation function can then be used to generate predictability and for understanding irregular rust behavior.

We begin our analysis of the vigor quality (VQ) plantings by inspecting the reaction norms of the susceptible controls (fig. 10). Rust incidence of the 1955 OP controls at Deception Creek reached 0.97 after 42 years of exposure (fig. 10). Even through the 1955 control lots exhibited significantly lower rust incidence at Priest River than at Deception Creek (fig. 10) we will assume that potential K = 1 at both sites. The significant across-environment means for all three populations [significance estimated by log-odds ratio 95 percent confidence limit according to Sokal and Rohlf (1995)] is taken as evidence of a genetic difference among the OP seedlots. This difference presumably arose from changes in pollen cloud during the 4 years (1951 to 1955) over which the OP seedlots were being collected. Further, the significant difference between the within-environment K means suggests either that expression of the putative accumulated resistance was affected by the environment or that the rust populations differ. The significant interaction among 1955 vs. 1959 controls across environments argues in favor of heritable variation in plasticity of the pine but against variation in virulence among these rust populations.

The full-sib GCA x GCA analysis was similar to the GCA x nonGCA resistance in that E was not significant and P was significant. These groups of families exhibited a nonsignificant G variance. Nevertheless, the downward slopes between the 1955 and 1959 plantings indicate the potential for a significant G (fig. 11). Full-sib crosses between GCA and other parents (mostly nonGCA) exhibited variable behavior across the sites (fig. 12) that are probably due to differential expression of genes. The families included in the 1955 and 1957 plantings show no distinctions at either site (fig. 12). The site-mean Ks do not differ (no E variance) but the K for the 1959 planting at PR is high and performance of the 1959 planting at PR and DC is significantly different, leading to significant G and P variances. The absence of a difference among sites for two family groups argues for similar rust at both sites.

An over-all conclusion from this analysis of K based on evidence of an overall reduction of the asymptote of disease incidence at the end of the epidemic of 0.16 in the OP controls in just 4 years (fig. 10) is that the WWP population in north Idaho may have the natural capacity to respond very quickly to rust pressure. Possible explanations are rapid and significant changes in frequency of resistant genes or expression of induced resistance that could even be partially transgenerational in areas of extreme WPBR impact. However, we also need to remember that the WPBR pathosystem is composed of other factors, such as epiparasites, that may have been subject to change, so that some alternative sort of disease attenuation or damping of the ability of *C. ribicola* to damage pine might be in play (Pfennig 2001, Ebert 1998, Levin and Bull 1994, Roy and Kirchner 2000).

Long-term performance of the vigor-quality plantations also indicates that overall early resistant populations obtained from the Phase I program performed equally well at both sites in that both kinds of crosses resulted in about 60 percent clean trees. Individual groups of families showed significant G x E for expression of resistance as well as differences among the groups. These differences are more than likely traceable to individual full-sib families that made up the groups. Results from the plantings also strongly indicate that perhaps site is more important than genetic background in determining whether local epidemics will behave in characteristically monocylic or polycyclic fashions. An explanation for the existence of two kinds of epidemics would add much insight to our understanding of rust epidemics. It is important to remember that well-characterized materials (full-sibs given 50 years of natural rust exposure and subjected to frequent examinations for rust behavior) are an experimental and developmental treasure that we should make every effort to protect and continue to develop.

Case 6: Changes in Rate “Constants” at Full-Sib Resistance Evaluation Plantations in Northern Idaho

One of the final tasks associated with completing the Phase I program was establishment of three test plantations: Merry Creek (MC), Gletty Creek (GLC) and Jaype Mill (JM) (Bingham and others 1973, McDonald and others 1994, McDonald and Dekker-Robertson 1998, Fins and others 2002). MC and GLC are replicate sites, each consisting of 36, 0.4 ha plantings laid out in a randomized complete block design for four stock types (Bingham and others1973). Each contains full-sib F_2, F_1, B_1 (backcross from F_1 to original parents), and local OP controls (that is, different seedlots representing different genetic backgrounds). MC, established in 1970, is located about 82 km east of Moscow, Idaho. GLC, established in 1972, is about 205 km northwest of Moscow near the town of Newport, Washington. JM, established in 1971, is about 125 km southeast of Moscow and contains only control and full-sib F_2 stock. At MC, a 225-tree subset of permanent sample trees from each resident stock had been selected, tagged, and inspected for rust incidence and severity at 2, 4, 6, 12, and 26 years. Many of the original 225 selected plants at MC have since been lost to animals and other unknown causes to the extent that the original design was destroyed (McDonald and Dekker-Robertson 1998). A hot site-preparation burn that stimulated a heavy stand of evergreen ceanothus (*Ceanothus velutinus* Douglas) further compromised the design. In response, the stand was divided for analysis into two site classes: brush and forest. This partition produced two classes of about equal size for all stock types, as described in an earlier report (McDonald and Dekker-Robertson 1998). The GLC and JM plantations remained largely intact, but each experienced lower rust incidence than MC and were inspected only 3 times (1973, 1983, and 1996). Incidence in 1973 was too low for analysis. The JM layout paired F_2 and control plots having 64 planting spots. In each plot, WWP seedlings alternated with those of grand fir (*Abies grandis* (Dougl.) Lindl.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.). All 32 WWP on each plot were inspected each time. Blocking at these plantations enabled use of ANOVA to construct reaction norms of infection rate and canker accumulation rate for two periods: plantation establishment to 1982, and 1982 to 1996. Infection rate was computed using Madden and Campbell’s (1990) equation 18 – the monomolecular equation where K is assumed to equal one and initial incidence is set to zero. Rate of infection from 1982 to 1996 was computed using the same equation with initial incidence set equal to the 1982 value. Canker accumulation rate was initially computed by dividing the average number of cankers in 1982 by the number of years of exposure, but for the final period, the number of cankers in 1982 was subtracted from the 1996 number and the result divided by 14 years. These values were subjected to ANOVA (Systat 9.1, SPSS) and reaction norms constructed to compare stocks growing at JM and GLC.

All variances for comparison of WPBR annual infection rate between the two periods of assessment were highly significant. The across-environment mean indicated that the local GLC control was almost 2 times more susceptible than the Jaype local control growing at Jaype, yet examination of F_2 material that was of the same genetic background at both sites indicated that the sites were of equal hazard (fig. 13). Could an explanation be found in natural selection operating at different levels of selection in different natural stands? The expectation is that WPBR had a much larger impact on WWP near the JM site on the Clearwater National Forest than in the GLC vicinity on the Colville National Forest. The significant interaction term is also of interest. The controls show no differential interaction as materials at both sites showed a proportional gain in infection rate for the second period. The interaction is generated by the nonsignificant plasticity test for the F_2 at both sites (fig. 13).

Cankering rate provides a different and unexpected result (fig. 14). In contrast with the increasing parallel reaction norms for infection rate in the controls over the two periods (fig. 13), cankering rate moved from a difference of 6x to a nonsignificant difference. While values were much smaller and differences not significant, the trend for the F_2 was the same. In addition, the JM cankering rate appeared flat and was not significantly different between periods. The GLC control illustrated that infection rate can increase while cankering rate is simultaneously decreasing. Does the JM control represent a pathosystem that is coming to a stable asymptote and therefore a stable and predicable K parameter? On the other hand, could the increasing cankering rate coupled with an increasing infection rate signify an impending unstable K?

Case 7: Anatomy of an Unexpected Epidemic—The Merry Creek Plantation

An expected epidemic at the MC site provides some insight into stability or instability of K, although the relatively small numbers of designated "sample" trees remaining from plantation establishment to the end of sampling removed any chance of computing significances in the standard fashion. The good news is that enough of the sampled population remained in records and on the ground to establish patterns and generate hypotheses. Infection rate and cankering rate were computed as for GLC except that the F_1 was used as a substitute control because none of the highly susceptible "true" control seedlings survived to the second period (fig. 15 and 16). All resistant stocks exhibited nearly equal infection rates during the first period when all the controls were becoming infected and dying (fig. 16) (McDonald and Dekker-Robertson 1998). Most infection rates increased 7 to 8x with very little differentiation between the brush and forest sites for the F_1 (fig. 16). In striking contrast, the F_2 stock growing in the forest site-type had rates of canker incidence increase by 20x (fig. 15). Infection rate at MC shows interesting patterns when displayed with the F_2 data from JM and GLC in a common format (fig. 16). Of note is the almost parallel nature of most reaction norms. The MC forest-site-type F_2 is most similar to the F_2 growing on the low hazard GLC and JM sites and is not distinguishable from the JM control in spite of the huge increase in cankering rate. This argues that the entire increase in number of cankers occurred on already infected trees! How can such an increase in amount of inoculum not result in an increase in number of previously clean trees becoming infected? An additional observation is that the highest overall rate increase (F_2 brush-site-type) was closely followed by the F_1 brush-site-type. Did the presumed large increase in aeciospore production by F_2 forest-site-type trees result in increased infection rate on F_1 and F_2-brush trees? Allowing ourselves to consider even "outside the box" possibilities, could this pattern signify spontaneous local appearance of a microcyclic form of WPBR capable of infecting pine to pine, and if so, might it recently have evolved, or might it be a new arrival? WPBR is known to encompass microcyclic forms or species (Imazu and others 1991, Imazu and Kakishima 1995). An intercontinental "river of dust" has been demonstrated to allow viable transport of spores of some fungi (Griffin and others 2002); could microcyclic aeciospores from Japan occasionally have survived such a journey?

These relationships can be studied in other ways. It has been argued (McDonald and Dekker-Robertson 1998) that the increase in infection at MC above the expected K of 0.34 in the F_2 was just the continuation of a constant rate that after 26 years resulted in the observed 0.89 incidence of infection. Since 7 out of 26 plantings of F_2 stock in north Idaho have exceeded the expected asymptote of 0.34 (McDonald and others 1994, Fins and others 2002) in the 12 years or less it took MC F_2 to exceed the threshold, a definitive answer about the causal dynamics is demanded. As a focal point for this discussion (not intended to convey statistical significance), we present a hypothetical rust progress curve for the MC F_2 based on some of the tools we have been discussing (fig. 17). By fitting the monomolecular equation (McDonald and Dekker-Robertson 1998 equation 1) using the first four data points from MC (Table Curve 2D, SPSS), a K of 0.5 is obtained. If we next assume the appearance of a variant that can overcome the resistance that was responsible for the K = 0.5 of the first 10 years of the epidemic and then simulate the behavior based on a new timeline, with the 10th year as year 0, then incidence in year 10 = 0, 12th = .5 and 26th year = .89. The perfect fit with K = .89 that these three points allow (fig. 17), illustrate the limitations of fitting a multiparameter function to a small data set but is also intriguing. This figure illustrates some important points. Control and F_2 stock are clearly delineated and the hypothetical curve is similar to the actual curve shown for SP at the Happy Camp site (Kinloch and Byler 1981). Interruption of the established cycle of inspections at MC by a bureaucratic decision to stop WPBR research in northern Rocky Mountains in 1983 precluded knowing the exact shape of

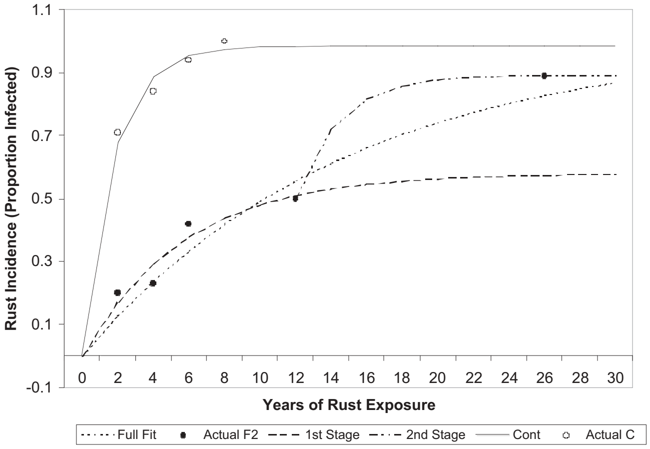


Figure 17—Expected rust progress curve for Merry Creek site (north Idaho) second generation resistant families (F_2) if epidemic asymptote were breached by *Cronartium ribicola* adjustment (plasticity or evolution?) compared with control lot. Both populations growing on forest site-type (Case 7 in text; data from McDonald and Dekker-Robertson 1998).

the MC progress curve. However, the hypothesized hump in the rust progress curve (fig. 17) might even now be verified or rejected by aging cankers at MC after the fact, in order to construct a reasonable progress curve.

Summary and Conclusions

The above discussion reinforces several significant lessons relating to resistance breeding, deployment, and management of all western five-needle pines. Of primary importance, pathosystems of long-lived plants demand long-term commitment in an atmosphere that fosters freethinking as well as commitment and focus to getting a big job accomplished efficiently. This probably means that lasting and workable relationships need to be forged between research and the practical breeding programs. Efforts in the past (Kinloch and Byler 1981, McDonald and others 1984, McDonald and others 1991, Kinloch and others 1999) exemplify such cooperation and sharing of data, and should be encouraged. The breeding programs generate large amounts of reliable data that are seldom subjected to peer review but could be of inestimable value, provided that such programs ensure that uniform and "adequate" control crosses are routinely included in artificial inoculations. Greater interregional cooperation may also help elucidate new resistance mechanism, for example mechanism "X" that USDA Forest Service Region 6's breeding and screening program at Dorena has observed in northern Cascade Mountain populations and that could potentially be related to resistance mechanisms in the northern Idaho WWP populations. Examination of this mechanism could be of critical importance in delineating seed zones and understanding the genetic structure of host populations. Recent studies of migration patterns of whitebark pine strongly suggest a north-south dichotomy whose boundary is in the southern Washington Cascades (Richardson and others 2002). This boundary could also be characterized by a significant change in the kinds and numbers of resistance genes in both WBP and WWP. Tools that are presently available to assess field performance are powerful, useful, and relatively inexpensive, if appropriate experimental designs and inspection intervals are maintained. Parting guidelines are as follows: Establish permanent plots so that adequate numbers of individuals can be tracked for at least 50 years. Ensure that repeated and blocked designs will provide sufficient numbers over time. Define measurement protocols to ensure continuity of data quality through changes in personnel. Ensure that canker incidence and severity, age, and size are recorded for whole trees to provide data appropriate for epidemiological analysis. Embrace the potential discriminatory power of pathosystem G x E by ensuring that genetic groupings to be tested (clones, full-sib families, half sib families and etc) are replicated in at least two environments. Finally, and perhaps most importantly, create customized control lots having known behavioral specifications and use them to link plantings in different geographic regions.

When dealing with long-term pathosystems, refrain from thinking "inside the box". For example, a strong correlation had been expected between density of *Ribes* populations and resulting WBBP impact (McDonald and Hoff 2001). Quite to the contrary, evidence is accumulating that such correlations are weak in the northern Rockies (Toko and others 1967, McDonald unpublished data) and southwestern Oregon (McDonald unpublished data). Two explanations are evident. First, western basidiospores may be more robust or travel further than assumed so that far fewer telial infections are needed to cause a corresponding amount of pine damage expected under the 300m "limit" of basidiospore spread. Second, other hosts could be involved; forms of *C. ribicola* are known in Japan and South Korea that alternate to *Ribes* spp and *Pedicularis*; in Japan and Germany, a form cycles to *Ribes* only; in South Korea, a form cycles only to *Pedicularis*; and in Canada it cycles to *Ribes* (Stephan and Hyun 1983). There is one report of a single branch of one plant of common red paintbrush (*Castillia miniata* Douglas, a North American Scropulariaceae) inoculated with WPBR that produced teliospores (Hiratsuka and Maruyama, 1976), and another report of artificial infection of this host but without spore production (Patton and Spear 1989). However, infections of artificially inoculated plants of this and other Scrophulariaceae were not obtained at multiple field sites by Hunt (1984), despite successful infection of "appropriate" *Castilleja* hosts after inoculation with stalactiform rust. Another potential problem with measuring WPBR susceptibility is the assumption that any old woods run collection of the pine host will make an adequate control. Custom controls should be developed and maintained for use by all breeding programs. This paper presented initial evidence that unknown mechanisms may cause rapid accumulation of "resistant" WPP. Theoretical discussions about disease attenuation are beginning to appear that invoke various kinds of evolutionary and plasticity (polymorphisms and polyphenisms) adjustments (Pfennig 2001, Ebert 1998, Levin and Bull 1994, Roy and Kirchner 2000). If the WPBR pathosystem is as dynamic as we have suggested, then an entirely new plan of attack may be needed to ensure that we can successfully restore and/or maintain ecosystems containing or requiring a five-needle pine component.

References

Bald, J.G. 1970. Measurement of host reactions to soil-borne pathogens. In: Toussoun, T.R., ed. Root diseases and soil-borne pathogens. Berkeley, CA: University of California Press: 37-41.

Bedwell, J.L.; Childs, T.W. 1943. Susceptibility of whitebark pine to blister rust in the Pacific Northwest. Journal of Forestry. 41: 904-912.

Bingham, R.T. 1966. Breeding blister rust resistant western white pine. Silvae Genetica. 15 (5/6): 160-164.

Bingham, R.T. 1972. Artificial inoculation of large numbers of *Pinus monticola* seedlings with *Cronartium ribicola*. In: Bingham, R.T., ed. Biology of rust resistance in forest trees: proceedings; 1969 August 17-24; Moscow, ID. Washington, D.C.: U.S. Department of Agriculture, Forest Service: 357-372.

Bingham, R.T. 1983. Blister rust resistant western white pine for the inland empire: The story of the first 25 years of the research and development program. Gen. Tech. Rep. INT-146. Odgen, UT: U.S. Department of Agriculture, U.S. Forest Service, Intermountain Forest and Range Experiment Station. 45 p.

Bingham, R.T.; Hoff, R.J.; McDonald, G.I. 1973. Breeding blister rust resistant western white pine – first results from field-testing of resistant planting stock. Res. Note INT-179. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 12 p.

Bingham, R.T.; Olson, R.J.; Becker, W.A.; Mardsen, M.A. 1969. Breeding blister rust resistant western white pine. Silvae Genetica. 18 (1/2): 28-38.

Bonello, P.; Gordon, T.R.; Storer, A.J. 2001. Systemic induced resistance in Monterey pine. Forest Pathology. 31: 99-106.

Bower, R.C. 1987. Early comparison of an Idaho and a coastal source of western white pine on Vancouver Island. Western Journal of Applied Forestry. 2: 20-21.

Buchanan, T.S. 1936. An alignment chart for estimating number of needles on western white pine reproduction. Journal of Forestry. 34: 588-593.

Campbell, R.K.; Sugano, A.I. 1987. Seed zones and breeding zones for sugar pine in southwestern Oregon. Res. Paper PNW-RP-383. Portland, OR: U.S. Department of Agriculture, U.S. Forest Service, Pacific Northwest Research Station. 18 p.

Campbell, R.K.; Sugano, A.I. 1989. Seed zones and breeding zones for white pine in the cascade range of Washington and Oregon. Res. Paper PNW-RP-407. Portland, OR: U.S. Department of Agriculture, U.S. Forest Service, Pacific Northwest Research Station. 20 p.

Chiron, H.; Drouet, A.; Lieutier, F.; Payer, H-D.; Ernst, D.; Sandermann Jr., H. 2000. Gene induction of stilbene biosynthesis in scots pine in response to ozone treatment, wounding, and fungal infection. Plant Physiology. 124: 865-872.

Covert, S.F. 1998. Supernumerary chromosomes in filamentous fungi. Current Genetics 33: 311-319.

Ebert, D. 1998. Experimental evolution of parasites. Science. 282: 1432-1435.

Enebak, S.A.; Carey, W.A. 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. Plant Diseases. 84 (3): 306-308.

Ficke, A.; Gadoury, D.M.; Seem, R.C. 2002. Ontogenic resistance and plant disease management: a case study of grape powdery mildew. Phytopathology. 92 (6): 671-675.

Fins, L.; Byler, J.; Ferguson, D.; Harvey, A.; Mahalovich, M.F.; McDonald, G.; Miller, D.; Schwandt, J.; Zack, A. 2002. Return of the Giants: Restoring western white pine to the inland northwest. Journal of Forestry. 100: 20-26.

Fluhr, R. 2001. Sentinels of disease, plant resistance genes. Plant physiology. 127: 1367-1374.

Fowler, T.J.; Mitton, M. F. 2000. Scooter, a new active transposon in *Schizophyllum commune*, has disrupted two genes regulating signal transduction. Genetics 156: 1585-1594.

Fracker, S.B. 1936. Progressive intensification of uncontrolled plant-disease outbreaks. Journal of Economic Entomology. 29 (5): 923-940.

Franceschi, V.R.; Krekling, T.; Christiansen, E. 2002. Application of methyl jasmonate on *Picea abies* (Pinaceae) stems induces defense-related responses in phloem and xylem. American Journal of Botany. 89 (4): 578-586.

Goddard, R.E.; McDonald, G.I.; Steinhoff, R.J. 1985. Measurement of field resistance, rust hazard, and deployment of blister rust-resistant western white pine. Res. Pap. INT-358. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 8 p.

Griffin, D.W.; Kellogg, C.A.; Garrison, V.H.; Shinn, E.A. 2002. The global transport of dust. American scientist. 90: 228-235.

Hatta, R.; Ito, K.; Hosaki, Y.; Tanaka, T.; Tanaka, A.; Yamamoto, M.; Akimitsu, K.; Tsuge, T. 2002 A conditionally dispensible chromosome controls host-specific pathogenicity in the fungal plant pathogen *Alternaria alternata*. Genetics. 161: 59-70.

Hiratsuka, Y.; Maruyama, P.J. 1976. *Castilleja miniata*, a new alternate host of *Cronartium ribicola*. Plant Disease Reporter 60:241.

Hirt, R.R. 1938. Relation of stomata to infection of *Pinus strobus* by *Cronartium ribicola*. Phytopathology. 28: 180-190.

Hirt, R.R. 1944. Distribution of blister-rust cankers on eastern white pine according to age of needle-bearing wood at time of infection. Journal of Forestry. 42: 9-14.

Hoff, R.J.; McDonald, G.I. 1980. Improving rust-resistant strains of inland western white pine. Res. Pap. INT-245. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 13 p.

Hoff, R.J.; McDonald, G.I. 1993. Variation of virulence of white pine blister rust. European Journal of Forest Pathology. 23: 103-109.

Hoff, R.J.; McDonald, G.I.; Bingham, R.T. 1973. Resistance to *Cronartium ribicola*: structure and gain of resistance in the second generation. Res. Note INT-178. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 8 p.

Hoff, R.J.; Ferguson, D.E.; McDonald, G.I.; Keane, R.E. 2001. Strategies for managing whitebark pine in the presence of white pine blister rust. In: Tomback, Diana F., ed. Whitebark Pine Communities. Washington, D.C.: Island Press: 346-366.

Hunt, R.S. 1984. Inoculations of Scrophulariaceae with *Cronartium ribicola*. Can. J. Bot. 62:2523-2524.

Hunt, R.S.; Meagher, M.D. 1989. Incidence of blister rust on "resistant" white pine (*Pinus monticola* and *P. strobus*) in coastal British Columbia plantations. Canadian Journal of Plant Pathology. 11: 419-423.

Imazu, M.; Kakishima, M. 1995. The blister rusts on *Pinus pumila* in Japan. In: Kaneko, S., ed. Proceedings of the fourth IUFRO rusts of pines working party conference; 1994 October 2-4; Tsukuba, Japan. Tsukuba: Forestry and Forest Products Research Institute: 27-36.

Imazu, M.; Kakishima, M.; Katsuya, K. 1991. Morphology and nuclear cycle of *Endocronartium yamabense*. Trans. Mycol. Soc. Japan. 32: 371-379.

Kegley, A., R.A. Sniezko, R. Danchok, J. Danielson, and S. Long. In press. Influence of inoculum source and density on white pine blister rust infection of whitebark pine: early results. In: B. Geils (comp.). Proceedings of the 51st Western International Forest Disease Work Conference. 2003 August 18-22. Grants Pass, OR. USDA Forest Service, Rocky Mountain Research Station. Flagstaff, AZ.

Kim, M-S.; Brunsfeld, J.J.; McDonald, G.I.; Klopfenstein, N.B. 2003. Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*). Theoretical and Applied Genetics 106: 1004-1010.

Kinloch Jr., B.B. 1992. Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Canadian Journal of Botany 70:1319-1323.

Kinloch Jr., B.B.; Byler, J.W. 1981. Relative effectiveness and stability of different resistance mechanisms to white pine blister rust in sugar pine. Phytopathology. 71: 386-391.

Kinloch Jr., B.B.; Comstock, M. 1980. Cotyledon test for major gene resistance to white pine blister rust in sugar pine. Canadian Journal of Botany. 58: 1912-1914.

Kinloch Jr., B.B.; Davis, D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine. In: Kinloch Jr., B.B., ed. Sugar pine status, values, roles in ecosystems: proceedings; 1992 March 30-April 1; Davis, CA. Davis, CA: University of California, Division of Agriculture and Natural Resources: 125-132.

Kinloch Jr., B.B.; Dupper, G.E. 1999. Evidence of cytoplasmic inheritance of virulence in *Cronartium ribicola* to major gene resistance in sugar pine. Phytopathology. 89: 192-196.

Kinloch Jr., B.B.; Dupper, G.E. 2002. Genetic specificity in the white pine-blister rust pathosystem. Phytopathology. 92: 278-280.

Kinloch Jr., B.B.; Littlefield, J.L. 1977. White pine blister rust: hypersensitive resistance in sugar pine. Canadian Journal of Botany. 55: 1148-1155.

Kinloch Jr., B.B.; Sniezko, R.A.; Barnes, G.D.; Greathouse, T.E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. Phytopathology. 89: 861-867.

Kitzmiller, J.H. 1976. *Tree improvement master plan for the California Region*. USDA Forest Service, San Francisco. 123 p.

Kitzmiller, J. 1982. Rust resistance sugar pine (*Pinus lambertiana*) in the pacific southwest region. In: Miller, D. ed. Breeding insect and disease resistant forest trees: proceedings; 1982 July 19-23; Eugene, OR. Washington, D.C.: U.S. Department of Agriculture: 92-108.

Kitzmiller, J.H.; Stover, P. 1996. Genetic variation in the growth of sugar pine progenies in California plantations. In: Kinloch Jr., B.B.; ed. Sugar pine status, values, roles in ecosystems: proceedings; 1992 March 30-April 1; Davis, CA. Davis, CA: University of California, Division of Agriculture and Natural Resources: 83-98.

Krokene, P.; Solheim, H.; Christiansen, E. 2001. Induction of disease resistance in Norway spruce (*Picea abies*) by necrotizing fungi. Plant Pathology. 50: 230-233.

Levin, B.R.; Bull, J.J. 1994. Shortsighted evolution and the virulence of pathogenic microorganisms. Trends in Microbiology. 2 (3): 76-81.

Liu, J.J.; Ekramoddoullah, A.K.M. 2004. Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. Ex. D. Don.) Molecular Genetics and Genomics. 270: 432-441.

Luck, J.E.; Lawrence, G.J.; Finnegan, E.J.; Jones, D.A.; and Ellis, J.G. 1998. A flax transposon identified in two spontaneous mutant alleles of the L6 rust resistance gene. The Plant Journal 16: 365-369.

Madden, L.V.; Campbell, C.L. 1990. Nonlinear disease progress curves. In: Kranz, J., ed. Epidemics of plant disease, mathematics and analysis. New York, Springer-Verlag 1: 181-230.

Mahalovich, M.F.; Eramian, A. 1995. Breeding and seed orchard plan for the development of blister rust resistant white pine for the northern Rockies. Development Plan. Coeur d'Alene, ID: U.S. Department of Agriculture, Forest Service, Northern Region. 59 p.

McDonald, G.I. 2000. Geographic variation of white pine blister rust aeciospore infection efficiency and incubation period. Hortechnology. 10 (3): 533-536.

McDonald, G.I.; Andrews, D.S. 1981. Genetic interaction of *Cronartium ribicola* and *Ribes hudsonianum* var. *petiolare*. Forest Science. 27 (4): 758-763.

McDonald, G.I.; Dekker-Robertson, D.L. 1998. Long-term differential expression of blister rust resistance in western white pine. In: Jalkanen, R., ed. First IUFRO Rusts of Forest Trees: proceedings; 1998 August 2-7; Saariselkä, Finland. Rovaniemi, Finland: The Finnish Forest Research Institute: 285-295.

McDonald, G.I.; Hoff, R.J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: early shedding of infected needles. USDA For. Serv. Res. Note INT-124.

McDonald, G.I.; Hoff, R.J. 1982. Engineering blister rust-resistant western white pine. In: Heybroek, H.M., ed. Resistance to disease and pests in forest trees: proceedings; 1980 September 14-21; Wageningen, The Netherlands. Wageningen: Centre for Agricultural Publishing and Documentation: 404-411.

McDonald, G.I.; Hoff, R.J. 1991. History and accomplishments of white pine blister rust research in the USDA Forest Service. In: Hiratsuka, Y., ed. Rusts of pine: proceedings; 1989 September 18-22; Banff, Alberta, Canada. Edmonton: Forestry Canada: 45-53.

McDonald, G.I.; Hoff, R.J. 2001. Blister rust: An introduced plague. In: Tomback, Diana F., ed. Whitebark Pine Communities. Washington, D.C.: Island Press: 193-220.

McDonald, G.I.; Harvey, A.E.; Tonn, J.R. 2000. Fire, competition, and forest pests: Landscape treatment to sustain ecosystem function. In: Neuenschwander, L.F.; Ryan, K.C., technical eds. The joint fire science conference and workshop: proceedings; 1999 June 15-17; Boise, ID. Moscow, ID: University of Idaho and The International Association of Wildland Fire:195-211.

McDonald, G.I.; Hoff, R.J.; Samman, S. 1991. Epidemiologic function of blister rust resistance: A system for integrated management. In: Hiratsuka, Y., ed. Rusts of pine: proceedings; 1989 September 18-22; Banff, Alberta, Canada. Edmonton: Forestry Canada: 235-255.

McDonald, G.; Evans, J.; Moeur, M.; Rice, T.; Strand, E. 2003. Using digital terrain modeling and satellite imagery to map interactions among fire and forest microbes. In: K.E.M. Galley, R.C. Klinger, and N.G. Sugihara (eds.) Proceedings of Fire Conference 2000: The First National Congress on Fire Ecology, Prevention, and Management. Misc. Pub. No. 13. Tall Timbers Research Station. Tallahassee, FL. p. 100-110.

McDonald, G.I.; Hansen, E.M.; Osterhaus, C.A.; Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Disease. 68: 800-804.

McDonald, G.I.; Hoff, R.J.; Rice, T.M.; Mathiasen, R. 1994. Measuring early performance of second-generation resistance to blister rust in western white pine. In: Interior cedar-hemlock-white pine forests: ecology and management: proceedings; 1993 March 2-4; Spokane, WA. Pullman, WA: Washington State University: 133-150.

Neuenschwander, L.; Byler, J.W.; Harvey, A.E.; McDonald, G.I.; Ortiz, D.S; Osborne, H.L.; Snyder, G.C.; Zack, A. 1999. White pine in the American west: a vanishing species—can we save it? Gen. Tech. Rep. RMRS-GTR-35. Ft. Collins, CO: U.S. Department of Agriculture, U.S. Forest Service, Rocky Mountain Research Station. 21 p.

Patton, R.F. 1961. The effect of age upon susceptibility of eastern white pine to infection by *Cronartium ribicola*. Phytopathology. 51: 429-434.

Patton, R.F.; Riker, A.J. 1966. Lessons from nursery and field-testing of eastern white pine selections and progenies for resistance to blister rust. In: Gerhold, H.D., ed. Breeding pest-resistant trees: proceedings; 1964 August 30-September 11; University Park, Pennsylvania. London: Pergamon Press: 403-414.

Patton, R.F.; Spear, R.N. 1989. Histopathology of colonization in leaf tissue of *Castilleja*, *Pedicularis*, *Phaseolus*, and *Ribes* species by *Cronartium ribicola*. Phytopathology. 79 (5): 539-547.

Pérez-Garcia, A.; Olalla, L.; Rivera, E.; Del Pino, D.; Cánovas, I.; De Vicente, A.T. 2001. Development of *Sphaerotheca fusca* on susceptible, resistant, and temperature-sensitive resistant melon cultivars. Mycological Research. 105 (10): 1216-1222.

Pfennig, K.S. 2001. Evolution of pathogen virulence: the role of variation in host phenotype. Proc. R. Soc. Lond. 268: 755-760.

Pierson, R.K.; Buchanan, T.S. 1938. Susceptibility of needles of different ages on *Pinus monticola* seedlings to *Cronartium ribicola* infection. Phytopathology. 28: 833-839.

Pigliucci, M. 2001. Phenotypic plasticity, beyond nature and nurture. Baltimore, MD: Johns Hopkins University Press. 328 p.

Rehfeldt, G.E.; Hoff, R.J.; Steinhoff, R.J. 1984. Geographic patterns of genetic variation in *Pinus monticola*. Botanical Gazette. 145 (2): 229-239.

Richardson, B.A.; Brunsfeld, S.J.; Klopfenstein, N.B. 2002. DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (*Pinus albicaulis*). Molecular Ecology. 11: 215-227.

Roy, B.A.; Kirchner, J.W. 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution. 54 (1):51-63.

Samman, S.; Kitzmiller, J.H. 1996. The sugar pine program for development of resistance to blister rust in the pacific southwest region. In: Kinloch Jr., B. B., ed. Sugar pine status, values, roles in ecosystems: proceedings; 1992 March 30-April 1; Davis, CA. Davis, CA: University of California, Division of Agriculture and Natural Resources: 162-170.

Schmidt, R. A.; Allen, J. E. 1998. Spatial stability of fusiform rust resistance in slash pine in the coastal plain of the southeastern USA. In: Jalkanen, R., ed. First IUFRO Rusts of Forest Trees: proceedings; 1998 August 2-7; Saariselkä, Finland. Rovaniemi, Finland: The Finnish Forest Research Institute: 219-229.

Sheppard, L.A.; James, S.G.; Delfino-Mix, A.; Bassoni, D.; Neale, D.B.; Kinloch, B.B. Jr. 2000. Resistance gene analogs in sugar pine. In: W 115, Plant Animal Genome VIII Conf., San Diego, CA.

Sniezko, R.A. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon. In: Kinloch Jr., B.B., ed. Sugar pine status, values, roles in ecosystems: proceedings; 1992 March 30-April 1; Davis, CA. Davis, CA: University of California, Division of Agriculture and Natural Resources: 171-178.

Sniezko, R.A.; Bower, A.; Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance of sugar pine and western white pine. HortTechnology 10: 519-522.

Sokal, R.R.; Rohlf, F.J. 1998. Biometry. New York: W.H. Feeman and Company. 887 p.

Steinhoff, R. J. 1971. Field levels of infection of progenies of western white pines selected for blister rust resistance. Res. Note INT-146. Ogden, UT: U.S. Department of Agriculture, Forest Service Intermountain Forest and Range Experiment Station. 4 p.

Stephen, B.R.; Hyun, S.K. 1983. Studies on the specialization of *Cronartium ribicola* and its differentiation on the alternate hosts of *Ribes* and *Pedicularis*. Journal of plant diseases and protection. 90 (6): 670-678.

Stokes, T.L.; Kunkel, B.N.; Richards, E.J. 2002. Epigenetic variation in *Arabidopsis* disease resistance. Genes and Development. 16: 171-182.

Teck, R.; Moeur, M.; Adams, J., compilers. 1997. Proceedings: Forest vegetation simulator conference.1997 February 3-Fort Collins, CO, Ogden, UT: U.S. Department of Agriculture, U.S. Forest Service, Intermountain Research Station. 222 p.

Tollrian, R.; Harvell, C. Drew, eds. 1999. The ecology and evolution of inducible defenses. Princeton, NJ: Princeton University Press. 383 p.

Toko, E.V.; Graham, D.A.; Carlson, C.E.; Ketcham, D.E. 1967. Effects of past Ribes eradications on controlling white pine blister rust in northern Idaho. Phytopathology. 57:1010.

Tomback, D.F.; Arno, S.F.; Keane, R.E. 2001. The compelling case for management intervention. In Tomback, D.F.; Arno, S.F.; Keane, R.E., eds. Whitebark Pine Communities. Washington, D.C.: Island Press: 3-25.

Waggoner, P.E.; Aylor, D.E. 2000. Epidemiology: A science of patterns. Annual Review of Phytopathology. 38:71-94.

Wilson, K.; Thomas, M.B.; Blanford, S.; Doggett, M.; Simpson, S.J.; Moore, S.L. 2002. Coping with crowds: density-dependent disease resistance in desert locusts. Proceedings of the National Academy of Science. 99 (8): 5471-5475.

Woo, K.S. 2000. Variation in needle morphology of blister rust susceptible and resistant western white pine. Ph.D. dissertation, University of Idaho, Moscow.

Woo, K.S.; Fins, L.; McDonald, G.I.; Wiese, M.V. 2001. Differences in needle morphology between blister rust resistant and susceptible western white pine stocks. Canadian Journal of Forest Research 31: 1880-1886.

Wu, R. 1998. The detection of plasticity genes in heterogeneous environments. Evolution. 52 (4): 967-977.

Wu, R.; Hinckley, T.M. 2001. Phenotypic plasticity of sylleptic branching: genetic design and tree architecture. Critical Reviews in Plant Sciences. 20 (5): 467-485.

Yan, W.; Kang, M.S. 2003. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. Boca Raton, FL: CRC Press. 271 p.

Yokota, S-I. 1983. Resistance of improved *Pinus monticola* and some other white pines to the blister rust fungus, *Cronartium ribicola*, of Hokkaido, Japan. European Journal of Forest Pathology. 13: 389-402.

Zadoks, Jan C.; Schein, Richard D. 1979. Epidemiology and Plant Disease Management. New York: Oxford University Press. 427 p.

Genetic Research and Development of Five-Needle Pines (*Pinus* subgenus *Strobus*) in Europe: An Overview

Ioan Blada
Flaviu Popescu

Abstract—An overview of genetic research on native and exotic five-needle pines (*Pinus* subgenus *Strobus*) in Europe, including the impact of white pine blister rust (*Cronartium ribicola*), is presented. The natural populations of *Pinus cembra* from the Alps and Carpathians are free from blister rust; even though the rust occurs throughout Europe on other five-needle pines and *Ribes* species. *Pinus strobus* was once considered to be an important exotic species for timber production in Europe, but plantations have been abandoned due to the high susceptibility to blister rust. Blister rust resistance has now been transferred to *P. strobus* through hybridization with Eurasian five-needle pines, and the potential for successful utilization of the species in Europe now exists. Other five-needle pine species are not of major interest for European forestry operations.

Key words: five-needle pines, Europe, genetics, *Cronartium ribicola*, genetic resistance, provenance, breeding, hybrid, heritability, genetic gain

Introduction

Only two species of five-needle pines (*Pinus* L. subgenus *Strobus* Lemm.) grow naturally in Europe: Cembran pine (*Pinus cembra* L.) and Balkan pine (*P. peuce* Gris.) (Critchfield and Little 1966). Exotic five-needle pine species have been used to enrich European forests due to the relatively low number of native species and have been the subject of much research and development. For this paper, we prepared a survey on genetic research and use of five-needle pine species in European countries (table 1). We used the responses of the survey in conjunction with published literature and personal communications to compile the following overview on five-needle research and development in Europe.

Cembran pine is distributed in the high-altitude forests in the Alps and the Carpathian region, including the Tatra Mts. (Georgescu and Ionescu-Barlad 1932, Critchfield and Little 1966, Holzer 1975, Contini and Lavarelo 1982). It is naturally distributed in the following countries: Austria, France, Germany, Italy, Poland, Romania, Slovakia, Switzerland, and Ukraine. Cembran pine is important for reforestation of subalpine forests, including restoration of forests near timberline to stabilize watersheds and reduce the risk of avalanches and flash floods (Holzer 1975). The species is used to create mixed Norway spruce (*Picea abies* (L.) Karst) – European larch (*Larix decidua* Mill)-Cembran pine stands at high elevations for increased wind resistance (Blada 1996). Cembran pine also contains high genetic resistance to white pine blister rust caused by *Cronartium ribicola* J.C. Fisch in Rabenh. (Bingham 1972a,b, Soegaard 1972, Holzer 1975, Hoff and others 1980, Blada 1987, 1994a) Cembran pine produces a dense-brown-reddish wood useful for handicrafts (Contini and Lavarelo 1982), and is an excellent landscaping tree due to the crown color, density, and a conical-oval shape (Blada 1997b).

Balkan pine naturally occurs in Albania, Bulgaria, Greece, and Macedonia and is confined to higher elevations in the Balkan and Macedonian regions (Nedjalkov 1963, Fukarek 1970, Mitruchi 1955, Popnikola and others 1978). Balkan pine is important for planting in severe mountain climates to prevent soil erosion, as well as timber production for furniture, barrels, and other purposes (Figala 1927, Nedjalkov and Krastanov 1962, Nedjalkov 1963, Popnikola and others 1978). The species has good tolerance to SO_2 pollution (Enderlein and Vogl 1966) and has shown high blister rust resistance in genetic tests containing both European and North American species (Delatour and Birot 1982, Blada 1987, 2000a, 2000b, Heimburger 1972, Hoff and others 1980). Balkan pine has been used in crossing with other white pines and is considered a good bridging species (Righter and Duffield 1951, Kriebel 1963, Patton 1966, Nikota and others 1970, Heimburger 1972, Blada 1987).

The Cembran and Balkan pines have and are still planted most countries where they naturally occur (table 1, columns 9, 10, 12, and 13). Cembran pine, however, has been less used in France, Germany, and Italy. These species are relatively slow growing and as more five-needle pine species from around the world became known to Europeans, foresters began to experiment with plantations of exotic species.

Exotic five-needle pine species have had a long history in Europe. The following five-needle pine species have been more frequently planted: *P. strobus* L., *P. monticola* Dougl., *P wallichiana* A. B. Jacks., *P. sibirica* Du Tour, *P. koraiensis* Sieb & Zucc., *P. armandii* Franch., *P. flexilis* James, and *P. lambertiana* Dougl. (Schmitt 1972, Soegaard 1972). Eastern white pine *(P. strobus)* was one of the first five-needle pines to be introduced to Europe. With minor exceptions,

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the Forest Research Institute, Sos. Stefanesti 128, Bucharest 11, Romania.

Table 1—Survey results for native white pine and *Pinus strobus* presence, past or present occurrence of white pine blister rust, past planting or breeding work, and current planting or breeding work in European countries. (Y= yes; N=no; ?=unknown)

		Native		**Introd.**	**PPBRO**[a]			**PP**[b]			**PPBW**[c]		
Rank	**Country**	**P.c**	**P.p**	**P.s**	**P.c**	**P.p**	**P.s**	**P.c**	**P.p**	**P.s**	**P.c**	**P.p**	**P.s**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	Albania	N	**Y**	?	?	?	?	?	?	?	?	?	?
2	Austria	**Y**	N	**Y**	N	N	**Y**	**Y**	N	**Y**	**Y**	N	N
3	Belarus	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
4	Belgium	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
5	Bulgaria	N	**Y**	**Y**	N	N	**Y**	N	**Y**	**Y**	N	**Y**	N
6	Croatia	N	Y?	**Y**	N	N	**Y**	N	N	**Y**	N	?	**Y**
7	Czech Republic	N	N	**Y**	N	N	**Y**	N	N	**Y**	?	N	N
8	Denmark	N	N	**Y**	N	N	**Y**	N	**Y**	**Y**	N	N	N
9	England	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
10	Estonia	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
11	Finland	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
12	France	**Y**	N	**Y**	N	N	**Y**	N?	N	**Y**	N?	N	N
13	Germany	**Y**	N	**Y**	N	N	**Y**	N?	N	**Y**	N?	N	**Y**
14	Greece	N	**Y**	**Y**	N	N	N	N	N	N	N	N	N
15	Hungary	N	N	N	N	N	**Y**	N	N	**Y**	N	N	N
16	Ireland	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
17	Italy	**Y**	N	**Y**	N	N	**Y**	Y?	N	**Y**	**Y**	N	N
18	Latvia	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
19	Lithuania	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
20	Luxembourg	N	N	?	N	N	?	?	N	?	N	N	?
21	Macedonia	N	**Y**	**Y**	N	N	**Y**	N	**Y**	**Y**	N	**Y**	N
22	Moldova	N	N	N	N	N	N	N	N	N	N	N	N
23	Netherlands	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	N	N
24	Norway	N	N	**Y**	N	N	**Y**	N	N	N	N	N	N
25	Poland	**Y**	N	**Y**	N	N	**Y**	**Y**	N	**Y**	**Y**	?	N
26	Portugal	N	N	N	N	N	?	N	N	N	N	N	?
27	Romania	**Y**	N	**Y**	N	N	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
28	Slovakia	**Y**	N	**Y**	N	N	**Y**	**Y**	N	**Y**	**Y**	N	N
29	Slovenia	N	N	**Y**	N	N	**Y**	N	N	**Y**	N	?	N
30	Spain	N	N	?	N	N	?	?	?	Y?	N	N	?
31	Sweden	N	N	N	N	N	**Y**	N	N	N	N	N	N
32	Switzerland	**Y**	N	**Y**	N	N	**Y**	**Y**	N	**Y**	**Y**	N	N
33	Ukraine	**Y**	N	**Y**	N	N	**Y**	**Y**	N	**Y**	**Y**	N	N
34	Yugoslavia	N	Y?	**Y**	N	N	**Y**	N	**Y**	**Y**	N	Y?	N

[a] PPBRO = past and present blister rust occurrence.
[b] PP = past planting or breeding work.
[c] PPBW = present planting or breeding work; P.c, P.p, P.s = *P. cembra, P. peuce, P.strobus*.

eastern white pine was introduced in all European countries (table 1, column 5) and planted for economic reasons (table 1, column 11) (Radu 1974). The species has demonstrated good qualities for timber production, is well adapted to the European climate (Schmitt 1972, Kriebel 1983) and has proven to be resistant to SO_2 pollution (Enderlein and Vogl 1966). The other exotic five-needle pine species have shown to be of minor importance, being used primarily for landscaping and in arboretums. Some Asiatic species have been introduced as genetic sources for blister rust resistance. However, the vast majority of research and development efforts on exotic five-needle pines in Europe have been conducted on eastern white pine

In Europe, eastern white pine was first recorded in 1553, in the Royal Gardens of Fontainebleau, France (Lanier 1961), followed by introduction at Badminton, Great Britain (MacDonald and others 1957) and in Germany in 1770 (Schenck 1949). The first records of the eastern white pine in other European countries were: Switzerland 1850 (Litscher 1908), Poland 1876 (Bialobok 1960), Slovakia 1773 (Musil 1969), Austria 1886 (Cieslar 1901), Romania 1894 (Davidescu 1894) and Bulgaria 1903 (Rusakoff 1936).

Small plantations of eastern white pine were established at intervals through the late 18th and 19th centuries, as the species exhibited good growth. This was especially true in Germany during the great reforestation period that took place through the 18th and 19th centuries (Borchers 1952, Schmitt 1972). Initially, it appeared that eastern white pine would become one of the most important trees of the European forests. In west central Germany, eastern white pine could outgrow all European coniferous species and keep a dominant position in stands for more than 80 years (Schmitt 1972). The species' growth performance also was remarkable under various site conditions in other countries such as Poland, Czechoslovakia, Romania, and Russia (Radu 1974). However, white pine blister rust has seriously impacted the survival and growth of this species. Blister rust attack has halted planting of eastern white pine for wood production in all countries in the last two decades (see table 2, column 14).

According to our survey (table 1 column 11), eastern white pine was planted over almost all Europe. Germany contains the largest planted area, approximately. 25,000 ha, of eastern white pine (Stephan, personal communication). In France, plantations with eastern white pine were established during the 19th century. The first plantations for wood production in Romania were established at the beginning of the 20th century, but much larger areas (about 1,360 ha) were established after 1960 (Radu 1974). Based on the initial results of provenance trials, Croatia planted approximately 1,500 ha (Gracan, personal communication).

White pine blister rust began to cause severe problems as early as 1865, when the rust was first noticed by H. A. Dietrich in Estonia (Leppik 1934). The rust spread throughout Europe by 1900 via the alternate host *Ribes nigrum* L. and susceptible *P. strobus* genotypes (Georgerscu and others 1957, Bingham and Gremmen 1971). It was not until 1926, however, that Germany recognized high mortality in eastern white pine due to the disease (compare Schmitt 1972). Correspondingly, planting eastern white pine in Germany was prohibited (Tubeuf 1927). Unfortunately, the species began to be planted again in Germany after 1935 (Wappes 1935). Other European countries followed the German lead (Radu 1974), and suffered serious economic losses due to blister rust. For example, the rust invaded almost all young stands in Romania after 1970, promoted by the simultaneous culture of eastern white pine and *Ribes nigrum* (Blada 1982). Blister rust attacks eastern white pine in all countries where the species has been planted (table 1, column 8). The rust does not attack *P. cembra* nor *P. peuce* in their natural habitats, however, in spite of concurrence with susceptible *P. strobus* and *Ribes* species (table 1, columns 6 and 7).

Research Studies

Provenance Trials

Once widespread planting with eastern white pine stopped, genetic research activities began to slow. Despite the problem of blister rust, Germany and Croatia still continue to make measurements in their provenance trials (table 1, column 14).

Provenance testing of eastern white pine has been conducted in Germany, where two trials with 69 provenances were established in 1966 and 1967, respectively (Stephan 1974). All provenances were originated from the natural range of the species. Growth rate, mortality, and infections by blister rust were assessed at age 11. (Stephan 1974). Height at age 11 was negatively correlated with provenance latitude ($r = -0.40$ to $r = -0.51$). Provenances from southern Appalachian mountain States of North Carolina, South Carolina, and Virginia (south of the 39th degree of latitude) showed better growth than the average in these trials. Provenances from areas north of the 45th degree of latitude (Manitoba, Quebec, Ontario, New Brunswick, Minnesota, and Wisconsin) had poor growth. Significant (p less than 0.5) and highly significant (p less than 0.1) height-height correlations among provenances at different ages were found. The correlations were not strong when the differences in age were great, but particularly strong between heights from the age of five onward. A high number of dwarfed trees from one provenance from Illinois were noticed. Under natural conditions, differences among provenances could be observed for mortality and infection by blister rust. *Pinus monticola* (one provenance) and *P. wallichiana* (two provenances), tested in the same German trial, showed only 85 percent and 40 percent, respectively, of the average height of *P. strobus*. In addition, high mortality could be observed in *P. wallichiana*.

The Croatian research program included 10 eastern white pine provenances, of which six from North America and four from established plantations in Croatia. The tests were established in 1970 and were measured at age 18 (Orlic 1993). For American provenances, the average values for survival, height, and diameter were 70.7 percent, 12.8 m and 20.7 cm, respectively. Similarly, the survival and growth estimates for local provenances were 73.2 percent, 13.7 m and 22.2 cm, respectively, which were higher (3.5, 7.0, and 7.2 percent, respectively) than the American provenances. In term of survival, the local source Hrvatska ranked first and the American provenance New Hampshire ranked second. The New York (USA) and the other two local sources exhibited the poorest survival. The average of total height ranged from 11.9 m to 14.0 m, with an average of 13.3 m, whereas the diameter from 17.7 cm to 23.2 cm with an average of 21.5 cm. The best and the poorest provenance in diameter were Georgia and Wisconsin, respectively. No information was given about blister rust resistance.

Croatia also conducted a species comparison test with eastern white pine, Scots pine (*P. sylvestris* L.), black pine (*P. nigra* Arnold), European larch, and Douglas-fir (*Pseudotsuga menziesii* (Mirb). Franco) was established in Tocak area. After 23 years of testing, eastern white pine was found to have produced the greatest about of volume per hectare (479 cu. m), in comparison to 214, 209, and 164 cu. m/ha produce by Scots pine, European larch and Douglas-fir, respectively (Orlic and Ocvirek 1993). In a 26 year-old trial at Slatki Potok, eastern white pine generated 549.1 cu. m/ha in comparison to only 270 cu. m/ha produced by black pine (Orlic and others 1997). These results demonstrate the superior performance in wood production of eastern white pine in European site conditions.

In Romania, a nursery provenance test of eastern white pine was planted with 45 provenances: 19 from North America and 26 of unknown origin taken from old stands planted across Europe. The weight of 1,000 seeds per provenance was assessed before sowing (Radu 1974). Seed weight means ranged from 11.4 g to 23.2 g, with a mean of 16.9 g. The North American provenances averaged 15.9 g, whereas European provenances weighted 8.8 percent more (17.3 g). Twelve traits including dry matter were measured at age 2. The total height ranged from 8.9 cm to 15.9 cm, with Romanian local seed sources surpassing the other groups of provenances. Significant differences were found among provenances for dry matter content. The provenances from northern Minnesota (USA) had the lowest average height, but the highest proportion of dry matter. In contrast, the fastest growing provenances (North Carolina, Kentucky and Ohio, all from the USA) had the lowest dry matter content even though their height was greater.

A nursery provenance test of Cembran pine was established in Romania. The test consisted of 12 provenances including seven from the Carpathian Mountains and five from the Alps. Blada (1997a) found significant differences among provenances for total height growth, annual height growth and root collar diameter. The top four provenances were Pietrele, Gemenele and Calimani from the Carpathians and Blunbach Grunalpe from the Austrian Alps, which exhibited faster height, height incremental growth, and root collar diameter than other provenances at age 6. Duncan multiple range tests (1955) for these traits suggested that major gaps separated provenances within the natural range of the species; that is, genetically distinct populations could be found in both the Alps and Carpathian Mountains. The same data suggested a discontinuous pattern of distribution suggesting the absence of a gene flow among populations. There were highly significant positive correlations between all traits, suggesting that indirect selection can be applicable. No significant correlations were found between any growth trait and geographic coordinates.

Provenance tests of Balkan pine were investigated in Bulgaria. Thirteen provenances, originating from 1,700 m – 2,100 m in the Pirin and Rila Mountains, were represented in each of the two tests. At age 3, it was found that the Balkan pine provenances from high elevations initiated growth earlier than the provenances from middle elevations (Dobrev 1997a). Approximately 90 percent of the seedlings had lammas shoots at the end of the August of the third growing season. Significant correlations were found between seed size with provenance latitude and elevation. Also, a significant positive correlation was found between height growth and needle length. No significant correlations were found between height growth and latitude or with elevation of provenances.

Dobrev (1997b) investigated the concentration of macro- and microelements in the needles of the 3-year-old seedlings from different Balkan pine provenances. Variation of concentration in macro- and microelements of needles was discontinuous among provenances. Provenances from the northern Pirin Mountains greatly differed in their relative concentration of nitrogen and calcium and copper as compared with provenances from southern Pirin and Rila and Central Balkan Mountains. A geographic differentiation in magnesium concentration of the needles was found. The Pirin Mountains provenances had a higher concentration of magnesium than provenances from Rila and Central Balkan Mountains. A significant positive correlation was found between phosphorus concentration and seedling height.

About three decades ago, various provenance trials were established in Ukraine (Yatsyk and Volosyanchuk, personal communication). A *P. cembra* trial with six provenances originating from Ukrainian Carpathian Mountains was established on 0.5 ha. At age 28, the mean height growth, diameter and volume were 5.0 m, 9.3 cm and 31 cu m/ha were achieved. Two tests of *P. koraiensis* comprising 19 provenances of unknown origin were planted on 2.4 ha. The latest measurement took place at age 22 and 30. The mean height growth, diameter, and volume in the first and second tests were 6.7 m, 11.8 cm, and 8.1 cu m/ha, and 3.9 m, 7.3 cm, and 8.5 cu m/ha, respectively. A trial with four *P. pumila* Regel provenances of Russian origin was established on 0.25 ha. The mean height growth and diameter at the ground level at age 30 were 11.3 m and 3.6 cm, respectively. A 2.5 ha test of *P. sibirica* with 36 provenances of Russian origin was planted. The height growth, diameter and volume at age 23 were 5.4 m, 10.9 cm and 2.4 cubic meters per hectare, respectively. A second trial with 31 provenances, of the same origin, was established on 13 ha. Until now, no other measurements were made. Two *P. peuce* provenances of unknown origin were planted on 0.1 ha. Height, diameter and volume at 22 years of age, measured 7.0 m, 12.5 cm and 13.2 cubic meters per ha, respectively. All provenances of the above species proved to be resistant to blister rust.

The Ukrainians also planted tests of North American species. A 0.1 ha *P. flexilis* plantation with one provenance of unknown origin was made about 33 years ago. The height growth and diameter at age 21 were 6.3 m and 10.8 cm, respectively. All provenances exhibited high susceptibility to blister rust and only 10 percent of trees survived after 21 years of testing. In the same timeframe, a *P. strobus* trial with three provenances of unknown origin was planted on 0.1 ha. After 23 years of testing, the height growth, diameter and volume were 9.7 m, 23.4 cm and 56.3 cu m/ha, respectively. Heavy blister rust attack occurred and only 1.5 percent of the trees survived.

Open-Pollinated (Half-Sibling) Progeny Tests

Blue pine—In 1971 an international program for testing white pines of known origin for resistance against blister rust was proposed (Bingham and Gremmen 1971). Another program dealt with seed collection and exchange (Kriebel 1976). As part of this program, Romania received 36 open-pollinated families of blue pine (*P. wallichiana*) from 16 provenances originated from Pakistani Himalayan Mountains. This material was tested for growth and blister rust resistance by artificial inoculation. Eight traits, including blister rust resistance and height growth were measured at age 11, and the results reported by Blada (1994b). Highly significant differences were observed among families for blister rust resistance (BR1), percentage of trees free of blister rust (BR2), tree survivors (BR3), total height growth (HT), and stem volume (V). BR1, BR2, BR3, HT, and V averaged 3.2 points (10 = the highest resistance), 9.7 percent, 49 percent, 10.4 dm, and 0.458 dm^3 respectively.

Genetic variance estimates for BR1, BR2, BR3, HT, and V accounted for 91, 99, 96, 79, and 38 percent, respectively, of phenotypic variance. Therefore, this high amount of genetic variance could be used in a blue pine breeding program. Narrow-sense heritability estimates at the family level for BR1, BR2, BR3, and HT were high: 0.909, 0.998, 0.960, and 0.974, respectively. Heritability for V was much lower (0.380). These estimates, coupled with the large amount of variation observed within blue pine population suggest a two-way selection program for rust resistance and growth would be rewarding. If the best 5, 10, or 15 families were selected and planted on sites more or less similar to that used in this trial, a genetic gain of 67, 51, and 40 percent in BR1 and 18, 14, and 11 percent in V, respectively, could be achieved.

A highly significant positive phenotypic correlation was found between blister rust resistance and latitude. All families that originated from above 35° N latitude exhibited a higher resistance to rust than families from lower latitudes. No significant phenotypic correlations were found among growth traits and latitude or between growth traits and blister rust resistance. Therefore, growth and blister rust resistances are independent traits, indicating that improvement using indirect selection is not possible.

Cembran pine—As part of the Romanian five-needle pine breeding program, a nursery test with 136 open pollinated families of *P. cembra* was established. Highly significant differences among families were found for height, root collar diameter (RCD), and total number of branches (TNB) (Blada in preparation). The genetic coefficient of variation for height, RCD, and TNB were 22, 14.6, and 26.3 percent, respectively, and the narrow-sense heritability estimates for height, RCD and TNB were 0.968, 0.938, and 0.966, respectively. Consequently, an improvement program with stone pine would be yield positive results.

Genetic correlations among height with TNB, RCD, and TNB were high: 0.881, 0.571 and 0.713, respectively. Selection in height or in RCD should lead to an indirect increasing of the TNB. However, an increased number of branches is a negative characteristic, as it lowers the quality of wood. Therefore, the number of branches in the next generation should be minimized by selecting fast growing trees with small number of branches.

If selecting the best 30, 35, 40, or 45 of the 136 families, genetic gains in height of 28.8, 26.8, 25.1, and 23.4 percent, and in RCD of 18.8, 17.6, 16.4, and 15.3 percent, respectively, could be expected. By using these early test results to guide an operational improvement program, two types of production seed orchards were planned: a seedling seed orchard using the fastest growing seedlings in the best 45 families and a clonal seed orchard using ortets from the best 45 trees.

Breeding and Seed Orchards

A few countries reported clonal seed orchards, such as: Cembran in Austria, Slovakia Romania and Ukraine; Balkan pine in Romania; and eastern white pine in Croatia and Romania.

A genetic improvement program was started in Romania in 1977 due to the potential importance of eastern white pine in consideration of blister rust. This program included both intra- and interspecific hybridization and has a final objective of establishing seed orchards composed of selections with high general combining ability for resistance to blister rust (Blada 1982). Due to the lack of official interest in five-needle pines, the scope of the initial program was restricted to continuing the measurements in the already established trials. These hybrid trials perform well, and additional details will be given elsewhere in these proceedings.

Another Romanian breeding program under way is concerned with *P. cembra*. The program was initiated in 1989 with the following objectives: (a) phenotypic selection of parents in natural populations; (b) provenance and half-sibling family testing; (c) full-sibling family (both from intra- and inter-specific crossing) testing and genetic parameters estimation; and (d) seed orchards establishment with the best combiners for both improved mass seed production and as a base population for advanced-breeding population (Blada 1990).

Full-Sibling Progeny Tests

Romaina was the only country that reported full sibling progeny tests in response to the survey. A 10 x 10 full diallel crossing experiment was conducted in Gemenele on native populations of *P. cembra* from Romanian Carpathian Mountains. The experiment was conducted to provide information on the genetic variation and inheritance of important breeding traits. Cotyledon number, total height, annual height increment, RCD, TNB, and lammas shoots formation were measured from age 2 to 6 (Blada 1999). The most prominent result from this experiment was that significant general combining ability (GCA), specific combining ability (SCA), and reciprocal effects for all traits were found. Also significant maternal effects occurred in number of traits, suggesting control by nuclear and extranuclear genes and by nuclear x extranuclear gene interactions.

Growth measurements indicated a progressive increase with age of the GCA variance within phenotypic variance. The GCA variance of the total height growth, increased from 2 percent at age 2 to 25 percent at age 6, while the GCA variance of the root collar diameter increased from 8 percent at age 4 to 14 percent at age 6. Similarly, the SCA variance of the total height growth ranged from 15 percent at age 2 to 27 percent at age 6. The diallel analysis showed that both GCA and SCA variances were important sources of variation. Dominance variance exerted a greater influence on 10 out of 17 traits as evidenced by SCA / GCA variance ratios. However, the magnitude of these ratios suggested that additive effects might be almost as important as nonadditive effects in the study. Consequently, the breeding strategy can employ both additive as well as nonadditive variations, indicating that considerable progress under direct selection is possible. If two out of 10 randomly selected parent trees exhibited significant GCA effects for total height, then it can be estimated that 20 percent of trees within the basic natural population could be selected as good combiners.

Heritability estimates were high enough to ensure genetic progress in improving growth and other traits. For height growth, heritabilities for both family and single tree level increased from age 2 (0.065 and 0.021 respectively) to age 6 (0.453 and 0.366 respectively). In the same manner, heritabilities for root collar diameter, for both family and individual level, increased from 0.228 to 0.321 and from 0.126 to 0.157, respectively.

Interspecific Hybridization

***P. strobus x P. peuce* hybrids**—In Romania a 7 x 4 factorial crossing was conducted between eastern white pine (female) and Balkan pine (male) to combine the rapid growth of eastern white pine with high resistance to blister rust of Balkan pine (Blada 2000a). The resulting families were artificially inoculated at age 2 and planted in the field at age 6. Blister rust resistance (BRR), trees free of blister rust (TFBR), tree survival (TS), tree height (H), diameter at 1.30 m (D), basal area (BA), stem volume (V), stem straightness (SS), and branch thickness (BT) were measured at age 17. Highly significant differences among hybrid families were found for all traits except stem straightness. Selection at family level, therefore, can be carried out for the most economically important traits, including BRR and V.

There was large genetic variation among the parents for all traits examined. The effects of eastern white pine female parents were significant not only for growth traits but for BRR, TFBR, and TS as well. This suggests that there were (1) additive genetic control for all traits and (2) parents with high GCA could be selected for breeding. The existence of resistance to the blister rust within eastern white pine (as a female parent) agreed with results found by Riker and others (1943), Riker and Patton (1954), Patton and Riker (1958), and Patton (1966) and were contrary to Heimburger (1972). Balkan pine (as a male parent) had significant effects on growth traits but no significant effects on BRR, TFBR, and TS. Therefore, all male Balkan pine parents exhibited the same level of resistance to blister rust in this study, as found by Blada (1989) at an earlier age of the study. This was contrary to Patton's study (1966), which found differences in blister rust resistance among his *P. peuce* selected parents. Male x female interaction effects were significant and highly significant for all traits except for stem straightness, suggesting a nonadditive gene action on most traits.

Significant, positive phenotypic correlations were found among growth traits. Such correlations imply significant genetic gains in these traits even if selection was practiced on only one trait. However, correlations between stem straightness and growth traits were low, ranging from 0.30 to 0.32. Low phenotypic correlations (0.01 to 0.33) were obtained between blister rust resistance and growth traits, thereby suggesting that the two traits were inherited independently and tandem selection cannot be applied.

Mid- and high-parent heterosis was calculated (MacKey 1976, Halauer and Miranda 1981). Balkan pine was found to be the best parental species for blister rust resistance and stem straightness, whereas eastern white pine was the best parent species for all growth traits. Mid-parent heterosis was positive for all but one trait and accounted for 34 percent for BBR, 55 percent for TFBR, and 53 percent for TS. Substantial mid-parent heterosis was also found in most growth traits, such as 26 percent in volume growth. The total height growth had the lowest (13 percent) positive mid-parent heterosis, while the stem straightness was the only trait displaying a negative mid-parent heterosis. High-parent heterosis was negative for all traits. For example, at age 17, this heterosis accounted for -5 percent for blister rust resistance, -8 percent for trees free from blister rust, and -9 percent for total height growth. Generally, hybrids were intermediate between the two parental species over all characteristics and incorporated desired characteristics from both parent species.

Genetic gain using the average of breeding values of the best parents was calculated. Selecting the best three eastern white pines (females) for blister rust resistance (average breeding value was 0.833) would result an increase of 9.5 percent for blister rust resistance. Similarly, using the best five Balkan pines (males - average breeding value was 21.6 dm^3) to cross with the above females for volume growth rate would result in a genetic gain of 18.3 percent in the overall mean (118.0 dm^3).

***P. strobus x P. wallichiana* hybrids**—In Romania, a factorial crossing was conducted among seven female trees of eastern white pine and four male trees of blue pine to combine the rapid growth of former species with high resistance to blister rust of the latter species (Blada 2000c). The hybrid families were artificially inoculated at age 2 and planted at age 6. Blister rust resistance (BRR), TS, H, annual height growth, D, BA, V, SS, and BT were the measured traits at age 17.

Factorial analysis indicated significant differences among hybrid families for all traits except branch thickness. The effects of eastern white pine (female) were significant not only for the growth traits, but for BRR and TS, again suggesting an additive genetic control in all growth traits and blister rust resistance and that high GCA parents could be selected for breeding. Blue pine (male) had significant effects on TS, H, tree survivors, annual height growth, BA, and SS, but no significant effects on BRR, D, V, and BT. It is important to note that blue pine male parents exhibited the same level of blister rust resistance. Male x female interaction effects were significant except for TS and SS, suggesting that nonadditive gene action had an influence on all economically important traits.

The contribution of GCA variance to the phenotypic variance was 87 percent for H, 53 percent for D, and 77 percent for V; whereas the contribution of SCA variance to the same traits was lower, that is, 10, 41, and 22 percent, respectively. Both additive and dominance variances could be used for improvement in wood production. The contribution of GCA variance to the phenotypic variance was 73 percent for BRR and 53 percent for TS, while the contribution of the SCA variance for the same traits was only 9 percent for the former and 0 percent for the latter trait. The GCA / SCA variance ratios demonstrated that there was additive genetic variation for all traits. The GCA-F / GCA-M variance ratios revealed that estimates of GCA variance of females were much greater than estimates of males for all traits, except BT. These results suggested that the greatest amount of additive variance associated with both blister rust resistance and growth traits was found within eastern white pine parent population. The blue pine male parent contribution to the additive variance was insignificant for blister rust resistance but significant for some growth traits.

The narrow-sense heritability estimates at the family level were high for all traits. For example, the estimates of 0.828 and 0.885 and 0.777 for BRR, H, and V, respectively, were obtained. The magnitude of heritability estimates was due to the high level of additive variance attributable to the female parents. In general, the individual-tree narrow sense heritabilities appeared to be high for blister rust resistance ($h^2_w = 0.421$), moderately high for total height growth ($h^2_w= 0.327$), low for diameter ($h^2_w= 0.122$), and very low for branch thickness ($h^2_w= 0.085$). In conclusion, heritability estimates were high enough to ensure progress in improving genetic blister rust resistance and growth traits by using *P. strobus x P. wallichiana* F_1 hybrids.

Both positive and negative GCA effects, which significantly ($p<0.05$) differed from zero, were generally found for both male and female parents for most traits. None of the blue pine male parents had significant GCA effects on BRR as these parents exhibited the same level of resistance. The range of estimated GCA effects among parents suggested that it may be possible to select parents with superior breeding values for BRR and growth traits.

Genetic and phenotypic correlations between SS and growth traits were relatively low, ranging between 0.143 and 0.288. Both types of correlations between BT and total height were high and negative (-0.543 and -0.533), indicating that larger trees produced thinner branches. High, positive genetic and phenotypic correlations were obtained between these two traits with BRR, $r_G = 0.928$ and $r_p = 0.916$ respectively. In addition, high, positive genetic correlations were obtained between BBR and D ($r_G = 0.680$), BA ($r_G = 0.655$), and V ($r_G =. 608$), suggesting that tandem selection can be applied.

Estimates of high-parent heterosis were positive only for TS and height and negative for all other traits. From these estimates, it is evident that hybrids combined their parental genes for both rapid growth and blister-rust resistance. These results may justify the use of F_1 *P. strobus* x *P. wallichiana* hybrid production. Genetic gains could be realized in increasing in both blister-rust resistance and timber production. Even smaller increases in resistance and volume growth would give appreciable improvement in yield, especially when considered in relation to large-scale plantation programs.

Host-Parasite Investigations

In order to investigate the pathogenic variation of *C. ribicola* a German-Korean joint inoculation experiment was conducted (Stephan and Hyun 1983, also in Stephan, these proceedings). *Cronartium ribicola* strains used in the German experiment were able to infect only *Ribes nigrum*, but not *Pedicularis resupinata* L., the alternate host species in Korea. Therefore, this host plant species cannot be considered as a host for the German fungus material. *Cronartium ribicola* strains used in the Korean experiment could infect only *P. resupinata*, thereby confirming pathogenic variation in *C. ribicola*.

The same joint experiment corroborated other reports, such as La and Yi (1976), that demonstrated a wider pathogenic variation of *C. ribicola* in eastern Asia. There is an increase of blister rust strains with a stronger virulence than had been observed in Korea since 1963 (La and Yi 1976)) and Japan since 1972 (Yokota and others 1975). Apparently, only the *Ribes* host-strain of *C. ribicola* had invaded Europe in the last century, and from Europe was subsequently introduced to North America. Introduction of the *Pedicularis*-host strain of *C. ribicola* into North America would be a potential disaster for endemic five-needle species (Stephan and Hyun 1983), as this strain is very virulent.

In 1971, the International Union of Forest Research Organizations (IUFRO) proposed an international program for testing white pine blister rust resistance. Parallel trials were carried out in the Western and Eastern United States, in France, South Korea, Japan and Germany (Bingham and Gremmeen 1971, Stephan 1986). In Germany, 14 species with a total of 63 provenances/progenies have been tested by artificial inoculation. After 10 years, Stephan (1986) found significant differences among the species investigated. Asian five-needle pines are generally more resistant to the blister rust than North American species. No or relatively weak symptoms were observed in *P. pumila, P. parviflora* Zieb. & Zucc., *P. sibirica*, and *P. koraiensis*. Surprisingly, a few provenances of *P. wallichiana* showed high infection rates. Most of native North American white pines showed a very high infection rate of more than 90 percent. *Pinus albicaulis* Engelm., *P. flexilis, P. lambertiana* and *P. monticola* were severely damage, although *P. aristata* Engelm. was relatively less infected. The fungus infected nearly 100 percent of the tested *P. strobus* provenances. This partially can be explained by the theory that the gene center of blister rust and blister rust resistance is in north central Asia (Bingham and others 1971).

There were no differences among provenances within species. The experiment also included a few F_1 and F_2 progenies between *P. lambertiana* and *P. monticola*, which possessed a certain level of improved resistance to blister rust under North American conditions. Only one provenance of *P. lambertiana* showed 20 percent less attack than in unimproved provenances. In *P. monticola*, the F_1 and F_2 progenies selected for rust resistance were severely attacked 7 years after artificial inoculation. The comparison of the German results with those obtained in France (Delatour and Birot 1982), Japan (Yokota 1983), and North American tests were interesting. Three years after inoculation there were significant correlations between the German and French results with respect to the percentage of rust-free trees in the seedlot BR n° 41 (*P. lambertiana*) and seedlot BR n° 43 (*P. monticola*). There were no significant correlations with the American results and the French seedlot BR n° 46 results, nor were there correlations with the Japanese results (Stephan 1986).

A similar IUFRO test was carried out in France. This French test demonstrated that the species from Europe and Asia proved to be less susceptible to rust than the American species. (Delatour and Birot 1982).

A survey on blister rust resistance in native (Cembran pine) and introduced five-needle pine species was conducted in Romania (Blada 1982, 1990). After 1970, blister rust had caused severe attacks to all young stands of eastern white and western white pines. This severe outbreak was promoted by simultaneous culture of the five-needle pine species with *Ribes nigrum*. Mature populations and young seedlings from natural regeneration of *P. cembra* distributed throughout the Carpathian Mountains were free from blister rust. *Ribes alpinum* L. and *R. petraeum* Wulf. Populations coexist at high altitude with Cembran pine and were also free from blister rust, although they have been shown to be susceptible (Georgescu and others 1957, Blada, unpublished data). After approximately two decades of survey, blister rust still could not be found on Cembran pine nor the *Ribes* species (Blada, unpublished data). The absence of the infection on both Cembran pine and natural *Ribes* populations indicates that the Romanian Carpathian Mountains do not represent a gene center for *C. ribicola*, as suggested by Leppik (1967). The rust was recently introduced via eastern white pine seedlings from Germany and *Ribes* sp. collections from elsewhere (Blada, in preparation).

Research at Molecular Level

Studies concerning genetic differentiation and phylogeny of stone pine species based on isozyme loci are summarized in a previous publication and in this volume (Krutovskii and others 1992, Politov and Krutovskii in these proceedings). Another molecular study of a Russian five-needle pine (*P. sibirica*) was conducted by Goncharenko and others (1992). Enzyme systems in the seeds of natural populations from various parts of Siberia were analyzed by starch gel electrophoresis, and 36 alleles at 20 loci were defined. Of the genes controlling these enzyme systems, 55 percent proved to be polymorphic, with an average 17.6 percent of gene/tree being heterozygous. Interpopulation genetic diversity accounted for a little over 4 percent of the total genetic diversity. The Ney distance coefficient ranged from 0.008 to 0.051, with an average of 0.023. The data obtained suggested lack of any marked genetic differences between central and marginal Siberian populations.

Genetic diversity and differentiation among five populations of Cembran pine from the Italian Alps were studied by means of isoenzyme variation at 15 loci and contrasted with five Scots pine populations (Bulletti and Gullace 1999). The two species showed similar values for the mean number of alleles per locus and percentage of polymorphic loci, while the expected heterozygozity for Scots pine was higher than that for Cembran pine (0.332 vs. 0.281). All the populations studied showed an excess of homozygotes; the Allevet population of Cembran pine had the highest value of fixation index (0.206). Furthermore, the latter stand exhibited the lowest allelic richness index value. Only 2.7 and 3.5 percent, respectively, of the observed genetic diversity in Cembran and Scots pines was due to differentiation among populations. Therefore, the populations for each species studied share similar respective gene pools, and there were no barriers hampering gene flow. The results of the study provide useful information for *in situ* conservation of genetic variability. Moreover, the data obtained can also be used for the identification of the most valuable stands for the production of high quality seeds.

A comprehensive study concerning phylogenetic relationship among *P. cembra, P. sibirica* and *P. pumila*, using microsatellites and mitochondrial *nad1 intron 2* sequences was recently completed (Gugerli and others 2001). The three-chloroplast microsatellite loci combined into a total of 18 haplotypes. Fourteen haplotypes were detected in 15 populations of *P. cembra* and one of *P. sibirica*, five of which were shared between the two species, and the two populations of *P. pumila* comprised four species-specific haplotypes. Mitochondrial intron sequences confirmed grouping of the species. Sequences of *P. cembra* and *P. sibirica* were completely identical, but *P. pumila* differed by several mutations and insertions/deletions. A repeat region found in the former two species showed no intraspecific variation. These results indicate a relatively recent evolutionary separation of *P. cembra* and *P sibirica*, despite their presently distinct distributions. The species-specific chloroplast and mitochondrial markers of *P. sibirica* and *P. pumila* should help to trace the hybridization in their overlapping distribution area and to possibly identify fossil remains with respect to the still unresolved postglacial recolonization history of these two species.

Acknowledgments

The authors express their gratitude to all colleagues from several countries who supplied information concerning genetic activities in five needle pines in their own countries. Particularly, our thanks are due to Drs. R. Stephan (Germany), R. Yatsyk and R. Volosyanchuk (Ukraine), J. Gracan (Croatia), A. Nanson (Belgium) and R. Longauer (Slovakia). The thanks are extended to our technician, Cristiana Dinu, for her help during the preparation of this report.

References

Bialobok, S. 1960. Historia introdukgi I aklimatyzacji drzew I zrzewow w Arboretum Kornickim. Arboretum Kornickie, Roz V: 141-200.

Bingham, R. T. 1972a. Taxonomy, crossability and relative blister-rust resistance of 5-needled white pines. In: Bingham, R. T. and of a NATO-IUFRO Advanced Study Institute, August 17-24, 1969, USDA Forest Serv. Misc. Publ. 1221: Washington D. C.: 357-372.

Bingham, R. T. 1972b. Artificial inoculation of a large number of *Pinus monticola* seedlings with *Cronartium ribicola*. In: Bingham, R. T. and others, eds. Biology of rust resistance in forest trees; Proc. of a NATO-IUFRO Advanced Study Institute, August 17-24, 1969, USDA Forest Serv., Misc. Publ. 1221: Washington D. C.: 357-372.

Bingham, R. T., Hoff, R. J. and McDonald, G. I. 1971. Diseases resistance in forest trees, Ann. Rev. Phytopath (9): 433-452.

Bingham, R.T. and Gremmen, J. 1971. A proposed international program for testing white pine blister rust resistance in forest trees. Eur. J. For. Path. (1): 93-100.

Blada, I. 1982. Relative blister-rust resistance of native and introduced white pines in Romania. In: Heybroek, H. M. and others (Eds.): Resistance to disease and pests in forest trees. Workshop Gen. of Host-Parasite Inter. in Forestry, Wageningen, Holland. pp. 415-416.

Blada, I. 1987. Genetic resistance to *Cronartium ribicola* and height growth in some five needle pines and in some interspecific hybrids. PhD Thesis, Academy of Agricultural and Forestry Sciences, Bucharest: 146 p.

Blada, I. 1989. Juvenile blister rust resistance and height growth of *Pinus strobus* x *P. peuce* F_1 hybrids. Silvae Genetica 38 (2): 45-49.

Blada, I. 1990. Blister rust in Romania. Eur. J. For. Path. 20: 55-58.

Blada, I. 1994a. Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genetica 43 (1): 14-20.

Blada, I. 1994b. Performance of open–pollinated progenies of blue pine in Romania. Silvae Genetica 43 (4): 231-238.

Blada, I. 1996. Breeding of *Pinus cembra* and its nursery techniques and planting operations. Annual Report No. 26, For. Res. Inst., Bucharest: 5 p.

Blada, I. 1997a. Stone pine (*P. cembra* L.) provenance experiment in Romania: I Nursery stage at age 6. Silvae Genetica 46 (4): 197-200.

Blada, I. 1997b. Diallel crossing in *Pinus cembra:* II The nursery testing at age 6. In: White and others, eds. Proceedings of the 24 th Biennial Southern Forest Tree Improvement Conference, Orlando, Florida, 9 to 12 June: 143-161.

Blada, I. 1999. Diallel crossing in *Pinus cembra*. III Analysis of genetic variation at the nursery stage. Silvae Genetica, 48 (3-4): 179-187.

Blada, I. 2000a. Genetic variation in blister–rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17: (Experiment 1). Forest Genetics 7 (2): 109-120.

Blada, I. 2000b. Genetic variation in blister–rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17: (Experiment 2). Silvae Genetica 49 (2): 71-78.

Blada, I. 2000c. Genetic variation in blister - rust resistance and growth traits in a 17 year old *Pinus strobus* x *Pinus wallichiana* F1 hybrid population. Proc. Hybrid Breeding and Genetics, Noosa, Australia: 35-48.

Blada, I. 2003. Testing stone pine half-sib progenies in the Carpathians: genetic variation at the nursery stage. Silvae Genet. (In preparation).

Borchers, K. 1951. Folgerungen aus den bisheringen Aubauergebnissen mit fremdlanschen Holzarten im Gebiet des Landes Niedersachsen fur die Kunftige waldbauliche Planung. Mitt. Deutsch. Dendrol. Ges 57: 69-81.

Bulletti, P. and Gullace, S. 1999. Genetic variation and structure among Swiss stone pine (*P. cembra*) and Scots pine (*P. sylvestris*) populations in the western Italian Alps. Alberi Oggi (5): 11-16.

Cieslar, A. 1901. Uber anbauversuche mit fremdlandischen Holzarten in Osterich. Centr. Bl. Ges. Forstw. 27: 101-116.

Contini, L and Lavarelo, Y. 1982. Le Pin cembro (*Pinus cembra* L.) .INRA, Paris: 197 p.

Critchfield, W. B. and Little, E. L. 1966. Geographic distribution of the pines of the wold. USDA Forest Service, Misc. Publ. 991, Washington, D. C.: 96 p.

Davidescu, E. 1894. Darea de seama asupra excursiilor din Bucovina. Rev. Padurilor 1: 14.

Delatour, C. and Birot, Y. 1982. The international IUFRO experiment on resistance of white pines to blister rust (*Cronartium ribicola*): The French trial. In: Heybrock, H. M. and others, eds. Resistance to diseases and pests in forest trees. Workshop Gen. of Host-Parasite Interactions in Forestry, 14-21 Sept. 1980, PUDOC, Wageningen, the Netherlands: 412-414.

Dobrev, R. 1997 a. Growth and morphological characteristics of the three year-old seedlings of Macedonian pine (*P. peuce)* in half-sib progeny trials. Forest Science, (1 –2): 20-30.

Dobrev, R. 1997 b. Concentration of macro-and trace elements in the needles of the three-year-old seedlings of Macedonian pine (*P. peuce*) of different provenances. Forest Science, (1-2): 31-40.

Duncan, D. B. 1955. Multiple range and multiple F-tests. Biomertics 11: 1-42.

Enderlein, H. and Vogl, M. 1966. Experimentelle Untersuchungen uber die SO_2 empfindlicheit der Nadeln verschiedener Koniferen, Arch. Forstw. 15: 1207-1224.

Figala, H. 1927. Studien uber die Nordtiroler Zirbe. Ph.D. Dissertation, Hochschule fur Badenkultur, Vienna: 86 p.

Fukarek, P. 1970. Otkrice I danasnja rasprostranjenost molike (*Pinus peuce*). Zbornik na simpoziumot za molikata, Pelister–Bitola 2-9 September 1969: 17-25.

Georgescu, C. C. and Ionescu–Barlad C.D. 1932. Asupra statiunilor de *Pinus cembra* din Carpatii Romaniei. Rev. Pad. 8-9: 531-543.

Georgescu, C. C., Ene, M., Pertescu, M. Stefanescu, M. and Miron, V. 1957. Bolile si daunatorii padurilor. Editura agrosilvica de stat, Bucuresti. 638 p.

Goncharenko, G. G., Padudov, V. E. and Silin, A. E. 1992. Genetic structure, variation and differentiation in populations of *Pinus sibirica.* Genetika (Moskva) 28 (10): 114-128.

Gugerli, F., Senn, J., Anzidei, M., Madaghiele, A., Büchler, U., Sperisen, C., Vendramin, G. G. 2001. Chloroplast microsatellites and mitochondrial nad 1 intron 2 sequences indicate congruent phylogenetic relationships among Swiss stone pine (*Pinus cembra*), Siberian stone pine (*P. sibirica*), and Siberian dwarf pine (*P. pumila)*. Molecular Ecology 10: 1489-1497.

Halauer, A. R. and Miranda, F. O. 1981. Quntitative genetics in maize breeding. Iova State University, Ames: 468 p.

Heimburger, C. 1972. Breeding of white pine for resistance to blister rust at the interspecific level. In Bingham, R. T. and other, eds. Biology of rust resistance in forest trees; Proc. of NATO-IUFRO Advanced Study Institute, August 17-24, 1969, USDA Forest Serv., Misc. Publ. 1221, Washington D. C.: 541-547.

Hoff, R. J., Bingham, R. T. and McDonald, G. I. 1980. Relative blister-rust resistance of white pines. Eur. Low. Forest Path. 10 (5): 307-316.

Holzer, K. 1975. Genetics of *Pinus cembra* L. Annales Forestales 6/ 5, Zagreb: 139-158.

Kriebel, H. B. 1963. Verifying species hybrids in the white pines. Proc. Forest Genet. Workshop, Macon, Ga., October 25-27, 1962. Spons. Pub. No. 22: 49-54.

Kriebel, H. B. 1976. The IUFRO program for haploxylon pine gene pool exchange, selection and breeding. In. Proc. 16th IUFRO Wold Congress, Oslo: 239-245.

Kriebel, H. B. 1983. Breeding eastern white pine: a worldwide perspective. For. Ecol. Manage:, 6: 263-279.

Krutovskii, K., Politov, D. V., Altukhov, Y. P. 1992. Genetic differentiation and phylogeny of stone pine species based on isozyme loci. Proc. int. workshop on subalpine stone pines and their enviroument. St. Maritz, Switzerland, Sept. 5-11.

La, Y. J. and Yi, C. K. 1976. New developments in the white pine blister rust of Korea. XVI IUFRO Woid Congress, Oslo, Div. 2: 344-353.

Lanier, L. 1961. La rouille vesiculeuse du pin Weymouth due to *Cronartium ribicola* (Fischer). Station de Recherches et Experiences Forestieres; Notes Tech. For., Nancy 8: 11 p.

Leppik, E. E. 1934. Uber die geographische Verbreitung von *Cronartium ribicola.* Eesti Loodus (Estonia), 3: 52-55.

Leppik, E. E. 1967. Some viewpoints on the phylogeny of rust fungy. VI. Biogenic radiation. Mycologia LIX (4): 568-579.

Litscher, B. 1908. Die Weimouthskiefer in den Stadtwaldungen von Raperswie. Schwez. Ztschr. F. Forstw. 59: 7-14.

MacDonald, J., Wood, R. F., Edwards, M. v. and Aldhous, I. R. 1957. Exotic forest trees in Great Britain. Gr. Brit. For. Comm. Bull. 30: 167 p.

Mac Key, Y. 1976. Genetic and evolutionary principles of heterosis. Eucarpia 7: 17-33.

Mitruchi, I. 1955. Drust dhe shikuret e Shqiperiese. Dendrologija Albanije, Tirana.

Musil, I. 1969. Introdukce vejmutovky (*Pinus strobus*) na severni Morave a ve Slezku. Acta Musci Silesiae, Opava: 51-60.

Nedjalkov, S. 1963. Die *Pinus peuce* in Bulgarien. Schweiz. Zeitschrift f. Forstwesen 114: 654-664.

Nedjalkov, S. and Krastanov, K. 1962. [Establishing the rate of growth and productivity of *Pinus peuce*]. Izv. Inst. Gorata, Sofia, Bulgaria 8: 85-98.

Nikota, B., Stamenkov, M. and Djordjeva, M. 1970. Prvi rezultati od meguvidovoto vkrstsuvanje na molikata (*Pinus peuce*). Zbornik na simpoziumot za molikata, Pelister-Bitola, 2-9 September: 183-190.

Orlic, S. 1993. Research of Weymonth pine (*P. strobus*) provenances. Sumarski list, Zagreb. (9 - 10): 361-368.

Orlic, S. and Ocvirec, M. 1993. Growth of domestic and foreign conifer species in Zoung cultures on Fernery and Heath areas of Croatia. Radovi, Vol. 28 (1-2): 91-103.

Orlic, S., Komlenovic, N., Rastovski, P. and Ocvirec, M. 1997. Growth and biomass production of six coniferos species on luvisol. Sumarski list Zagreb 7-8, CXXI: 361-370.

Patton, R. F. 1966. Interspecific hybridization in breeding for white pine blister rust resistance. In: Gerhold, H. D. and others, eds. Breeding pest – resistance trees. Proc. NATO and NSF Advanced Study Institute, Pensylvania 1964: 367-376.

Patton, R. F. and Riker, A. J. 1958. Blister-rust resistance in eastern white pine. Proc. 5th Northeast. For. Tree Improv. Conf., pp. 46-51.

Popnikola, N., Jovancevis, M. and Vidakovic, M. 1978. Genetics of *Pinus peuce* Gris. Annales Forestales Vol.VII.

Radu, S. 1974. Cultura si valorificarea pinului strob. Editura Ceres, Bucuresti: 304 p.

Righter, F. I. And Duffield, J. W. 1951. Interspecies hybrids in pines: a summary of interspecific crossings in the genus *Pinus* made at the Institute of Forest Genetics. J. Heredity, 42: 75 – 80.

Riker, A. J., Kouba, T. F., Brener, W. H. and Byam, L. E. 1943. White pine selections tested for resistance to blister rust. J. For. 41: 753-760

Riker, A. and Patton, R. F. 1954: Breeding of *Pinus strobus* for quality and resistance to blister rust. Univ. Wis. For. Res. Notes. No. 12.

Ruskoff, M. 1936. Uber den Anbau der Weymountskiefer in Aussland und bei uns. Jahrsb. Der Universitat Sofia, Land. Und Forstw. Fafultat. Ed. 16: 155-198.

Schenck, C. A. 1949. Fremdlandische Wald – und Parkbaume.Vol. I, II, III. Berlin.

Schmitt, R, 1972. Intrinsic qualities, acclimatization and growth potential of white pines introduced into Europe, with emphasis on *Pinus strobus*. In: Bingham, R. T. and others eds. Biology of Rust resistance in forest trees; Proc. of NATO-IUFRO Advanced Study Institute, August 17-24, 1969; USDA Forest Serv. Misc. Publ. 1221, Washington D. C.: 111-123.

Soegaard, B. F. 1972: Relative blister rust resistance of native and introduced white pine in Europe. In: Bingham, R. T. and others, eds. Biology of rust resistance in forest trees: Proceedings of a NATO-IUFRO Advanced Study Institute, August 17-24, 1969; USDA Forest Serv. Misc. Publ. 1221, Washington D. C.: 233-239.

Stephan, B. R. 1974. Zur geographisc Variation von *Pinus strobus* aufgrund erster Ergebnisse von Versuch-sflächen in Niedersachsen. Silvae Genetica, 23 (6): 214-220.

Stephan, R. B. 1986. The IUFRO experiment on resistance of white pines to blister rust (*Cronartium ribicola*) in northern Germany. Proc. 18th IUFRO Wold Congress, Ljubliana; Div. 2, Vol.: 80-89.

Stephan, B. R. and Hyun, S. K. 1983. Studies on the specialization of *Cronartium ribicola* and its differentiation on the alternate hosts *Ribes* and *Pedicularis*. Journal of Plant Disease and Protection 90 (6): 670-678.

Tubeuf, C. 1927. Das Schicksal der Strobe in Europa. Jahresber. Deutsch. Forstver.: 330-348.

Yokota, S. T. 1983. Resistance of improvement *P. monticola* and some other white pines to the blister rust fungus, *Cronartium ribicola*, of Hokkaido, Japan. Eur. J. For. Path. (13): 389-402.

Yokota, S. T. Vozumi, T., Endo, K. and Matsuzaki, S. 1975. *Cronartium* rust of strobe pine eastern Hokkaido, Japan. Pl. Dis. Reptr. 59: 419-422.

Wappes, L. 1935. Bericht der Weymouthskiefen-Kommission. Reichsnahrstand Verl. Abt. Der Deutsche Forstwirt, Berlin: 63 p.

Genetic and Conservation Research on *Pinus peuce* in Bulgaria

Alexander H. Alexandrov
Roumen Dobrev
Hristo Tsakov

Abstract—Macedonian (Balkan or Roumelian) pine (*Pinus peuce* Griseb.) is a five-needle pine native to the Balkan Peninsula, occupying in Bulgaria an area of 14,223 ha. Genetic investigations made in Bulgaria include determination of the monoterpene composition of oleoresins, the delineation of geographic and ecological races, detailed analysis of progeny tests and other genetic studies. Many of the natural stands have the status of national parks and reserves with a total area of 5,250 ha, including 65 seed stands with an area of 709 ha. In addition, 152 candidate-elite trees have been selected. *Ex situ* methods for conservation of the genetic resources of this species include 40 clones in seed orchards (10 ha), six half-sib progeny trial plantations (5.6 ha), five provenance trial plantations (7.2 ha), and a forest seed bank. The indigenous populations of Macedonian pine in Pirin are a valuable genetic resource available for the introduction of this species into other countries of Europe, and also North America and Asia.

Key words: *Pinus peuce* Griseb., genetic resources, *in situ* conservation and *ex situ* conservation.

Species Distribution

Pinus peuce Griseb. is found only in the Balkan Peninsula, occurring in some of the high mountains of Bulgaria, Serbia, Macedonia, Montenegro, Albania and Greece in the range between 41° and 43° northern latitudes. In Bulgaria, the natural range of this species consists of two parts separated by the valley of the Vardar River. The eastern part is in southwestern Bulgaria and includes Pirin Mountain, Slavyanka Mountain (Ali Botush), Rila Mountain, the western Rhodopes, Vitosha Mountain, and the Central Balkan Range. The western part includes Macedonia, southwestern Serbia, southeastern Montenegro, eastern Albania, northeastern Greece and some spurs of the Dinar Alps, including Prokletija, Kom, Sekiritsa, Sar, Pelister, Kozhuh, Nidje, Korab, Rudoka, and Tsena (Dimitrov 1963).

Distribution in Bulgaria

The easternmost occurrence of Macedonian pine is in the Central Balkan Range. The westernmost, which is also the northernmost population, is on Sekiritsa Mountain, and the southernmost is in the Pelister, Nidje and Tsena Mountains (Dimitrov 1963). The areas occupied by the species in Bulgaria, listed by mountain, are as follows: Pirin 7,175 ha, Rila 6,230 ha, Central Balkan Range 193 ha, Western Rhodopes 170 ha, Vitosha 104 ha and Slavyanka 57 ha. Within these areas, *P. peuce* stands are scattered like islands, the most compact ones being those in the Pirin, Rila, Prokletija and Pelister Mountains.

There are two complexes of the species on Pirin Mountain, one in the northeast with an area of 3,775 ha, where the altitudinal distribution of the trees ranges from 1,600 to 2,200 m, and one in the southwest with an area of 3,400 ha with an elevation range from 1,700 to 2,200 m.

On Rila Mountain there are three *P. peuce* complexes, one in the southern part (1,635 ha in area and 1,700 to 2,000 m in elevation), one in the central part along the Rilska river (911 ha and up to 2,100 m elevation), and one in the northern part (3,684 ha with a 1,600 to 2,100 m range in elevation).

In the Central Balkan Range, two populations separated by the main ridge have been differentiated, one of 188 ha on the northern slope, from 1,500 m to 1,900 m elevation, and the other on the southern slope with an area of 5 ha and from 1,300 m to 1,400 m elevation.

On Slavyanka Mountain, *P. peuce* occurs in groups and as solitary trees, while on Vitosha Mountain and in the Western Rhodopes the species occurs mainly in plantations. On Sredna Gora it occurs only in plantations totalling 88 ha (Alexandrov 1998).

In 2000, the total wood volume of the 14,223 ha of Macedonian pine stands in Bulgaria was 4,198 000 m^3, distributed by age class from I (1-20 years) to VIII (141-160 years) and following approximately the normal curve. The stands of age classes V (81-100 years) and VI (101-120 years) had the largest area, totalling 6,037 ha (42.5 percent of all stands) with a growing stock of 2,160 000 m^3 (51.5 percent of the total wood volume).

The overall average volume of the Macedonian pine forests in Bulgaria is 295 m^3/ha, the average quality class is III (medium) and the rotation period is 160 years (Tsakov 2001). The average stand volume exceeds that of *Picea abies* (L.) Karst and is considerably higher than that of *Pinus silvestris* L. (Krastanov 1970).

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the Forest Research Institute, 132, St. Kliment Ohridski Blvd. 1756 Sofia, Bulgaria. E-mail: forestin@bulnet.bg

Genetic Research

Genetic studies of the Macedonian pine in Bulgaria, which were performed during the last 10 years, included a seed stand in each of the following areas, except for the larger number of stands in the Pirin and Rila regions, as indicated:

1. Pirin (3 Forestry Estates) – 1,900 m altitude, 10 seed stands
2. Gotse Delchev Forestry Estate – 1,800 m alt.
3. Bansko Forestry Estate – 1,700, 1,800, 1,900, 2,000 m alt.
4. Razlog Forestry Estate – 1,700, 1,900, 2,000, 2,100 m alt.
5. Rila (7 Forestry Estates) – 1,800, 2,000 m alt., 9 seed stands
6. Belitsa Forestry Estate – 1,900 m alt.
7. Yakoruda Forestry Estate – 2,000 m alt.
8. Rila Monastery Forestry Estate – 1,800 m alt.
9. Kostenets Forestry Estate – 1,900 m alt.
10. Samokov Forestry Estate – 1,800 m alt.
11. Doupnitsa Forestry Estate – 1,800, 1,900 m alt.
12. Central Balkan (Ribaritsa Forestry Estate – 1,700 m alt.

Analyses were made of variation in monoterpene composition and in morphological and physiological characteristics.

Monoterpene Variation

Monoterpene composition was determined from apical buds, 2-year-old needles, wood samples and bark from 2-year-old branches collected from representative Macedonian pine populations in the northern Pirin Mountains, the northern Rila Mountains and the northern slopes of the Central Balkan Mountain. Twelve monoterpenes were identified, eight of them (α-pinene, camphene, β-pinene, Δ-3-carene, myrcene, limonene, β-phellandrene and terpinolene) having relative proportions above 0.5 percent, regardless the origin of the samples or the investigated tissue. It was shown that the populations studied differ statistically in their monoterpene compositions. This made possible the division of the Macedonian pine populations from Northern Pirin, Rila and Central Balkan into separate geographical races based on the monoterpene composition of the oleoresins (Dobrev 1992).

Variation in Morphological and Phenological Characteristics

Measurements were made on cones, seeds and seedlings. On the basis of these results, the Macedonian pine population from Southern Pirin could be distinguished as a separate geographic race. It was established that the repeatability coefficients for these traits, i.e. repeatability in different areas, are relatively high for the origins from the central parts of the species natural range in Bulgaria, whereas the reproductive materials (cones and seeds) from the marginal parts of the natural range have lower coefficient values (Dobrev 1995).

With respect to 20 characteristics reflecting the morphology of the cones, cone scales, seeds and the sizes, morphology and phenology of 1-year-old seedlings in half-sib progeny trials, a phenotypic similarity was established between mature trees of 13 representative provenances of Macedonian pine from Pirin, Rila and Central Balkan Range. Based on of the calculated similarity matrix and the dendrogram of grouping pattern of populations from these mountains, the taxonomic distances were shown to be large and not proportional to the geographic distances between them. The results were comparable with those from preliminary studies of monoterpenes from sample trees in several *P. peuce* populations in Bulgaria (Dobrev 1996).

Identification of Geographic/Ecological Races

From analysis of 44 morphological, growth, phenological and chemical traits characterizing the populations of Macedonian pine and their progenies, it was possible to distinguish five geographic and ecological races of this species in Bulgaria, as follows: Central Balkan, Rila, Southern Pirin, and Northern Pirin (where one middle mountain and one high mountain ecotype could be separated).

In an evaluation of growth rate differences, as determined from total tree height, statistically significant differences ($p = <0.01$) were found among six-year-old families from 13 Bulgarian seed sources in five half-sib progeny trials distributed over a diversity of sites. Test locations included Stara Reka Forestry Estate at 1000 m elevation, Sliven Forestry Estate at 1000 m elevation, Yakoruda Forestry Estate at 1450 m elevation, Belitsa Forestry Estate at 1650 m elevation and Kostenets Forestry Estate at 1850 m elevation Family heritability estimates for height growth were statistically significant, varying with site from 0.220 to 0.574 (Dobrev 1998).

A 10th-year evaluation of these 13 progenies growing in four of these locations (excluding Kostenets) showed that the fastest-growing trees came from the Northern Pirin region at 1,900 m elevation. This population was consistently superior in growth in different tests at elevations from 1,000 to 1,450 m.

Genetic Considerations in Reforestation and Afforestation

Macedonian pine is one of the species most suitable for restoring the upper forest zone below the tree limit, which, in many mountains, has been moved down as a result of human interference. The trial plantations of *P. peuce*, which have been successfully established in the high parts of the mountains, provide a reason for expanding these plantings. However, satisfactory growth is possible only if transfer of genotypes is from lower to higher altitudes, with a maximum vertical seed transfer distance of 300 m (Alexandrov 1998).

Conservation of Genetic Material

In situ Conservation

National parks, nature parks, reserves, seed stands and plus trees provide *in situ* conservation of genetic resources of Macedonian pine. The total area of the natural forests, which are included in Pirin, Rila and Central Balkan National Parks and Bayuvi Dupki – Djindjiritsa, Yulen, Rilomanastirska Gora, Parangalitsa, Ibar, Tsarichina and Central Rila Reserves, amounts to 5,250 ha. The gene pool of this species as a whole is preserved through the genetic diversity inherent in these different ecological regions.

The preservation of gene resources in permanent seed stands provides the basis for implementing a breeding programme. There are 65 *P. peuce* seed stands in Bulgaria, totalling 709 ha, or approximately five percent of the Macedonian pine forests. These seed stands are sufficient to meet anticipated needs for the species (Alexandrov 2000). In addition, the 152 candidate-elite Macedonian pine trees showing phenotypically superior growth, form and resistance (trees showing no damage from insects and diseases) have been selected by the Forest Seed Testing Stations in Sofia and Plovdiv. This *in situ* selection in natural stands provides a base of information and material for future genetic investigations, breeding improvement and greater utilization of *P. peuce* for reforestation and afforestation.

Ex situ Conservation

The genetic resources of Macedonian pine are also being preserved *ex situ* in Bulgaria through provenance testing plantations, progeny trial plantations, seed orchards and gene banks for seeds. Provenance trials at ages of 28-31 years include plantations on Rila Mountain at 2,050 m, the Western Balkan Range at 1,650 m and 1,700 m, and the Rhodopes at 2,050 m and 2,100 m, with a total area of 7.2 ha (Dakov and others 1980).

There are six half-sib progeny trial plantations, age 12 at the time of writing of this paper, distributed in various parts of the species range at elevations from 1,000 to 1,850 m. In all, 170 half-sib families from 13 provenances are being tested in these plantations on a total of 5.6 ha.

A 32-year-old, 10 ha clonal seed orchard in the Western Rhodopes (1450 m elevation) includes 40 clones (Bogdanov 1970).

Assessing the relative advantages of *in situ* and *ex situ* conservation of the genetic resources of Macedonian pine, the first seems to be a more reliable method for Bulgaria, because of the growth and health of the species under a diversity of ecological conditions. Our results indicate that the native populations of Macedonian pine in Pirin are an especially valuable genetic resource for the introduction of this species into many countries of the Northern Hemisphere.

References

Alexandrov, A. 1998. *Pinus peuce* Grisb. – *In* Enzyklopädie der Holzgewächse, ECOMED, Landsberg, Germany, III-1:1-10.

Alexandrov, A. 2000. Genetic conservation of conifers in Bulgaria – *In*: The Balkan Ecology 3:5-10.

Bogdanov, B. 1970. Seed Orchards ? from *Pinus peuce*. -*In*: Proc. Symp. on the Macedonian pine, Skopje, 211-220 (in Serb.).

Dakov, M., I. Dobrinov, A. Iliev, V. Donov, S. Dimitrov. 1980. Raising the upper forest limit. Zemizdat, Sofia, 220 pp (in Bulg.).

Dimitrov, T. 1963. Roumelian pine (*Pinus peuce* Grisb.), Sofia, 116 pp (in Bulg.).

Dimitrov, M. 1980. The Macedonian pine (*Pinus peuce* Grisb.). Zemizdat, Sofia, 180 pp (In Bulg).

Dobrev, R. 1992. Monoterpene composition of the essential oil of some populations of Macedonian pine (*Pinus peuce* Grisb.) in Bulgaria. Forest Science (Sofia) 2:8-16 (In Bulg.).

Dobrev, R. 1995. Intraspecific variation in *Pinus peuce* in Bulgaria: cone characteristics. *In*: Carying for the Forest: Research in a Changing World. Abstracts of Invited Papers. IUFRO XX World Congress, Tampere, Finland, p. 146.

Dobrev, R. 1996. Phenotypic similarities of representative populations of Macedonian pine (*Pinus peuce* Grisb.) in Bulgaria. Forest Science (Sofia) 4:16-23 (in Bulg).

Dobrev, R. 1998. Variance, genotypic stability and family mean heritability of the growth in height of 6-year-old seedlings of Macedonian pine (*Pinus peuce* Grisb.) in a series of half-sib progeny trial plantations. Forest Science (Sofia)1-2:5-23. (in Bulg).

Krastanov, K. 1970. Wachstum, Leistung und Technisches Haubarkeitsalter von *Pinus peuce* - Bestände in Bulgarien. Berichte. Symposium über *Pinus peuce,* Skopje, pp 277-289 (In Serb.).

Tsakov, H. 2001. Composition and productivity of natural Macedonian Pine endrocoenoses in Northern Pirin Mountain and created anthropogenic ones in Vitosha and Sredna Gora Mountains. D.Sc. Thesis, Sofia, 137 pp. (in Bulg.).

Five-Needle Pines in Russia: Introduction and Breeding

Anatoly I. Iroshnikov
Dmitri V. Politov

Abstract—*Pinus sibirica* Du Tour, *P. pumila* (Pall.) Regel and *P. koraiensis* Sieb. *et* Zucc. are primarily located in Russia and occupy about 36.69, 38.30 and 2.87 million ha in the Asian part of Russia, respectively. These species, together with *P. strobus* L., *P. peuce* Gris., *P. cembra* L. and *P. monticola* Doug. ex D. Don, have been introduced in the European part of Russia. In all genetic tests *P. strobus* was found to have a high growth rate, but it was severely affected by white pine blister rust (*Cronartium ribicola* J.C. Fisch.). An intensive breeding program that would incorporate disease resistance genes from *P. peuce* is suggested to address this problem. *P. sibirica* is resistant to blister rust and has highly nutritious edible seeds and, thereby, is considered the most promising five-needle pine species for propagation in the European part of Russia. Its disease resistance and high intrapopulation variation in reproductive and growth traits provide a good basis for selecting new varieties. More than 2,500 plus trees have been selected throughout the entire *P. sibirica* area, and 6,100 ha of genetic reserves have been established. Growth traits of 12 to 32 year old plantations were studied on six common garden test sites in Siberia and on three sites in the European part of Russia. Seed sources that exhibited optimum growth were from the Altai and Sayan Mountains in southern Siberia and the southern taiga in western Siberia and are considered to contain the most valuable gene pool. Progeny from northern and sub-alpine populations had growth rates 20-40 percent lower than average, and progeny from transitional sub-zones and elevations had intermediate growth rates. There was no significant east-to-west difference in the growth rate. The similarity in growth traits corresponds well with the relatively low interpopulation differentiation demonstrated by isozyme data. The existing network of common garden tests of *P. sibirica* is insufficient for complete characterization of the gene pool and revision of existing seed zones. Certain adjustments, such as merging of some seed zones, can be suggested.

Key words: *Pinus*, *P. sibirica*, *P. koraiensis*, *P. pumila*, *P. strobus* five-needle pines, stone pines, introduction, breeding, Russia

Background

Three species of five-needle pines (*Pinus* L. subsection *Cembrae* Loudon) are naturally distributed in Russia: *Pinus sibirica* Du Tour, *P. koraiensis* Sieb. *et* Zucc. and *P. pumila* (Pall.) Regel. Each species has distinctive morphological, genetic, and, sometimes, biological traits and are adapted to specific ecological niches. *P. sibirica* naturally occurs in areas with a humid continental climate: northeastern European Russia, the Urals Mountains, Western Siberia taiga, and the northern macroslopes of mountains in southern Siberia and Transbaikalia. In these forests *P. sibirica* occupies a total area of 36.69 million ha. Forests containing *P. koraiensis* occupy a much smaller area (2.87 million ha) and occur in a monsoon climate, predominantly in the Primorskii territory and the southern part of the Chabarovskii territory. Thickets formed by *P. pumila* occupy a total area of 38.3 million ha and are located in the cold climate zones of eastern Siberia and the Far East. This species is usually found in localities characterized by a high level of snow cover.

Total stem volumes for *P. sibirica* and *P. koraiensis* are estimated as 7.4 billion m^3. Total stem volume of *P. pumila* is estimated at 1.1 billion m^3. Specific traits of these Russian five-needle pines, site descriptions, and relationships with other forest tree species have been reviewed in numerous publications (Tikhomirov 1949; Iroshnikov 1974; Pravdin and Iroshnikov 1982; Krilov and others 1983; Semechkin and others 1985; Kolesnikov 1954, 1966).

Under optimal site conditions, the high productivity of *P. sibirica* and *P. koraiensis* stands resulted in clearcutting of many large and valuable stone pine forests between the 1930s and the 1980s. Forests in the Altai, Western Sayan, and Sikhote-Alin Mountains, southern taiga in Western Siberia, and lower Amur basin were subjected to intensive exploitation. The cutting of these forests caused widespread changes in species composition, soil erosion, and declines in harvests of stone pine seeds (= nuts) and commercial fur hunting. Due to public efforts, stone pine forests were recognized for their exceptional environmental values and have been protected in Russia since 1989. Clearcutting was prohibited, and management of these forests has since been oriented to provide multiple values. Intensive reforestation efforts for these species have occurred including introduction in regions outside the respective natural ranges.

Early efforts in the breeding and introduction of native and exotic *Pinus* section *Strobus* species and other economically valuable conifers in the former Soviet Union occurred in the 1920s through 1950s (Kern 1934; Georgievski 1931, 1941; Nesterov 1935; Pogrebnjak 1938; Eitingen 1938, 1946; Tikhomirov 1949; Grozdov 1952; Girgidov 1955). Specific studies evaluated the acclimatization of different species in

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Anatoly I. Iroshnikov is with the Research Institute of Forest Genetics and Breeding, Voronezh, Russia. Dmitri V. Politov is Senior Research Associate, Laboratory of Population Genetics, N.I.Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 Gubkin St., GSP-1, Moscow 119991, Russia. Phone: 7 (095) 135-5067. Fax : 7 (095) 132-8962. E-mail: dvp@vigg.ru.

different environments (Sukachev 1922; Maleev 1933; Gurski 1957). Throughout the 1960s through 1980s, several important studies evaluated the introduction of coniferous species in botanical gardens, arboreta, experimental forests, and private estates during second half of 1800s (Shin 1961; Vekhov and Vekhov 1962; Timofeev 1965; Maurin 1970; Shkutko 1970; Mashkin 1971; Nekrasov 1980; Redko and Fedorov 1982; Ignatenko 1988; Logginov 1988; Plotnikova 1988; Lapin 1971). Unfortunately, these materials were often of unknown geographic origin, which limited the value of the studies' conclusions.

In the 1960s and 1970s, broad-scale experimental studies on the introduction and breeding of prospective conifer species were undertaken by the Institute of Forestry in the Siberian Branch of the Russian Academy of Sciences, All-Union Research Association "Soyuzlesselektsia," and Moscow State Forest University. These studies were designed to: (1) review results from past plantings and the current state of introductions of section *Strobus*, subsection *Strobi* pines to Russia; and (2) evaluate introduction and breeding experiments on *P. sibirica* conducted in different regions of Russia.

Introduction of Subsection *Strobi* Species

Four of the 17 pine species classified in subsection *Strobi*, *P. flexilis* James, *P. monticola* Douglas ex D. Don, *P. peuce* Grisebach and *P. strobus* L., have been introduced, over time, into arboreta and botanical gardens in European Russia. Among these species, *P. flexilis* and *P. monticola* were found to be unfit for planting in Russia and are not widely distributed. Since the early 19th century, most introduction efforts were of *P. strobus*, initially as ornamental tree and later as a forest tree that could rapidly produce high quality wood. Between 1860 and 1890, *P. strobus* was planted in the Baranovichi forest management unit of Byelorussia (Shkutko 1970), Forest Experimental Station of Petrovsko-Razumovski Academy of Agroforestry in Moscow region (Nesterov 1935; Eitingen 1938, 1946), and in Shatilov's estate (now the Mokhovskoi forest management unit of Orlovskaia region) (Pankov and others 2000; Kapper 1954). *P. strobus* was widely planted in Byelorussia, Latvia, Lithuania, Ukraine and several regions of Russia in the late 1800s and early 1900s (Grozdov 1952; Girgidov 1955; Shin 1961; Sevalnev 1966; Fedoruk 1969, 1980; Maurin 1970; Shkutko 1970; Redko and Fedorov 1982; Usachev 1983; Sirotkin and Gvozdev 1987; Logginov 1988; Kapper 1954). Later in the 20th century, the species was evaluated in Kazakhstan (Rubanik 1974), the Far East (Samoilova 1972), Georgia, the northern Caucasus Mountains (Holjavko 1981), and Estonia (Kasesalu 2000).

Recommendations for the introduction of *P. strobus* have been adjusted in recent years, as new information on productivity and disease resistance was accumulated for different vegetation zones of the former USSR. Initially, it was recommended that the species be planted in forests, steppe-forests, and the northern part of steppe zones eastwards up to the Yenissey River. These recommendations were altered to advise planting of *P. strobus* in the following regions: northwestern Ukraine, southern Moscow region, Kurskaia, Voronezhskaia, Orlovskaia, Belgorodskaia, Lipetskaia, Tambovskaia, western Saratovskaia region, and the Republic of Moldova. It was specified that *P. strobus* should be planted only in areas where the species would be higher in growth and value than *P. sylvestris* L.

It is noteworthy that Byelorussia, Latvia, Lithuania, Estonia and the northwestern regions of Russia were not designated for planting *P. strobus*. These regions have a relatively wetter climate where extensive blister rust (*Cronartium ribicola* J.C. Fisch) infection has been observed in both pure and mixed stands of *P. strobus* at different ages (Nesterov 1935; Eitingen 1946; Girgidov 1955; Maurin 1970; Shkutko 1970; Grozdova 1975; Potapova 1984; Kasesalu 2000). However, there are environments in these regions where *P. strobus* can be successfully grown. In Byelorussia, stands with different levels of tolerance to this pathogen have been noted (Shkutko 1970; Fedoruk 1980; Sirotkin and Gvozdev 1987). In the central Chernozem regions of Russia (Kurskaia, Voronezhskaia, Orlovskaia, Belgorodskaia, Lipetskaia, Tambovskaia), blister rust causes only minor damage to *P. strobus* (Shin 1961; Pismenni 1967; Kapper 1954). *P. strobus* plantings in the Ukrainian steppe zone are rarely attacked by blister rust (Logginov 1988). Pismenni (1967) concluded that the threat of *C. ribicola* to *P. strobus* plantations is largely overestimated, based on observations of 30 plantings established throughout European part of the former USSR. Despite the wide distribution of alternate hosts – black currant (*Ribes nigrum* L.) and gooseberry (*Grossularia* Mill.) – in this area, Pismenni concluded that there was a higher degree of damage associated with soil pH, relative air humidity (at 1 p.m.), mean air temperature, and number of days with cloudy weather in August in the regions of growing than with proximity of alternate hosts (table 1). Additionally, Pismenni found that stands with faster growing trees and a higher productivity class were more susceptible to the pathogen. There are some problems, however, with Pismenni's (1967) conclusions. Pismenni was not aware that diseased trees had been removed in sanitation cuttings and that there were well-known cases of extensive blister rust infections (especially on thin sandy soils) and even mortality in some of the plantations (Nesterov 1935; Grozdov 1952; Kapper 1954).

Valuable information on the intensity and dynamics of *P. strobus* mortality due to blister rust has been obtained from long-term observations (1880 to 1920) on *P. sylvestris* L. plantations established at the Forest Experimental Station in the vicinity of Moscow (Nesterov 1935, Eitingen 1946). These studies revealed extensive damage of Scots pine in several regions of Russia (Rudzski 1874, Sobichevski 1875). Long-term (130 plus years) investigations of the dynamics of Scots pine populations, in different parts of the natural range, showed populations with a high incidence of *Peridermium pini* (Pers.) Lev., and *Cronartium flaccidum* (Alb. *et* Schw.) Wint. had periodic epidemics caused by these pathogens in association with solar activity. The studies also revealed different types of interactions between the pathogen and trees, the influence of growth conditions on the metabolism of each organism, and respective levels of tree resistance and pathogen virulence. These studies suggested a similar mode of interaction between the host-pathogen pair of *P. strobus* and *C. ribicola*. Definitive conclusions, however, can not be made as *P. strobus* – *C. ribicola* interac-

Table 1—Damage of *P. strobus* stands by white pine blister rust *Cronartium ribicola* (Cr.r%) in regions differing by air humidity (W, %), mean air temperature (T, °C) and percentage of cloudy days (V,%) in August (Pismenni 1967)

Location of plantations: republic, region (forest management unit)	Age, years	Cr.r, %	W,%	T °C	V,%
Lithuania (Valkinskii, Smalininskii)	48; 45	14; 24	68	15	55
Byelorussia (Bobruiskii, Uzdenskii, Prilukskaia Dacha)	50; 56; 48	14; 12; 8	65	16	55
Ukraine, Sumskaia (Trostianetskii)	69; 52; 28	6; 4; 1	55	16.4	40
Russia, Orlovskaia (Mokhovskoi)	10; 28; 80	0; 6; 9	54	17	39
«-», Lipetskaia (LOSS, Leninskii)	28; 28	3.2; 1	54; 52	17.2	39
«-», Kurskaia (Rylskii)	16; 54; 54	0; 1; 1.5	52	17.2	39
«-», Penzenskaia (Yursovskii)	67	0,5	51	17.6	38
«-», Voronezhskaia (Vorontsovskii, Savalskii)	30; 32	0	50	18; 18.2	38
Moldova (Kalarashskii)	24	0	49	19	30

tions have been viewed for only a relatively short time in Russia, and there is a lack of comparative studies of homogenous progeny over different sites. In addition, Minkevich's (1986) observations on the lack of correlation between solar activity and *C. ribicola* outbreaks in Europe (r=0.09±0.18) over a 31-year period suggests a different interaction between *P. strobus* – *C. ribicola* than *P. sylvestris* and its pathogens.

The low resistance of *P. strobus* plantations to *C. ribicola* in different regions of the former USSR stimulated selection of individuals resistant to the pathogen and the introduction of *P. peuce* Grisebach, as a species highly tolerant to blister rust (Eitingen 1946; Grozdov 1952; Maurin 1970; Shkutko 1970). However, the limited availability of seeds of this Balkan species has prevented widespread field testing.

Tables 2 and 3 present growth data of different subsection *Strobi* species in plantations throughout the country. Plantations older than 20 to 30 years are reviewed; that is, those that have reached the "critical age when fitness of exotics at particular conditions is finally elucidating" (Maleev 1933, p. 108).

Toward the end of the 20th century, the total area occupied by *P. strobus* plantations reached 259 ha (Beloborodov and others 1992). Some stands were 60 to 120 years old, highly

Table 2—Growth indices of some five-needle pine species in the points of their introduction in Russia.

Region	Age year	Height, m	Diameter, cm	Source
		Pinus peuce		
Moscow. LOD MSHA	53	19	28	Eitingen, 1946
Moscow. Ivanteevka	33	9.8	13	Grozdova, 1975
Lipetsk. LOSS	31	11	15	Kuzmin, 1969
Orjol. Mokhovoe	70-80	22-24	40-46	Vekhov, Vekhov, 1962
Orjol. Shestakovo	75-80	26	50	Mashkin, 1971
		P. flexilis		
Lipetsk. LOSS	44[a]	14.2	31	Vekhov, Vekhov, 1962; Kuzmin, 1969
		P. monticola		
Lipetsk. LOSS	40[a]	11.4	26.8	Kuzmin, 1969
		P. koraiensis		
Altai. G.-Altaisk	32	6-10	11-15	Luchnik, 1970
Lipetsk. LOSS	40	9.4	20.2	Kuzmin, 1969
Mari. Ioshkar Ola	30	7-8	10-12	Alimbek, 1991
Moskow. Ivanteevka	35-38	6-8	6-9.5	Yablokov, Dokuchaeva, 1976
		P. cembra		
Lipetsk. LOSS	30	5.4	8	Vekhov, Vekhov, 1962
Moscow. Chlebnikovo	80	16.6	25.5	Ignatenko, 1988
Smolensk. Dugino	35	11	6	Grozdov, 1952
		P. pumila		
Altai. G.-Altajsk	30	3-4	2-6	Luchnik, 1970
Lipetsk. LOSS	36	3.3	-[b]	Kuzmin, 1969

[a] Very sensitive to fungal disease *Cronartium ribicola*

[b] Not measured

Table 3—Growth indices of *Pinus strobus* artificial stands on the territory of the former USSR.

Republic region	Age, year	Height m	Diameter cm	Number of trees per ha	Growing stock, m-3/ha	Source
The Ukraine						
Ivano-Frankovsk	70	30.5	37.6	700	960	Usachev, 1983
Trans-Carpathians	68	38.5	35.3	633	915	” - ”
Right-bank forest steppe	60	27	28	-[a]	570	Logginov, 1988
Byelorussia						
Brest	54	25	42.2	429	650	Usachev, 1983
Minsk, Uzdenski	66[b]	25.5	32.5	550	386	Shkutko, 1970
Latvia						
Shkedovskoe	70[b]	23.9	39.7	-	348	Maurin, 1970
Skriverskaya	70[b]	24	36.8	-	360	” - ”
Lithuania	60	25	40	-	-	Jankauskas, 1969
Estonia						
Agali arboretum	33[b]	16.6	22	900	282	Kasesalu, 2000
Kazakhstan						
Alma-Ata	23[b]	5	7	-	-	Rubanik, 1974
Russia						
Bryansk	84	22.3	23.1	940	468	Smirnova, 1997
Kaliningrad, Nagornoe	67	29.8	37.2	699	1199	Redko, Fedorov, 1982
Kaliningrad, Novo-Bobruyskoe	93	29.3	41.8	294	630	” - ”
Kursk, Rilsky	55	22	18	-	250	Sevalnev, 1966
Leningrad, Viborgsky	45[b]	14.9	26	-	-	Girgidov, 1955
Lipetsk, LOSS	41	15.6	37	-	-	Kuzmin, 1969
Moskow, LOD MSHA	47[b]	16.5	20	-	-	Eitingen, 1946
” - ”	45[b]	14	14	-	-	Timofeev, 1965
Moskow, Ivanteevka	40[b]	15.5	19.2	-	-	Grozdova, 1975
Orjol, Mokhovoe	125	31.4	35.2	103	383	Pankov et al., 2000
Penza	67	18.4	28	750	425	Usachev, 1983
Voronezh	40	18	28	-	-	Dorofeeva, Sinitsin, 1996

[a] Not measured
[b] Very sensitive to fungal disease *Cronartium ribicola*

productive, free from blister rust, and produced large seed yields with high quality (Kapper 1954; Maurin 1970). In order to conserve gene pools and create a permanent seed base of *P. strobus* in the USSR, a gene reserve (1.6 ha), plus stands (5 ha), 180 plus trees, 17 ha of permanent seed orchards, and 2.5 ha of clonal plantations were established in the early 1990s (Beloborodov and others 1992). In addition experiments on hybridization of this species with *P. wallichiana* A.B. Jackson and *P. ayacahuite* Ehrenberg *ex* Schlechtendahl, were initiated in the Ukraine, and 11 candidates for varieties were selected (Patlaj and others 1994). Currently, a spontaneous hybrid between *P. wallichiana* and *P. strobus* is under observation at the Sochi Arboretum, Institute of Mountain Forestry and Ecology in the northern Caucasus Mountains. The hybrid tree measured 23 m in height and 42 cm in diameter at the age of 39 years (Soltani 2001).

Genetic plantings and orchards of *P. strobus* have been established at different locations. Since the 1980s, the Research Institute of Forest Genetics and Breeding in Voronezh has been evaluating seed and clonal progenies of plus and phenotypically superior *P. strobus* trees selected in old plantations in three forests from the Kaliningrad region, Borskoie forests of Voronezh Natural State Reserve, Glushkovskoie forests of Lipetskaia region, and Mokhovskoie forests of Orlovskaia region (Beloborodov and others 1993, 1994). Testing of 29 families was initiated in 1984 in a 1.2 ha area within the Homutovskoie forest, and 96 families were established in 3.8 ha planting at Mezen Pedagogical College in Orlovskaia region in 1986 and 1987. An additional 33 families are being evaluated in a 1.2 ha planting that was established in 1989 in the Davydovskoie forest (Voronezh area). An archive (0.7 ha) of 41 clones was established in the Gremyachenskoie forest within the Voronezhskaia region, and a clonal seed orchard consisting of 25 plus trees (1.4 ha) has been established in Zagon forest (Smolenskaia region).

The 10 year-old results of these progeny testing experiments have shown superior growth of eight families from the Mokhovskoie and Glushkovskoye forests, while progenies from Kaliningradskaia region and Voronezh State Reserve show significant variability in growth rate. Only 25 percent of the tested families have shown resistance to white pine blister rust (Beloborodov and others 1993, 1994). Further studies of these plantations and clonal archives indicate that the stands become more susceptible to blister rust with advancing age. At age 16, only 7.7 percent of families and 36 percent of clones were free from blister rust. In some families, all trees are infected, and up to 67 percent of the ramets were susceptible in the clonal plantings (Shirnina and Beloborodov 1999).

A blister rust resistance study on subsection *Strobi* species was conducted by Bsaibes (2000) in northwest Russia (St. Petersburg and Leningradskaia region) and in Central-Chernozem regions. This study confirmed the high resistance of *P. peuce* to *C. ribicola* and the extremely high susceptibility of *P. monticola* and *P. strobus* to the pathogen. To ensure resistance of *P.* strobus, Arefiev and Bsaibes (2000) recommended combining selection of resistant provenances and progenies with site selection and silvicultural practices to form plantations with a genetic composition and environmental conditions not favorable for the pathogen. To some extent, these plantations represent a synthesis of earlier recommendations (Maleev 1933; Nesterov 1935) but do not assume blister rust resistance through interspecific hybridization, which was shown to be effective in the case of *P. strobus* and *P. peuce* in Romania (Blada 1994, 2000a,b), or inoculation with pathogen spores at early stages of pine ontogenesis that became a common practice in North America (McDonald and Hoff 2001).

Strategies for establishment of future *P. strobus* plantations will benefit from knowledge of the modes and mechanisms for coadaptation of *P. strobus* and *C. ribicola* (Millar and Kinloch 1991). Understanding host-blister rust interactions in spatially and temporarily heterogeneous environments will provide a significant contribution to the theory and practice of species introduction, as well as to forecasting and development of preventive measures for decreasing the negative effects of pathogen epidemics.

Introduction of Stone Pines, Subsection *Cembrae* Loudon

Stone pines with all uncertainty about their phylogeny and taxonomy have always been attractive objects for botanists and foresters. Concern about protection of *P. sibirica* stands against fire and unwarranted clear-cutting is reflected in early publications, such as before the 19th century, which also discussed their diversity and biological, and aesthetical resources (Pallas 1786; Dmitriev 1818). In the 19th century, data on testing of stone pines in botanical gardens, arboreta, and various experiment stations (Lisinskoie forest near St. Petersburg and the Petrovsko-Pazumoskaia Forest Station in Moscow) stimulated a broader introduction of Siberian stone pine in parks and orchards in European Russia. These plantings were studied to evaluate species variability and ecology (Gomilevski 1909). At the same time, there was a shift from seed collections in natural stands in remote taiga regions to seed production and collection in seed orchards formed from progenies of highly productive trees of foothill (low mountain) populations in optimal growth conditions (Barishentsev 1917).

Numerous publications and documents have been focused on broad-scale popularization of seed production in seed orchards (Georgievski 1932; Vekhov and Vekhov 1962; Yablokov 1962; Nekrasov and Tvelenev 1970; Shkutko 1970; Potapova and Potapova 1984; Ignatenko 1988; Usmanov and Korolkova 1997; Drozdov and Drozdov 2002; Titov 1999; Iroshnikov and Titov 2000). A number of methods have been developed for seed and vegetative reproduction of stone pines.

The limited number and area occupied by old plantations of *P. sibirica* in European Russia, as well as the unexplained mortality of 55 to 68 year-old stands in Lisino and Petrovsko-Razumovskaya Experiment Station has hindered planting of the species outside of its native range. Establishment of *P. sibirica* plantings also would be hindered by competitive ability in sites outside of the native range. Comparison of growth between 22 and 32 year-old stone pines with local forest tree species in common garden tests in the Leningradskaia region (forest-steppe site), Moscow region (subtaiga site), and Yaroslavskaia region (foothill site) has shown inferior growth of *P. sibirica* by 20 to 40 percent (Iroshnikov 2000).

The results discussed above as well as data from subsequent studies (summarized in tables 4 and 5) show that there is substantial interspecific variation in *P. sibirica.* The tests show that the best growing seed sources are from low and middle mountain belts in the south Siberia mountain ridges and the sub-taiga and southern taiga zones in western Siberia. Progenies from northern and subalpine regions had growth rates 20 to 30 percent lower. Meridian (west-east) differences in origin within corresponding zonal and altitudinal complexes of stone pine forests had little effect on growth of progenies.

Common garden and test plantations of *P. sibirica* have been established in different vegetation zones, as well as a study of variation in natural populations of *P. sibirica* (Iroshnikov 1974). These tests confirmed the high variability of the species and also revealed unique morphological tree forms and variation in reproductive processes. Significant variation was detected in needle size, shape, and position on shoots, duration of juvenile phase, long-term dynamics of macrostrobili formation, macrostrobili maturation dates, and in premature ripening of cones within the first year after “flowering” with formation of 40 to 60 percent of unsound seeds.

Investigations on natural populations of *P. sibirica* have been limited to Altai and Sayan Mountains and do not allow conclusions about the distribution of blister rust across the species range. Lebkova (1964, 1967) found a high frequency of *Cronartium* species, including *C. ribicola* in a 10 to 15 year-old stone pine understory in the subalpine and middle mountain zone in the western Sayan Mountains and the northeastern Altai Mountains. Tovkach (1968) also reported severe damage to the *P. sibirica* understory by *C. ribicola* (up to 36 percent of trees) in the eastern Sayan Mountains (Nizhneudinskii forest management unit of Irkutskaia region). Ulcerous cankers putatively caused by *Biatoridinia pinastri* Colov. *et* Stzedr. were also detected in the *P. sibirica* understory in the Shestakovskii and Kyrenskii forest management units of the Irkutskaia region (Osipova 1968).

The only *P. sibirica* common garden study damaged by *C. ribicola* was in the Dmitrovskii forest management unit in the the Moscow region (table 5). Aeciospores were detected in 1993 and 2001 on seedlings of 17 of the 24 families. Approximately 2 to 11 percent of the trees were infected. After 40 years of observation, *C. ribicola* damage was rarely detected in Siberian plantations or the seed orchards and tests planted at the Yemelyanovskii, Ermakovskii and Uzhurskii forest management units of the Krasnoyarskii territory.

The introduction of *P. cembra* and *P. sibirica* occurred in the 19th century in botanical gardens in the Ukraine, Latvia,

Table 4—Growth indices of 37-year-old descendants of Siberian pine of various origins in Krasnoyarsk forest-steppe (Emelyanovsky Forest of Krasnoyarskii Territoty). (Iroshnikov A.I.).

Origin of seeds			Height		Diameter
Region	Forest management unit	Altitude above sea level, m, zone	cm	% of control	cm
E. Kazakhstan	Leninogorsky	1200-1500	731±37	83	8.3±0.9
Altai	G.-Altajsky	1200-1500	712±55	81	8.7±0.8
Kemerovo	Kuzedeevsky	300-700	930±27	106	11.8±0.8
	Myskovsky	1000-1300	856±40	98	9.9±0.9
	Tisuljsky	700-1000	897±17	102	10.6±0.4
	Mariinsky	subtaiga	876±24	100	10.0±0.6
	Yurginsky	subtaiga	875±19	100	10.3±0.6
Khakasia	Tashtypsky	900-1000	931±14	106	10.9±0.4
	Birikchuljsky	1000-1300	885±17	101	11.9±0.6
	Balyksinsky	900-1000	877±38	100	9.4±0.6
	Khakassky	900-1000	900±14	103	11.4±0.4
	Oktyabrsky	1100-1300	822±26	94	12.6±1.1
	Sonsky	1100-1400	891±23	102	12.6±1.2
Krasnoyarsk	Ermakovsky	400-500	877±57	100	13.1±1.3
	” - ”	1500-1600	594±53	68	7.8±1.3
Irkutsk	Cheremkhovsky	1200-1300	893±24	102	10.8±0.7
	Ikejsky	900-1000	932±33	106	13.7±0.8
	Slyudyansky	600-900	930±29	106	11.8±0.8
	Oljkhonsky	1000-1300	768±22	88	10.3±0.6
Buryatia	Zakamensky	1100-1300	800±19	91	12.5±0.6
	Dzhidinsky	1300-1400	809±21	92	13.4±0.9
Chita	Kr.-Chikojsky	900-1100	807±40	92	11.4±1.1
	Khiloksky	900-1100	888±20	101	10.9±0.4

Table 5—Indices of growth and damage with *Cronartium ribicola* of Siberian and Korean pine geographic cultures in Dimitrovsky Forest in Moscow Region (Iroshnikov and Tvelenev 2002).

Origin of seeds				Height[a]			
	Forest	Latitude/longitude			% of	Diameter,[b]	
Region	management unit	°N	°E	cm	control	mm	Stem rust[b], %
Siberian pine							
Komi	Troitsko-Pechersky	63	57	522±16	71	99±4	8.9
Sverdlovsk	Novo-Lyalinsky	59	60	533±18	68	110±5	0.0
	N.-Tagiljsky	58	60	572±17	73	125±4	0.0
Tomsk	Tymsky	60	80	651±18	84	123±5	2.4
	Chainsky	58	83	671±18	86	118±4	0.0
	Shegarsky	57	84	644±22	83	119±7	3.2
	Zyryansky	57	86	705±18	90	141±5	2.4
Tver	Kalininsky	57	36	605±16	78	118±5	11.1
		Elevation above Sea Level, m					
Altai	Baygolsky	1300-1800		655±17	84	124±7	2.3
	Kyga	430		779±24	100	146±6	0.0
	” - ”	1250		730±20	94	150±6	2.1
	” - ”	1500		635±16	82	129±5	2.4
Krasnoyarsk	Shushensky	550		745±26	96	146±8	0.0
	” - ”	800		816±18	105	170±4	2.4
	” - ”	1300		621±16	80	113±5	0.0
Irkutsk	Slyudyansky	1000		722±13	93	160±3	2.0
Buryatia	Dzhidinsky	1000		612±19	79	115±5	0.0
	Bichursky	1000		678±16	87	146±4	5.1
Korean pine		°N	°E				
Khabarovsk	Bikinsky	47	134	804±23	103	159±8	0.0

[a] At 32-year age (2001)
[b] At 30-year age (1999)

Lithuania, and Central-Chernozem regions of Russia (table 2). Due to lack of information about geographic origin, combined data on the state of these stands has been often presented in literature (Maurin 1970; Shkutko 1970; Fedoruk 1980). Plantations established in Khlebnikovskii Park near Moscow in 19th century (described by Ignatenko as *P. sibirica*), evidently originated from seeds obtained from the Balkans, as the planting also contains several *Abies alba* Mill. trees and three cone-bearing *Pinus peuce* individuals. Single trees were observed to be infected by *C. ribicola*, although natural stands of *P. cembra* in eastern Carpathia have been reported to be highly resistant to pathogens up to age 400 to 500 years (Smagljuk 1969).

Progenies of two *P. cembra* clones originating from the Carpathians were planted in the forest-steppe zone in Krasnoyarsk territory. Testing of 40-year-old clones grafted in 1963 onto *P. sylvestris* stocks showed the same growth characteristics as even-aged *P. sibirica* grafts originating from the western Sayan Mountains and bore few cones (10 to 50 per tree). Grafts of two other Carpathian clones (20 to 28 years-old) planted in the Dmitrovskii forest management unit (Moscow region) are characterized by exclusively high level of micro- and macrostrobili formation, up to 300 to 500 per each tree with a well-developed crown.

P. koraiensis was introduced in Russia after *P. sibirica* and *P. cembra*. In many regions of European Russia, Korean pine grows quickly during the first few decades (table 2) and has early formation of female cones (macrostrobili). For instance, in the Dmitrovskii forest management unit, 32 year-old plantations originating from seeds (brought from the Khabarovskii territory) grew as fast as the best *P. sibirica* stands (table 5). However, some trees were infected by *C. ribicola*. Azbukina (1974, 1984) describes white pine blister rust outbreaks across the whole Korean pine range. Additional studies are required to evaluate the different perspectives of broad-scale introduction of this species in Russia.

Dwarf Siberian Pine

P. pumila is relatively rare in botanical gardens and arboreta. Tikhomirov (1949) concludes that cultivation is possible, as there has been successful cultivation in the subalpine zone of in the Urals and at other locations. However, the species has been somewhat ignored due to a comparatively low economic value. Semechkin and Semechkina (1964) concluded that *P. pumila* is sensitive to mechanical squeezing when planted in loamy and loose soils, and sown seeds are often destroyed by rodents and nutcrackers (*Nucifraga caryocatactes* L.). In our (A. I. Iroshnikov) experiments in the western Sayan foothills, *P. pumila* did not survive longer than 15 years. Low survival of this species was also observed in Khakasia (Likhovid 1994) and in the Lipetsk region (Vekhov and Vekhov 1962). Additionally, grafts of *P. pumila* to *P. sylvestris* and to *P. sibirica* at the Dmitrovskii forest management unit did not survive past 20 to 25 years.

Susceptibility to blister rust is another factor that should be considered when planting *P. pumila*. Azbukina (1974, 1984) observed intense blister rust damage of *P. pumila* in the Far East. A subsequent study (Azbukina and others 1999) demonstrated that *Cronartium kamtschaticum* Joerst., which had been described as a pathogen of *P. pumila*, is really *C. ribicola*.

In conclusion, it appears that successful introduction of *P. pumila*, as well as other stone pines, is largely dependent of number and origin of individuals. Genetic differentiation within the range of pines of subsection Cembrae is reviewed in this volume (Politov and Krutovskii, this proceedings).

Conclusions

Effectiveness of using exotic woody plants for increasing forest biodiversity, productivity, quality and sustainability to biotic and abiotic factors depends on genotype, adaptive potential, and competitiveness. Approximately 200 years of studies in Russia suggest that the success of an introduced species can be evaluated only through long-term testing of progenies representing a broad spectrum of provenances and tested in different ecological conditions. Information from such studies increases when more genotypes are involved.

Pinus sibirica progeny tests established in diverse ecological conditions, inside and outside the species' natural range, have revealed intra- and interpopulation diversity, high breeding potential, and have helped to specify a geographic zone that is optimal for *P. sibirica* growth with maximal genetic variation. Long-term experiments with *P. sibirica* and *P. strobus* have allowed for development of current forest seed zone recommendations for *P. sibirica* (in 1982) and help direct the planting program for introduction of *P. strobus*.

To increase successful introduction of exotic five-needle pine species requires the development of international collaboration, coordination of corresponding studies within Russia, wider publication of results in peer-reviewed journals and monographs, maintenance of available and establishment of new experimental objects, and *in situ* and *ex situ* conservation of genetic resources.

References

Alimbek, B.M. 1991. Outlooks for Korean pine introduction in the central Volga Region. Lesnoe Hozjaistvo. 9: 20-22.

Arefiev, Yu. F. and Bsaibes, F.E. 2000. Does *Pinus strobus* have a future in Russia? In: Reproduction of Forests, Energy and Resource Saving Technologies of Forest Complexes: Proc.Scientific and Practical Conference, Devoted to the 70th Anniversary of VGLTA (Voronezh, 27-29 September, 2000). Voronezh: 153-156.

Azbukina, Z.M. 1974. Rust fungi of the Far East. M. Nauka. 527 pp.

Azbukina, Z.M. 1984. Glossary for determination of rust fungi of the Soviet Far East. M. Nauka. 288 pp.

Azbukina, Z.M., Hiratsuka, Y., Kakishima, M., Imazu, M., Kaneko, S., Katsuya, K., Oho, Y. and Sato, S. 1999. The blister rusts on *Pinus* spp. in Russian Far East. In: Forests and forest formation process in the Far East: Proc. of International Conference (23-35 August, 1999), devoted to the 90th anniversary of B.P. Kolesnikov. Vladivostok: Institute of Biology and Soil Science FEB RAS: 161-162.

Barishentsev, V.V. 1917. Siberian stone pine forests-fruit gardens // Lesnoi Zhurnal. Issue 1-3: 35-55.

Beloborodov, V.M. and Shirnina, L.V. 1994. Growth of *Pinus strobus* trees in connection with attack by blister rust. In: Selection and its use for improvement of forest species: Collected Book of Scientific Works. Voronezh. NIILGiS. Kvadrat: 75-82.

Beloborodov, V.M., Shirnina, L.V., Beljaev, A.B. and Shirjaev, V.I. 1993. Growth and sanitation of *Pinus strobus* in experimental stands. In: Genetic and ecologic Bases for Increasing of Forest Productivity: Proceedings. Voronezh: NIILGiS: 76-84.

Beloborodov, V.M., Shirjaev, V.I. and Patlai, I.N. 1992. Exotics in artificial stands of the European parts of Russia. Lesnoe Hozjaistvo. N8-9: 38-39.

Blada, I. 1994. Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genet. 43: 14-20.

Blada, I. 2000a. Genetic variation in blister rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17 (Experiment 1). For. Genet. 7(2): 109-120.

Blada, I. 2000b. Genetic variation in blister rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17 (Experiment 2). Silvae Genet. 49: 2: 71-78.

Bsaibes, F. E. 2000. Ecologic and phytopathological aspects of five-needle pines. Introduction in the European part of Russia. Author's Abstract of Thesis for a Doctor's Degree. Speciality 03.00.16. Ecology. Voronezh. VGLTA. p. 22.

Dmitriev, V. 1818. Siberian stone pine // Sibirski Vestnik. Sankt-Peterburg. Part 1: 134- 145.

Dorofeeva, V.O. and Sinitsin, E.M. 1996. Exotics in the VGLTA Arboretum and potential use in forestry. In: Complex Productivity of Forests and Organization of Multipurpose Forest Use. Theses of All-Russian Conference. Voronezh, 13-14, Dec. 1995. Voronezh: 157-158.

Drozdov, I.I. and Voitjuk, M.M. 1988. Introduction of Korean pine plantations in the central European Part of the USSR. In: Forest Geobotany and Biology of Woody Plants: Collected Book of Scientific Works. Brjansk: 27-33.

Drozdov, I.I. and Drozdov, Y. I. 2002. Introduction of valuable exotic trees. Forestry Information. VNIILM. N10: 30-53.

Eitingen, G.R. 1938. Foreign species in the experimental forest of Timirjazev, Agricultural Academy. Lesnoe Hozjaistvo. N5 (II): 53-60.

Eitingen, G.R. 1946. Experimental forest 1865-1945. Moscow. Goslestechizdat, 176 pp.

Fedoruk, A.T. 1969. Coniferous exotics of the Brest Region // Izvestija BSSR. Set of Biologic Sciences. Minsk. N1: 29-37.

Fedoruk, A.T. 1980. Woody plants in gardens and parks of Byelorussia. Minsk. Nauka and Tehnika. 208 pp.

Georgievski, S.D. 1931. Dendrological examination of parks near Moscow. In: Proc. on Applied Botany, Genetics and Breeding. V. 27 Issue 3. Leningrad. VIR: 297-407.

Georgievski, S.D. 1932. Stone pines of the USSR // Lesopromishlennoe Delo. N4: 241-249.

Georgievski S.D. 1941. Introduction of woody species in the Far East Territory of the European part of the USSR. Nature and Socialist Economy: Collected Book. M. N.8. Part I: 12-33.

Girgidov, D. Ya. 1955. Tree species introduction in the northwest USSR. Moscow-Leningrad. Goslesbumizdat, 48 pp.

Gomilevski, A.I. 1909. Siberian pine as a garden and decorative tree // Progressivnoe Sadovodstvo I Ogorodnichestvo. Sankt-Peterburg. N44; 522-523.

Grozdov, B.V. 1952. Dendrology. Moscow-Leningrad. Goslesbumizdat, 436 pp.

Grozdova, N.B. 1975. Growth peculiarities of introduced species in the Ivanteevka Arboretum. In: Genetics, breeding, seed improvement and introduction of forest species. Proceeding VNIILM, Moscow: 3-8.

Gurski, A.V. 1957. The main results of introduction of woody plants in the USSR. M.-L. Publishing House USSR AN, 304 P.

Holjavko, V.S. 1981. Fast-growing exotics. M. Lesnaja Promishlennost. 274 pp.

Ignatenko, M.M. 1988. Siberian stone pine: biology, introduction, and culture. Moscow, Nauka, 160 pp.

Iroshnikov, A.I. 1974. Polymorphism in Siberian stone pine populations. In: Variability of Woody Plants of Siberia. Krasnojarsk: 77-103.

Iroshnikov, A.I. 2000. Problems of study, gene pool protection and selection of stone pines. In: Korean pine-broadleaved forests of the Far East: Proc. from the International Conference. Sept. 30 - Oct. 6, 1996. Khabarovsk, Russian Federation. USDA FS PNW-GTR-487: 92-113.

Iroshnikov, A.I. and Titov, E.V.M. 2000. Recommendations on selection and evaluation Siberian pine plus trees for seed productivity. 36 p.

Iroshnikov, A.I. and Tvelenev, M.V. 2002. Selection and evaluation of Siberian stone pines according to sexual differentiation. In Genetic base of evolution and breeding. Proc. of Inter-Regional Conference Devoted to 90-year Celebration of S.I. Mashkin, 16-18 Oct. 2002, Voronezh: 144-152.

Jankauskas, M.A. 1969. Investigations on the ecology and acclimatization of tree species. Forest assessment and forestry organization in the Lithuanian SSR. A Report in Proc. for a Doctor's Degree. Vilnius, 56 p.

Kapper, O.G. 1954. Coniferous species. Silvicultural characteristics. M.-L. Goslesbumizdat. 304 p.

Kasesalu, H. 2000. Cultivation of introduced pines (*Pinus* spp.) at Järvselja. In: Forest Studies. XXXII, Tartu: 63-72.

Kern, E.E. 1934. The most important foreign woody species suitable for growing in the USSR. Leningrad. Publishing House VIR: 177 pp.

Kolesnikov, B.P. 1954. Korean pine on the Soviet Far East. In: Komarov's Lectures, Vladivostok. Issue IV: 23-67.

Kolesnikov, B.P. 1966. Stone pines and stone pine forests of the USSR. In: Papers for the VI World Congress: Forestry and industrial consumption of wood in the USSR. M. Lesnaja Promishlennost': 395-403.

Korjakin, V.N., Grek, V.S., Romanova, N.V. and Nechaev, A.A. 2002. Growth of mixed stands of Korean pine and Manchurian walnut in the Hekhtsirski Forestry Enterprise. In: Dynamics and State of Forest Resources in the Far East: Proc. of Regional Conference. Khabarovsk, December, 2002: DalNIILH:165-169.

Kozhenkova, A.A. 1989. Problems in cultivation of *Pinus strobus* in the Moscow region. In Proc. MLTI. Issue 211: 125-129.

Krilov, G.V., Talantsev, N.K. and Kozakova, N.F. 1983. Stone pine. Lesnaja Promishlennost: 215 pp.

Kuzmin, M.K. 1969. Trees and bushes in the Forest-Steppe Experimental and Breeding Station, Voronezh. Central-Chernozem Book Publishing House. 116 pp.

Lapin, P.I. 1971. Theory and practice of introduction of woody plants in the central region of the European part of the USSR. Bulletin of the Main Botanical Garden. Issue 81: 60-69.

Lebkova, G.N. 1964. The phytopathological state of stone pine stands in western Sayan. Izvestia SO AN USSR. Series of Biologic and Medical Science N8. Issue 2: 77-82.

Lebkova, G.N. 1967. Phytopathological state of stone pine stands in northeastern Altai. In: Diseases of Forest Stands in Siberia. M. Science: 73-80.

Likhovid, N.I. 1994. Introduction of trees and bushes in Khakasia. Novosibirsk. NIIagrar. of Khakassia Problems: 330 pp.

Logginov, V.B. 1988. Optimization of introduction to forest ecosystems. Kiev. Naukova Dumka. 164 pp.

Luchnik, Z.I. 1970. Introduction of trees and bushes in the Altai. Moscow, Kolos, 656 pp.

Maleev, V.P. 1933. Theoretical basis of plant acclimatization. M. Selhozizdat. 168 p.

Mashkin, S.I. 1971. Dendrology of the central Chernozem Region. V. 1. Voronezh. VGU, 344 pp.

Matveeva, R.N, Butorova, O.F. and Romanova, A.B. 2000. Introduction of plants to SibGTU Arboretum, Krasnoyarsk. SibGTU. 194 pp.

Maurin, A.M. 1970. Experiments with woody plant introductions to Latvian SSR. Riga. Zinatis, 259 pp.

McDonald, G.I. and Hoff, R.J. 2001. Blister rust: an introduced plague. In: Whitebark Pine Communites. Ecology and Restoration. Ed. by D.F. Tomback, S.F. Arno and R.E. Veak. Island Press. Washington-Covelo-London: 193-220.

Millar, C.I. and Kinloch, B.B. 1991. Taxonomy, phylogeny and coevolution of pines and their stem rusts. In: Y. Hiratsuka, J.K. Samoil, P.V. Blenis, P.E. Crane, and B.L. Laishley ed. Rusts of Pine. Proc. 3rd IUFRO Rusts of Pine Working Party Conference. Sept. 18-22, 1989 Banff, Alberta. For. Can, Northwest Reg., North. For. Cent., Edmonton, Alberta. Inf Rep. NOR-X-317: 1-38.

Minkevich, I.I. 1986. Outbreaks of fungal diseases of woody trees. Leningrad. Publishing House LGU. 115 pp.

Nekrasov, V.I. 1980. Vital problems of the development of acclimatization theory. M. Nauka. 102 p.

Nekrasov, V.I. and Tvelenev, M.V. 1970. On introduction of Pinus sibirica in the European part of the USSR // Bulletin of the Main Botanical Garden M. Issue 75: 25-27.

Nesterov, N.S. 1935. Experimental forests in Petrovsko-Razumovski near Moscow. M.-L. Ogiz-Selhozgiz. 560 pp.

Osipova, L.S. 1968. Ulcerous cankers of conifers in the Irkutsk Region. Scientific treatise LTA.Leningrad. I. 115. Issue I: 171-179.

Pallas, P.S. 1786. Description of the plants of Russian state with their pictures. Sankt-Peterburg. V. 1. 204 p.

Pankov, Ya.V., Sushkov, M.M. and Sushkov, L.M. 2000. State, productivity and natural regeneration of the stands in the Shatilovski Forest. In: Regeneration of forests, resources and energy-saving technology of a forest complex. Proc. of Scientific and Practical Conference, Devoted to 70-year Celebration of VGLTA (Voronezh, 27-29, Sept. 2000). Voronezh: 226-231.

Patlaj, I.N., Molotkov, P.I., Gajda, Yu. I. and others 1994. Permanent forest-seed stability of the main forest forming species in the Ukraine based on breeding and genetics. Lesovodstvo and Lesorazvedenie: Review Information. M. VNIITslesresurs. Issie I: 1-32.

Pismenni, N.R. 1967. Peculiarities of spreading *Pinus strobus* rust. Lesnoi Zhurnal. 3: 20-22.

Plotnikova, L.S. 1988. Scientific bases of introduction and protection of woody plants. In: Introduction and Protection of Woody Plants of the USSR Flora. M. Nauka. 263 p.

Pogrebnjak, P.S. 1938. Exotic trees in mixed stands. V Zaschitu Lesa. 6: 10-15.

Potapova, S.A. 1984. Soil conditions as one factor when introducing pine species. Bulletin GBS AN USSR. M. Issue 131: 39-43.

Potapova, S.A. and Potapova, A.D. 1984. *Pinus cembra* in natural and planted stands within the USSR Lesovedenie. 1: 64-67.

Pravdin, L.F. and Iroshnikov, A.I. 1982. Genetics of *Pinus sibirica* Du Tour, *P. koraiensis* Sieb. *et* Zucc. and *P. pumila* Regel. Ann. Forestales. 9(3): 79-123.

Redko, G.I. and Fedorov, E.A. 1982. Artificial regeneration of introduced forest species from North American origin. A Lecture. Leningrag LTA, 52 pp.

Rubanik, V.G. 1974. Introduction of gymnosperms in Kazakhstan. Alma-Ata. Nauka, 271 pp.

Rubanik, V.G. and Zeronkina, T.A. 1980. Introduction of trees and bushes from Europe into Kazakhstan. Alma-Ata. Nauka. 192 pp.

Rudzski, A.F. 1874. What is the origin, evolution and importance in forestry of the pine disease called 'jarinnik' or 'vertnik'in the Vladimir region? In: Collected Papers on the Problems of Suggestions for Discussion by the Members of Congress II of Forest Owners in Lipetsk (August, 16-26, 1874). SPb. Issue of Lesnoe Obschestvo: 51-54.

Samoilova, T.V. 1972. *Pinus strobus*_in Primorski Territory. In: Komarovskie chtenia. Issue 19: 5-14.

Semechkin, I. V., Polikarpov, N.P., and Iroshnikov, A.I. 1985. Stone pines of Siberia. Novosibirsk: Nauka: 257 pp.

Semechkin, I.V. and Semechkina , M.G. 1964. Results of seeding *Pinus pumila* in the southern part of the northern Ural Mountains. In: Nature and Forest Vegetation of the Northern Part of Sverdlovsk Region. Proc. of Commissions on Nature Control UF AN USSR. Sverdlovsk. Issue I: 171-181.

Sevalnev, V.M. 1966. Artificially regenerated *Pinus strobus* stands in the Rilski Forestry Enterprise. Lesnoe Hozjaistvo. 2: 66-67.

Shin, T.-S. 1961. Some peculiarities of *Pinus strobus* growth in forest-steppe conditions. Author's Abstract. Kiev. 23 pp.

Shirnina, L.V. and Beloborodov, V.M. 1999. Evaluation of disease in seed and clonal progeny of *Pinus strobus*. In: Genetic and Breeding Bases of Forest Improving. Voronezh. NIILGiS: 260-274.

Shkutko, N.V. 1970. Exotic coniferous species in Byelorussia and their economic importance. Minsk, Nauka I Technika, 272 pp.

Sirotkin, Yu. D. and Gvozdev, V.K. 1987. *Pinus strobus* in forest stands of the USSR. Lesovedenie I Lesnoe Hozjaistvo: Republican Inter-department Collected book N22: 38-42.

Smagljuk, K.K. 1969. *Pinus cembra* L. in the Ukrainian Carpathians. Lesovedenie. 1: 3-15.

Smirnova, M.Yu. 1997. Coniferous exotic plants as experimental forestry crops. Lesnoi Zhurnal. 1-2: 48-53.

Sobichevski, V.T. 1875. Modern state of plant pathology of forest trees and the importance of plant parasitic fungi in developing forests. Forest Journal Issue V: 1-33. Issue VI: 58-93.

Soltani, G.A. 2001. Addressing the problem of use of exotic species in forest stands of the northern Caucasus. In: Forest of EuroAsia in the Third Millenium: Proc. of International Conference of Young Scientists (June, 26-29, 2001). M. MGUL. V.1: 104-105.

Sukachev, V.N. 1922. Next tasks of Russian dendrology // Proc. of All-Russian Forest Conference. 10-17 November 1926 in Moscow. Issue 1: 46-58.

Tikhomirov, B.A. 1949. *Pinus pumila*: Biology and Use. M. Publishing House MOIP. Issue 6(XIV). 106 pp.

Timofeev, V.P. 1965. Natural and artificial stands in the experimental forest of TSHA for over 100 years. Moscow. Lesnaya Promishlennost. 168 pp.

Titov, E.V. 1999. Stone pines breeding. Educational Supply. Voronezh. VGLTA, 58 p.

Tovkach, L.N. 1968. Primary pests and diseases of stone pine reproduction in the Tofalarsk Division of Forestry, Nizhneudinsk Forestry Enterpise, Irkuts Region. In: Proceeding LTA. L. N115. Issue 1: 120-125.

Usachev, A.I. 1983. *Pinus strobus* L. in artificially regenerated stands in the European part of the USSR. In: Forest Introduction. Proc. of TsNIILGiS. Voronezh: 24-31.

Usmanov, D.D. and Korolkova, A.V. 1997. Study of Korean pine in the natural areas of Russian Mixed Forests. In: Forest Use and Reproduction of Forest Resourses: Proc. MGUL. M. Issue 286: 87-95.

Vekhov, N.K. and Vekhov, V.N. 1962. Coniferous species of Forest-Steppe Station. Moscow. Publishing House of the Ministry of Municipal Economy RSFSR, 149 pp.

Yablokov, A.S. 1962. Breeding of maternal stands and trees of stone pines in the forests of Siberia and the Far East Territory. V.: MLTI. 12 p.

Yablokov, A.S. and Dokuchaeva, M.I. 1976. The Ivanteevski Arboretum of VNIILM. (Catalogue). Moscow, 88 pp.

Genetic Resources, Tree Improvement and Gene Conservation of Five-Needle Pines in East Asia

Huoran Wang
Jusheng Hong

Abstract—East Asia is very rich in the genetic resources of five-needle pines, including 11 species and three varieties. Of these taxa, *Pinus armandii* is the most widely distributed, ranging from Taiwan and Korea to central and western China. The natural range of *P. koraiensis* includes northwestern China, North and South Korea, Japan, inland Siberia and the Russian Far East. Because they are the most commercially important pines for wood and nut production, most genetic improvement research has been carried out with these two species. *Pinus fenzeliana* and *P. dabeshanensis* are used in plantations in some areas. However, along with the other species of five-needle pines, they are mainly of importance in studies of taxonomy and ecophytogeography. In addition to a review of genetic resources, this review paper also gives a brief overview of tree improvement and disease and insect pests of the five-needle pines in east Asia.

Key words: East Asian pines, five-needle pines, gene resources, tree improvement, tree pathogens, tree insects

Introduction

This paper attempts to give a brief review of the status of genetic resources, tree improvement and breeding programs and gene conservation of five-needle pines in east Asia, including China, Japan, the Democratic People's Republic of Korea (North Korea), Republic of Korea (South Korea), inland Siberia and the Russian Far East region.

The genus *Pinus* L. is generally divided into two subgenera, Subgenus *Strobus* (Haploxylon pines), and Subgenus *Pinus* (Diploxylon pines), commonly known as soft pines and hard pines respectively. Of the soft or five-needle pines native to east Asia, *P. armandii* Franch. and *P. koraiensis* Sieb. & Zucc. are as important for timber production as such hard pines as *P. massoniana* Lamb. and *P. yunnanensis* Franch. in China and *P. densiflora* Sieb.& Zucc. in Japan. The rest of the five-needle pines are of more limited economic importance due to their restricted gene resources. In this paper, most emphasis is placed on the research in the genetic resources, tree improvement and gene conservation of the two most important species, *P. armandii* and *P. koraiensis.* In fact, very little information other than that on taxonomy and ecology is available for other species.

Genetic Resources

Of 24 species of *Pinus* in East Asia, 11 including three varieties of *P. armandii* are five-needle pines (Wu 1956; Mirov 1967; AASE 1978; Zheng 1983, Price and others 1998). They are listed here in conformity with the system standardized for all papers presented at this conference (Price and others 1998) and so listed in the conference program, which differs in a few cases from the taxonomic designations used in China. There is no universal agreement on the taxonomy of east Asian five-needle pines. In Chinese literature, for example, *kwangtungensis* is a separate species morphologically related to *P. wangii.* The east Asian list follows, with more detail in table 1:

P. armandii Franchet var. *armandii,* widely distributed and planted in China;

P. armandii Franch. var. *mastersiana* (Hayata) Hayata, in Taiwan of China;

P. armandii Franch. var. *amamiana* (Koidzumi) Hatusima, includes isolated populations in Japan;

P. dabeshanensis Cheng & Law, very restricted in a small area in central south China;

P. dalatensis de Ferré, central Vietnam;

P. fenzeliana Handel-Mazzetti, (including *P. kwangtungensis* Chun & Tsiang) southern China to northern Vietnam;

P. wallichiana Jackson (*P. griffithii* McClellan), Himalayan chains;

P. koraiensis Siebold & Zuccarini, China, Japan, Korea, Siberia and Russian Far East;

P. morrisonicola Hayata, central Taiwan;

P. parviflora Siebold & Zuccarini, native to Japan and exotic in China and Korea;

P. pumila (Pallas) Regel, China, Japan, Korea and Russian Far East;

P. sibirica Du Tour, northwestern China, Siberia and Russian Far East;

P. wangii Hu & Cheng, scattered and limited populations in southwestern China.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are Research Professors at the Chinese Academy of Forestry, Beijing,100091, China. E-mail: wanghr@rif.forestry.ac.cn

Table 1—Classification and phytogeography of five-needle pines in east Asia.

Species	Natural distribution	Characterization
Pinus armandii var. *armandii* Franch. (Armand pine, Huashan Mountain pine)	China in Shanxi, Henan, Shaanxi, Gansu, Sichuan, Hubei, Guizhou and Yunnan provinces and Tibet; elevation range 1000-3300 m.	Tree up to 35 m high and 1 m dbh. Widely planted for forestry and landscape. Flowers April- May; cones mature September-October of the following year; cone size 10-20 cm x 5-8 cm; seed 1.0-1.5 cm long,edible; 3 medial resin canals; wood density 0.43-0.48.
P. armandii var. *mastersiana* (Hayata) Hayata (Taiwan Armand pine)	Central Taiwan at 1800-2800 m elevation.	Tree up to 20 m high and 100 cm dbh; leaves 15 cm. Mature cones peduncled, ovoid, up to 10-20 cm long and 8 cm in diameter. Seeds ovoid, compressed, wingless, with a sharp edge all around, 8-12 mm long. Wood density 0.46.
P. armandii Franch. var. *amamiana* (Koidz.) Hatusima (Japanese Armand pine)	On two isolated islands, Tanegashima and Yakushima, Japan. Ecologically and genetically isolated populations, vulnerable.	Tree up to 25 m height, 1 m dbh; dark gray shoots; leaves 5-8 cm long; cones short stalked, oblong-ovoid, 5-8 cm long; seeds wingless, about 12 mm long.
P. dabeshanenesis Cheng & Law (Dabeshan Mountain white pine)	Anhui and Hubei provinces of China at elevations between 900 and 1400 m; range very restricted.	Tree over 20 m, dbh 50 cm. Wood similar to that of *P. armandii*, 2 external resin canals. Wood density 0.43.
P. dalatensis de Ferré Dalat or Vietnamese white pine	Very restricted range in evergreen subtropical forests of Vietnam at elevations of over 1500 m. Often in mixed stands, very sparsely distrubuted; species surtvival threatened.	Tree to 15-25 m, 60-100+ cm dbh, crown conical, somewhat open. Female cone has 20-30 scales, yellowish-brown maturing to dark gray. Cones mature October-December. No data on resin canals or wood density.
P. fenzeliana Hand.-Mzt. (Hainan white pine)	South China in Hainan, Guangxi, and Guizhou provinces; central Vietnam; elevation range 1000-1600 m.	Tree to 50 m in height, 2 m in dbh; leaves 10-28 cm long. Seed cones ovoid-ellipsoid, 6-9 cm long; seeds chestnut brown with a wing 2-4 mm long. No data on resin canals .Wood density 0.55-0.59.
P. koraiensis Sieb. & Zucc. (Korean pine)	Northeast China, also in Korea, Japan and Russian Far East, elevation range 150-1800 m. Dominant species in mixed stands with broadleaved trees, pure stands can be found.	Tree to to 50 m and over 1 m dbh; flowering in June; cones mature September-October in the following year, seed edible; 3 medial resin canals; wood density 0.38-0.46; major timber species in northeast forest region of China.
P. kwangtungensis Chun & Tsiang (South China white pine). In China considered a separate species; in this conference, a part of *P. fenzeliana* (Price *et al.* 1998).	China, geographically disjunct from *P. fenzeliana* proper; found in southern Hunan, northern Guangxi and Guangdong provinces, with outliers in Guizhou and Hainan provinces. Elevation range 700-1600 m. Forms pure stands or mixed stands with *Tsuga*, *Fagus, Quercus.*	Tree to 30 m high and 1.5 m dbh; flowers April-May, cones mature in October of the following year; seed 8-12 mm long. Planted trees grow fastest 10-30 years after planting, reaching 25 m in height and 45 cm dbh at about age 60; 2-3 resin canals; wood density 0.50.
P. morrisonicola Hayata (Taiwan white pine)	Central mountains in Taiwan, no pure stands; mixed with other conifers or hardwoods	Tree up to 30 m tall and 1.2 m in dbh.; 2 dorsal resin canals.
P. parviflora Sieb. & Zucc. (Japanese white pine)	Naturally distributed in Japan and introduced to China.	A tree up to 25 m tall and 1 m in dbh, 2 external resin canals. Ornamental tree, often grafted as bonsai
P. pumila (Pall.) Regel (Japanese stone pine)	Northeastern China with altitudes ranged 1 000-1 800m and extended in Russia, Japan and Korea; forms dense and low community on top of mountains and exposure sites.	Shrub or small tree 2-8 m, often multi-stemmed; 2 dorsal resin canals. Good for ground cover and ornamental
P. sibirica Du Tour (Siberian stone pine)	Northwestern Altai in Xinjiang and the Great Xingan Range in China and extended to Sibirica, ranged 66° 25'-46° 40'N in latitude and 49° 40'-127° 20'E in longitude, altitudinaly 1,600-2,350 m; dominated in the stands mixed with *Larix sibirica*	A tall tree, up to 35 m and over 1.8 m in dbh; 3 medial resin canals; flowering in May and cones mature in September-October in the following year. Wood density 0.45. Timber is as good as that of *P. koraiensis*, an important forest tree in Siberia, Russia.

(con.)

Table 1—(Con.)

Species	Natural distribution	Characterization
P. wallichiana Jacks. (Himalayan white pine)	Southern Tibet and northwestern Yunnan in China, Bhutan, Burma, Nepal, India, Pakistan and Afghanistan. at 1600-3300 m elevation.	Tree to 50+ m with straight trunk and short, down-curved branches. Branches longer in solitary trees, creating a dome-like crown. Leaves 15-20 cm, usually pendant but in some trees spreading. Cones 20-30 cm, bluish-green when young, maturing to light brown.
P. wangii Hu & Cheng (Yunnan white pine)	Restricted area in southeastern Yunnan Province in China altitude 1100-2 000 m. Occurs in or mixed stands with oaks on limestone slopes. Endangered status.	Tree to 20 m high and 60 cm dbh; 3 medial resin canals.

From an ecological standpoint, five-needle pines are, in contrast to hard pines, mostly adapted to cold or temperate and moist environments. There exists a latitudinal gradient and a trend of species replacement from north to south within their natural occurrences in East Asia (Wu 1956; Wu 1980; Kuan 1982; Zhao, G. 1991; Ma 1992). In the north, *P. sibirica*, *P. pumila* and *P. koraiensis* occur at relatively lower elevations; southward in the temperate and subtropical zones, they are replaced by *P. armandii;* still farther south, *P. fenzeliana* and *P. dalatensis* occur discontinuously at higher elevations in subtropical and tropical areas. *P. dabeshanensis* and *P. wangii* occur as relicts on difficult sites for tree growth and survival in central south and southwestern China respectively as restricted populations or scattered individuals.

From a geographical standpoint, all the five-needle pines in east Asia are discontinuously distributed. *P. sibirica* (Zhao, G. 1991) and *P. armandii* (Ma 1989,1992) are typical examples. *P. armandii* is extensively distributed on the mainland of China from temperate to subtropical regions, with its two varieties, *P. armandii* var. *mastersiana* extending to Taiwan Island and *P. armandii* var. *amamiana* appearing in Japan (Nakashima and Kanazashi 2000). The populations of *P. fenzeliana* and its form recognized in China as *P. kwangtungensis* Chun & Tsiang are also geographically isolated from each other. The ecological and geographic patterns have introduced great genetic variability into these five-needle pines, making some of them very difficult to classify taxonomically. For instance, *P. koraiensis* and *P. sibirica* were confused with each other in taxonomic status for long time (Zhao 1991).

Tree Breeding and Improvement

Tree improvement in East Asian countries is mainly focused on commercially important species. Five-needle pine research activities are mostly undertaken with *P. koraiensis* and *P. armandii,* although *P. fenzeliana* and *P. dabeshanensis* have been used locally in planting programs.

Pinus koraiensis

Pinus koraiensis is by far the most important species in conifer tree improvement programs in northeast China, North Korea and South Korea. Kim and others (1994a) estimated that more than 250,000 ha of plantations had been established with this species in South Korea by 1994. In North Korea 305,000 ha were established with this species for wood and nut production. For nut production, 250 clones were selected. It is estimated that the plantation area of Korean pine in North Korea is increasing at a rate of about 30,000 ha per year.

In China, tree improvement and breeding research programs were launched in the early 1980s with emphasis on provenance trials, plus tree selection and gene conservation. A provenance trial was established in 1986 with 12 seedlots collected from natural stands throughout the range and one seedlot from a plantation. Tenth-year results showed that there were significant differences in growth rates between provenances, and that the seedlots collected from areas around Changbaishan Mountain performed best (Zhang and Wang 2000).

Wang and others (2000a) reported tenth-year results of progeny tests established on three sites using open-pollinated seeds of 557 parents from natural stands. Significant differences were found in growth performance between individual families and between provenance zones, and there were also large genotype x environment (GxE) interactions. Based on the progeny testing, a genetically improved seed orchard was established by grafting (Wang and others 2000b). Observations indicated that the average interval between every two good seed crops was five years. Strobilus abortion rate reached 46.5 percent in the seed orchard, but cone yield could be increased by 20 percent through crown pruning and controlled pollination (Wang and others 1992).

Although seed orchard clones varied in fertility level, leading to an increased level of relatedness in the offspring, genetic diversity in seed orchard progenies was nevertheless only slightly depressed compared with that of the reference populations from which the plus trees were selected (Kang and Lindgren 1998).

Variation in effective number of clones in the seed orchards of *P. koraiensis* was examined. The mean number of clones averaged about 70 in each orchard, but the average effective number (N_c) was 43 (Kang and others 2001).

Pinus armandii

P. armandii is the most widely and discontinuously distributed of the five-needle pines in China, occurring in 12 provinces ranging in latitude from 23°30' to 36°30' N, in longitude from 85° 30' to 113° 00' E and in altitude from 1,000 to 3,500 m, which suggests that a large amount of geographically-related genetic variation may exist within the species.

Range-wide provenance trials of *P. armandii* were established on 9 experimental sites in 1980. The trials were coordinated by the Research Institute of Forestry of the Chinese Academy of Forestry, using seed collected from 30 provenances. Because the seedlings of southern seedlots were all killed by frost at northern experimental sites, successive trials were established on 12 sites in the following year using all northern seedlots. The provenance trial results indicated that not only did GxE interactions exist but also that the differences in morphological characteristics and growth rates among provenances were so significant that two provenance zones, southern and northern, could be clearly distinguished (Ma and others 1992a,b; Cooperative 1992). Moreover, Ma (1992) was of the opinion that these two population groups should be considered for recognition as two varieties, namely, *P. armandii* var. *armandii* and *P. armandii* var. *yunnanensis*. Ma noted that many plantations of *P. armandii* have failed, especially in central China in the 1960s and 1970s, due to the wrong provenances being used in afforestation programs. He cautioned that great attention must be paid in plantation forestry to provenance selection, and *P. armandii* should not be grown for commercial purposes north of 40°N and beyond its natural altitudinal limits in central subtropical China.

To establish clonal seed orchards by grafting, 850 superior trees were selected in the southern provenance zone, covering Yunnan, Sichuan and Guizhou provinces. Research in reproductive biology showed that *P. armandii* starts flowering at 5-7 years of age, but over 70 percent of female strobili abort (Wu 1992; Zhang and others 1992). Generally, seed yields of *P. armandii* are very low, averaging 15kg/ha for the 133 ha of seed orchards in the whole of China. In addition to much rain and wind during flowering in May, genetic variation in reproductive ability between clones has been recorded (Liao and others 1998).

Genetic Diversity and Gene Conservation

Genetic diversity has been studied in recent years using analysis of isoenzyme gene markers. Kim and Lee (1995) found that the overall mean proportion of polymorphic loci was 66.7 percent in *P. koraiensis* compared with 86.2 percent in *P. densiflora* , the most widely distributed native pine in South Korea. They also found that although many populations of *P. koraiensis* were small in size, distributed at high elevations and composed of closely related individuals, gene flow between these isolated populations still remained high in this species. To study genetic variation, eight populations of *P. koraiensis* were sampled within its range in South Korea. Research results suggested that seven of the eight populations should be included in gene conservation programs (Kim and others 1994b; Kim and Lee 1998).

Politov and others (1999) reported genetic evidence of natural hybridization and possible gene exchange in Siberia between *P. sibirica* and *P. pumila*.

In northeastern China, where the natural forest resources were over-exploited in the last several decades, the establishment of natural reserves with total area of 56,000 has protected the remaining forest of P. koraiensis ha. In addition, 88 natural stands totaling over 40,000 ha have been identified and conserved *in situ* as gene resources for sustainable forest management (Li 1997). However, due to the increasing number of plantations of *P. koraiensis* being established, necessitating the proper seed sources, more than 30 ha of seed stands in the natural forest were documented for seed supply on several locations, with about 1,000 individuals selected for *ex situ* gene conservation (Niu and others 1992).

In South Korea, three outstanding stands of *P. koraiensis* and one of *P. pumila*, with areas of 55 ha and 2 ha respectively, have been identified and reserved for *in situ* gene conservation. In the last 30 years, 91 ha of Korean pine seed orchards have been established, the seed orchards also serving as *ex situ* gene conservation (Lee 1997).

Diseases and Insect Pests

A survey of the literature indicates that little breeding for resistance to diseases and insect pests has been done on any species of five-needle pine in east Asia. However, major pathogen and insect pests attacking *P. koraiensis* and *P. armandii* have been investigated.

Histopathological research has shown that the stem rust disease of *P. koraiensis* is caused by *Cronartium ribicola* J.C. Fischer in Raben. (Xue and others 1995). Occurrence of *C. ribicola* attack on *P. koraiensis* depends on the coexistence of *Pedicularis* sp. (lousewort), the alternate host of the pathogen (Jia and others 2000).

A dieback fungus isolated from *P. koraiensis* was identified as *Cenangium abietis* (Pers.) Duby (*Cenangium ferruginosum* Fr.). When it was inoculated on five-year-old seedlings of *P. koraiensis*, 80 percent of the seedlings were infected with the same symptoms and signs as those of naturally infected trees (Lee and others 1998)

P. armandii in the Qinling Range and Dabashan Mountains have been attacked since 1956 by D*endroctonus armandii* Tsai & Li and also by 20 other species of beetles, causing mortality in many trees over 30 years old in the natural forest (Tang and Chen 1999). *Ophiostoma* sp. and L*eptographium* sp., which are fungi symbiotic with the insects, attack the host prior to the beetle invasion.

Chen and others (1999) studied the ecology of the insect pests in natural stands of *P. armandii* at middle elevations (1,600-2,000 m) in the Qinling Range. In this study, 19 species of beetles were listed as follows:

Dendroctonus armandii Tsai & Li
Xyloterus lineatum Olivier
Hylurgopus longipilis Reitter
Polygraphus sinensis Eggers
Pityogenes sp.
Ips acuminatus Gyllenhal
Ips sexdentatus Borner
Tomicus piniperda Linnaeus
Cryphalus lipingensis Tsai & Li
Cryphalus chinlingensis Tsai & Li
Cryphalus pseudochinlingensis Tsai & Li

Hylastes paralellus Chapuis
Hylastes techangenesis Tsai & Huang
Hylurgopus major Eggers
Polygraphus rudis Eggers
Polygraphus verrucifrons Tsai & Yin
Dryocoetes luteus Blandford
Dryocoetes uniseriatus Eggers
Dryocoetes autographus Ratzeburg

Of these listed species, the first 11 coexist, since they possess different spatial and temporal niches in the stand, enabling an equilibrium between living space and nutrient availability among these insects.

Cone damage in seed orchards of *P. koraiensis* caused by *Dioryctria mendacella* Staudinger and *Rhyacionia pinicolana* Doubleday was reported by Liao and others (1998).

Another type of injury to *P. koraiensis* is terminal shoot attack leading to forking of the main stem (Zhao and others 1999). *Pissodes nitidus* Roelofs and *Diorictria splendidella* H.S. are the main agents responsible for the damage.

Blister rust usually occurs in association with *Pineus armandicola* Zhang on *P. armandii,* necessitating control of both the pathogen and the insect (Li and others 2000). Other diseases found by Liao *et al* .(1998) in a study of seed orchards include *Pestalotia funereal* Desm., *Hypoderma desmazieri* Duby, *Coleosporium solidaginies* (Schw.) Thum., *Capnodium* sp. and *Lophodermium pinastri* (Schrad. *ex* Fr.)

Conclusions

P. koraiensis and *P. armandii* are important forest species for wood and nut production. Traditional breeding programs for advanced generations should be maintained and strengthened by using molecular genetic markers, especially for *P. armandii,* which has such a wide geographic range. No resistance breeding research is yet under way for any species of five-needle pine in east Asia.

Currently genetic structure and genetic diversity in these five-needle pine species in China is not well understood. Therefore priority in research programs should be given to studies of genetic variation, a critical prerequisite for long-term breeding and gene conservation of the two most important species. There is a trend, however, to use more Japanese larch, [*Larix kaempferi* (Lamb.) Carr.], to establish plantations in China, since Japanese larch grows much faster in early years than *P. armandii* growing under similar ecological conditions.

Biochemical and molecular genetic markers have proven to be a powerful tool in biosystematic and biogeographic studies of forest trees (Adams 1992; Strauss and others 1992). Molecular biology can also help with understanding of the scattered patterns of occurrence of the less-known five-needle pines, providing information on evolutionary history and intraspecific variation, fields of investigation not yet undertaken in China. Without this information, conservation strategy cannot be established on a sound scientific basis.

In China, the remaining genetic resources of *P. koraiensis* are mostly protected in nature reserves, whereas the outstanding seed stands of *P. armandii* identified in earlier provenance trials have not yet been integrated into forest management activities. Gene conservation is a crucial component of sustainable forest management (Eriksson and others 1993; Palmberg-Lerch 1999). Therefore, a strategy for gene conservation and utilization of five-needle pines should be developed and integrated into a regional action plan for the conservation of the forest genetic resources in east Asia, as recommended by the FAO and other concerned organizations (Palmberg-Lerch 2000; Sigaud and others 2000).

Acknowledgments

The senior author wishes to sincerely thank Professor Scott Schlarbaum of the University of Tennessee, Coordinator of five-needle Pines Working Party, IUFRO, for his kind invitation and Dr. Safiya Samman of Forest Service, USDA, for making all the arrangements for his attention to the conference, and also thanks the USDA/FS for their generous support. The authors also thank Professor Zhao Guangyi, Northeast Forestry University, and Professor Ma Changgeng, Chinese Academy of Forestry, for useful discussions during the preparation of this paper.

References

Adams, W.T. 1992. Gene dispersal within forest tree populations. In: Population Genetics of Forest Trees, *Edited* by W. T. Adams, S. H. Strauss, D. L. Copes and A. R. Griffin. Kluwer Academic Publishers, pp. 217-240.

Agendae Academiae Sinicae Etida (AASE). 1978. Flora, Republicae Popularis Sinicae. Tomus 7. China Science Press, Beijing. 211-236.

Chen, H.; Tang, M.; Ye, H.; Yuan, F.; 1999. Niche of bark beetles within *Pinus armandii* ecosystem in inner Qinling Mountains. Scientia Silvae Sinicae, vol. 35(4), 40-44.

Cooperative research group of *Pinus armandii*. 1992. Studies on selection of suitable seed sources for reforestation of *Pinus armandii*. In: Ma Changgeng, ed. Symposium on selection of optimum seed sources for plantations of *Pinus armandii* Franch. Beijing Agricultural University Press. 24-35.

Eriksson, G.; Namkoong, G.; Roberds, J.H. 1993. Dynamic gene conservation for uncertain futures. Forest Ecology and Management, 62:15-37.

Jia, Y.; Chen, Z.; Zhang, L.; Yu, J.; Wang, J.; Hao, Y.; Zhou, Y. 2000. The survey and study on *Cronartium ribicola* to Korean pine plantations. Journal of Northeast Forestry University, vol. 28(3), 43-47.

Kang, K. and Lindgren, D. 1998. Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora, P. thunbergii* and *P. koraiensis* clonal seed orchards. Silvae Genetica, 47(4), 196-201.

Kang, K.; Harju, A.M.; Lindgren, D.; Nikkanen, T.; Almqvist, C.; Suh, G.U. 2001. Variation in effective number of clones in seed orchards. New Forests, 21: 17-33.

Kim, Z.; Lee, S.. 1995. Genetic diversity of three native *Pinus* species in Korea. In: Ph. Baroda, W.T. Adams & G. Muller-Stack (eds.), Population genetics and genetic conservation of forest trees. Academic Publishing, The Netherlands. 211-218.

Kim, Z. and Lee, S. 1998. Genetic diversity in East-Asian *Pinus* species. Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. 207-219.

Kim, Z.; Yi, C.; Lee, S. 1994b.Genetic variation and sampling strategy for conservation in *Pinus* species. In: Z.S. Kim and H.H. Hatemmer (eds.) Conservation and Manipulation of Genetic Resources in Forestry. Kwang Moon Kong, Seoul. 294-321.

Kim, Z.; Lee, S.; Lim, J., Hwang, J.; Kwon, K.. 1994a. Genetic diversity and structure of natural populations of *Pinus koraiensis* Sieb. et Zucc. in Korea. Forest Genetics, 1(1): 41-49.

Kuan, C. 1982. The geography of conifers in Sichuan. Sichuan People's Publishing. 106 pp.

Lee, S.; Joo, H.; Lee, J. 1998. Cultural characteristics and pathogenicity test of a dieback fungus, *Cenangium ferruginosum* isolated from *Pinus koraiensis*. Journal of Korean Forestry Society, 87(4), 557-561.

Lee, S. 1997. Forest genetic resources and conservation in the Republic of Korea. Diversity, Vol.13 (1). 277-287.

Li, J. 1997. Ecology and Management of the Mixed Korean Red Pine Forests in NE China. Northeast Forestry University Press. 312 pp.

Li, Y.; Xie, K.; Cao. K.; Gan, Y. 2000. A study on the compound control threshold of *Pinus armandii* blister rust and *Pineus armandicola* . Scientia Silvae Sinicae, vol. 36 (6), 77-81.

Liao, M.; Jin, T.; Wu, X.; Zhou, Y. 1998. Analysis of low seed production of *Pinus armandii* Franch. Guizhou Forestry Science and Technology, vol. 26(3), 36-41.

Ma, C. 1989. Geographic variation in *Pinus armandii* Franch. Silvae Genetica 38:81-90.

Ma, C. 1992. Natural distribution and historical researches of *Pinus armandii*. In: Ma Changgeng, ed. Symposium on selection of optimum seed sources for plantations of *Pinus armandii* Franch. Beijing Agricultural University Press 1-12.

Ma, C.; Hu, X.; Zhang, Y. 1992a. Geographic variation in *Pinus armandii* Franch. In: Ma Changgeng, ed. Symposium on selection of optimum seed sources for plantations of *Pinus armandii* Franch. Beijing Agricultural University Press 13-23.

Ma, C.; Zhang, Y.; Xiao S.; Peng S. 1992b. Geographic grouping and seed zone division of *Pinus armandii* Franch. In: Ma Changgeng, ed. Symposium on selection of optimum seed sources for plantations of *Pinus armandii* Franch. Beijing Agricultural University Press. 37-45.

Mirov, N.T. 1967. The genus *Pinus*. The Ronald Press Company. New York. 602 pp.

Nakashima, K.; Kanazashi, A. 2000. Inbreeding owing to isolation restricts regeneration in vulnerable species growing on isolated small islands in Japan. In: Krishnapillay B. ed. Forests and society: the role of research. XXI IUFRO World Congress, 7-12 August 2000 Kuala Lumpur, Malaysia. Abstracts of Group Discussions, vol. 2, p. 42.

Niu, Y.; Shu, F.; Ning, Y. 1992. Korean red pine. In: Shen Xihuan, ed. Seed Orchard Techniques. Beijing Science & Technology Publishing House. 66-71.

Palmberg-Lerch, C. 1999. Conservation and management of forest genetic resources. Journal of Tropical Forestry Research, 11(1): 286-302.

Palmberg-Lerch, C. 2000. International action in the management of forest genetic resources: status and challenges. Forest Genetic Resources Working Papers FGR/1, FAO, Rome.

Peng, Z.; Jiang, Z. 1999. *Pinus dabeshanensis* and its origin. China Forestry Publishing House. Beijing. 86 pp.

Politov, D.V.; Belokon, M.M.; Maluchenko, O.P.; Belokon, Y.S.; Malozzhinikov, V.N.; Mejnartowicz, L.E.; Krutovskii, K.V. 1999. Genetic evidence of natural hybridization between Siberian stone pine, *Pinus sibirica* du Tour, and dwarf Siberian pine, *P. pumila* (Pall.) Regel. Forest Genetics 6(1): 41-48.

Price, R.A., Liston, A. and Strauss, S.H. 1998. Phylogeny and systematics of *Pinus*. In: Richardson, D.M. (ed.). Ecology and Biogeoghraphy of *Pinus*. Cambridge University Press, pp.49-68.

Sigaud, P.; Palmberg-Lerch, C.; Hald, S. 2000. International action in the management of forest genetic resources: status and challenges. In: Forests and Society: the Role of Research. XXI IUFRO World Congress, 7-12 Aug. 2000. Kuala Lumpur, Malaysia. Ed. by B. Krishnapillay. Vol.1. Sub-Plenary sessions, 91-99.

Strauss, S.H., Bousquet, J., Hipkins,V.D. and Hong Y.P. 1992. Biochemical and molecular genetic markers in biosystematic studies of forest trees. In: W. T. Adams, S. h. Strauss, D. L. Copes and A. R. Griffin ed. Population Genetics of Forest Trees. Kluwer Academic Publishers. 125-158.

Tang, M.; Chen, H. 1999. Effect of symbiotic fungi of *Dendroctonus armandii* on host trees. Scientia Silvae Sinicae, vol. 35 (6), 63-66.

Wang, G.; Ning, Y.; Jin, J.; Zhang, S.; Yi, H. 2000a. Study on the establishment technique of seed orchard for Korean pine improved generations. Journal of Northeast Forestry University. 28(3), 68-69.

Wang, X.; Zhang, A.; Wang, W.; Wu, P. 1992. A Study of increasing cone yield of clonal seed orchard of *Pinus koraiensis*. In: Shen Xihuan, ed. Seed Orchard Techniques. Beijing Science & Technology Publishing House. 208-213.

Wang, G.; Zhang, S.; Ning, Y., Zhang, S and Yi, H. 2000b. Synthetic analysis on multi-site determination of filial generation for Korean pine elite tree. Journal of Northeast Forestry University. 28(3), 51-53.

Wu, C.1956. Taxonomy and distribution of the genus *Pinus* L. in China. Journal of Plant Taxonomy 5(3):131-163.

Wu X. 1992. *Pinus armandii*. In: Shen X., ed. Seed Orchard Techniques. Beijing Science & Technology Publishing House, pp 32-36.

Wu, Z. 1980. The Vegetation of China. China Science Press, Beijing. 211-249.

Xue, H.; Shao, L.; Jin, G.; Cui, Y. 1995. Study on the histopathology of three pine stem rust diseases. Journal of Northeast Forestry University, 23:(6), 1-7.

Zhang, R.; Hu, X.; Wu, X.; Wang, Y. 1992. Study of reproductive biology of clonal seed orchard of *Pinus armandii*. In: Shen Xihuan, ed. Seed Orchard Techniques. Beijing Science & Technology Publishing House.185-191.

Zhang, L. and Wang, X. 2000. Behaviour of northeast natural Korean pine forest provenance in Liaoning. Journal of Northeast Forestry University. 28(3), 48-50.

Zhang, L.; Wang, X.; Wang, W.; Liu, J.; Meng, G. 1992. Study of crown shaping of trees in seed orchard of *Pinus koraiensis*. In: Shen Xihuan, ed. Seed Orchard Techniques. Beijing Science & Technology Publishing House, pp 201-207.

Zhao, G. 1989. Morphological study of *Pinus sibirica* in the Great Xingan Range. Scientia Silvae Sinicae, vol. 25 (8), 252-256.

Zhao, G. 1991. Study on *Pinus sibirica* in the Great Xingan Range, China. Northeast Forestry University Press. 103 pp.

Zhao, X., Zhang, J., Fan, C., Su, F. and Zhang L 1999. Study on formation and control of defective branch of Korean pine: form and characteristic of injured leave at the trunk top. Forest Science and Technology, No. 8, 17-18.

Zheng, W. 1983. Chinese Flora of Woody Plants. Vol.1. China Forestry Publishing House. Beijing. 258-276.

Genetic Variation in Blue Pine and Applications for Tree Improvement in Pakistan, Europe and North America

Shams R. Khan

Abstract—Stands of blue pine (*P. wallichiana* A.B. Jacks. syn. *P. griffithii* McClelland) are highly diverse throughout its range of distribution in the Himalayan Mountains where the species grows under varying geographic, climatic, and edaphic conditions. The species occurs in two distinctly different ecotypes (mesic monsoon and dry nonmonsoon), and strict avoidance of germplasm transfer between the ecotypes is necessary for survival and productivity in Pakistan, India, and Nepal. The role of these ecotypes in enhancing productivity and in establishing large-scale plantations resistant to blister rust is presented and compared with plantations in India and Bhutan. An alternate management strategy to establishing a pure species stand is to interplant with other native conifers. Testing of blue pine in other countries is discussed, notably the superior performance of blue pine hybrids in the USA at specific sites, which indicates its breeding value as a parent for volume growth rate. In Germany and Japan, the species does not appear to be useful for forest plantations. Further international cooperation in testing of blue pine for rust resistance is proposed, given the variable resistance to blister rust within the species. Research needs to be conducted, especially in neighboring countries, to further assess the extent, nature, and pattern of geographic variability.

Key words: genetic diversity, blue pine, Himalayan pine, *P. wallichiana,* blister rust, pine hybrids, ecotypes

Introduction

Blue pine (*Pinus wallichiana*) is a highly variable species that occurs in the mountainous region of lower Asia. Natural stands of blue pine, generally known outside of southern Asia as Himalayan pine, are distributed in Afghanistan, Pakistan, India, Nepal, Bhutan, Tibet, China, and Burma. The species spans a longitudinal range between 68° and 100° E, a latitudinal range between 25° and 37° N, and an altitudinal range between 1,500 and 3,800 m (Critchfield and Little 1966, Khan, 1986). In the Himalayan Mountains, blue pine occurs under two rainfall regimes, within and outside the monsoon region. Dogra (1972) and Ahsan and Khan (1972), along with several earlier investigators including Brandis (1906), Osmaston (1927), and Shebbeare (1934), have recognized this variable site distribution of the species occurring in several countries of the region. Pure and mixed patches at varying altitudes are found, but the species grows well at an optimum elevation of 2,000-2,500 M. Although this pine occurs over a wide altitudinal range, there is no evidence of altitudinal races that could be given subspecific or specific taxonomic ranking.

This species has been known by a number of scientific names since first described. The taxonomy of blue pine has been a subject of controversy, probably corresponding to the diversity in the species on the wide range of ecotypes where it occurs. The older scientific names of blue pine were *P. excelsa* Wall. ex Lamb. (1824), *P. chylla* Lodd. (1836), *P. nepalensis* De Chambr. (1845), *P. griffithii* McClelland (1854), and *P. dicksonii* Hort. ex Carriere (1855). *Pinus griffithii* persisted in international botanical acceptance until the mid-twentieth century (Rehder 1940, Little and Critchfield 1969), and in China it is still the accepted taxonomic name (Wang, pers. comm.). Elsewhere, *P. wallichiana* A.B. Jacks. (1938) is the latest revision and is now the internationally accepted name (see citation in the list of five-needle pine species in this volume).

Although ecotypic variation has been observed (Khan 1986, 1994, 1995a), no detailed taxonomic studies of blue pine have been made at the ecotypic level. Patschke (1913), Wilson (1916), Kew Bulletin (1938), Dallimore and Jackson (1961), Ouden and Boom (1965) have mentioned varieties/forms of blue pine. Grierson and others (1980) reported "var. *parra*" as a blue pine variety. Critchfield and Little (1966) also made this distinction and described "var. *parra*" from Arunachal Pradesh (India) as the mesic monsoon zone ecotype of blue pine.

Blue pine has also been found to occur at high altitudes in low rainfall areas and at low altitudes (less than 2,800 m) in high rainfall areas (Khosla and Raina 1995). The soils of mesic habitat were found to be different from those of xeric sites in pH, maximum moisture holding capacity, and Ca and Mg concentrations throughout the species' distribution in Pakistan (Khan 1986). There were also differences exhibited in the phenology of male and female flowers in the two distinct ecological zones of the species (Khan 1995b).

In view of the importance of geographic variability to any tree improvement program, provenance studies were undertaken in Nepal, India, Bhutan, and Pakistan. In Pakistan, the species has been extensively studied for genetic variability and timber utilization, as well as the species' role in protection of the fragile ecosystems in the Himalayan Mountains. Such studies have further led to the delineation of

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Shams R. Khan is Forest Geneticist, Pakistan Forest Institute, 145/HJ3, Street No. 8, Phase 2, Hayatabad, Peshawar, Pakistan. E-mail shamspfi@yahoo.com.

seed zones with strict prohibition of transfer of germplasm between ecological zones (Khan 1991, 1994). Two geographic ecotypes were identified on the basis of differences in mean annual increment and growth in Pakistan, accounting for 56 percent of the variation among stands (Khan 1997, 2000). These two ecotypes also occur in India, Nepal, and Bhutan under similar geoclimatic conditions and consequently have similar ecological requirements and distribution patterns. The present paper outlines the extent to which genetic diversity in blue pine could be utilized for forest plantings in these countries.

Genetic Research on Blue Pine in Pakistan

Pakistan has sampled the genetic variation in blue pine more extensively than has any of the other countries in a cooperative project with the USDA Forest Service. Detailed studies of genetic diversity and its utilization in the natural stands of blue pine in Pakistan were conducted in several phases during the past 25 years. Studies of 32 provenances sampled throughout its range of distribution in Pakistan were undertaken to assess genetic variation in several morphological and anatomical traits of the needles, cones, and seeds.

Seed Movement

The potential for further genetic improvement in blue pine was shown by studies that showed evidence of ecotypic differentiation and suggested strict avoidance of transfer of germplasm from xeric to mesic habitat and vice versa (Khan 1991, 1994).

Comparison of Blue Pine with Other Species

Paudel and others (1996) tested *P. patula* Schiede & Deppe ex Schlechtendal & Chamisso and blue pine in Nepal. Of these species, blue pine had better survival at age six. Wallace (1989) compared the growth of *P. strobus* L. and blue pine in eastern Nepal and observed little difference in survival, growth, and form. However, he suggested blue pine was a better species overall. Siddiqui and others (1989) explored variability in the wood characteristics of three forest tree species, including blue pine, and found marked differences in percentage of late wood, smaller tracheid dimensions, higher density, and better strength in wood of drier areas when compared to wood from moist areas. Ashley and Fisk (1980) tested 45 species in 15 trial plots and observed that blue pine growing well in Kathmandu Valley, Nepal. Of several exotic temperate pines tested in the past two decades, none seems to thrive better than native blue pine in Pakistan (Annual Progress Reports, Pakistan Forest Institute, Peshawar). The consensus from these reports is that exotic pines have little potential in large-scale plantations in the native habitat of blue pine.

Interplanting Blue Pine to Increase Overall Yield

In Pakistan, interplanted mixtures of blue pine and other coniferous species in different stands have led to studies that identified the best combinations of associated conifers for high productivity, in addition to further quantifying genetic diversity in blue pine. Differences in mean annual increment between two species habitats were compared with those of the highly associated conifer *Cedrus deodara* (D. Don.) G. Don (Khan 1997, 2000). These studies suggest that blue pine should be planted as a monoculture in the xeric areas (less than 750 ± mm per annum), whereas it should be planted in mixtures with *C. deodara* in mesic habitats. Similar recommendations were also made by Khan (1979) for moist coniferous forests of northern Pakistan, although he did not report on the dry, nonmonsoon zone. Further research is under way on growth differences of the two ecotypes of blue pine in combination with other associated conifers, for example, *Abies pindrow* (Lamb.) Royle and *Picea smithiana* (Wall.) Boiss., to assess the possibility of improving productivity of mixed stand plantations in the Himalayan Mountains.

Performance of Blue Pine as an Exotic Species

Blue pine has been tested for variation in growth rate and in breeding for blister rust resistance (*Cronartium ribicola* Fisch. ex Rabenh.) in a few countries of Asia, Europe, and the USA. Stephan (1974) observed differences in rust resistance between trees of two provenances of blue pine in Lower Saxony, Germany at age 11, although there was a high level of mortality in trees from both provenances. In a species comparison test, he found that only 40 percent of the blue pine seedlings were infected by blister rust compared with an infection rate of 100 percent in *P. strobus, P. monticola* Dougl. ex D. Don, and *P. flexilis* James (Stephan 1985). Borlea (1992) and Blada (1994) have also used blue pine in Romania in experimental breeding for growth and blister rust resistance. These authors found that blue pine has potential value for genetic improvement in rust resistance.

The growth and blister rust data recorded in Romania indicated that half-sib families from low rainfall areas in Pakistan were more resistant to blister rust at age 11, compared with those originating from high rainfall areas (Blada 1994). Blada reported a correlation between latitude and blister rust ($r=0.66^{***}$), but could not include rainfall data of the native habitat in his studies, as they were in remote locations. To aid in interpretation of results, rainfall of the nearest meteorological station was included. Two of the most important phenomena affecting rainfall in the Himalayas, namely inversion of temperature and rainshadow effect, were also not taken into consideration, as no reliable data were available for such sites. In spite of these limitations, the moderately high correlation between blister rust and rainfall suggests the adaptive potential of the two ecotypes of this pine. The correlation would have been more reliable, however, if the annual rainfall data had been included from the actual collection sites, as the distribution of rainfall is highly erratic in the Hindukush-Karakorum region. It was concluded that differences in the adaptation of the species to varying moisture regimes in its native habitat, along with morphological and phenological differences between the two ecotypes, might be casually associated with corresponding differences in resistance to blister rust in blue pine. These results, therefore, suggest that the selection of trees/stands for rust resistance should be primarily based upon the origin of seed lots of the native habitat of this pine.

Comparative studies on genetic diversity in natural stands for various traits and the degree of resistance involving blue pine and other five-needle pines have also been reported by Dogra (1972), Ahsan and Khan (1972), and Bakshi (1972). The comparative growth of blue pine as an exotic in Europe, USA and Canada has been studied by Gremmen (1972), Kriebel (1983), Heimburger (1972), and other scientists. These studies emphasized mass production of rust resistant stands through hybridization. However, this approach can be expensive, cumbersome and time-consuming and may not be practical for developing countries. A more feasible strategy would be to collect seed from the xeric habitat of the species range for mass production of desirable trees in Pakistan and Romania. Insufficient cold hardiness is a deterrent in the USA and Canada, where hybridization might be feasible. The authors cited agree that conventional intraspecific breeding may not be the best method due to long generation time, mode of pollination, highly diverse populations due to cross pollination, haploid nature of infecting rust and the presence of an alternate five-needle pine host(s).

A two-way seed exchange of plant material was conducted between Pakistan and the USA. This exchange was part of a worldwide program to test haploxylon pines for resistance to the white pine blister rust and to test other five-needle pine species in Pakistan. This project yielded useful information on the nature of resistance, growth potential of some white pines, inoculation techniques, and breeding schemes for mass production of resistant trees.

Garrett (1992) reported that Himalayan white pine was not resistant to the white pine weevil (*Pissodes strobi* Peck) and suggested that it should only be planted in weevil-free regions of the USA. However, the species has not performed well where tested in weevil-free regions (Ohio and Tennessee), and commercial planting cannot be recommended anywhere in the USA on the basis of present knowledge. In 20-year-old provenance tests in Ohio of trees from India, Pakistan, and Nepal, new terminal shoots on trees of all seed sources were repeatedly killed back by late spring frosts. No trees from Nepal survived the winter climate. Field testing of *P. strobus x wallichiana* hybrids in Ohio over two decades demonstrated superiority in wood volume growth over most *P. strobus* genetic selections (Kriebel 1983). The hybrids also have a higher wood specific gravity than *P. strobus* (Kriebel, pers. comm.). In Tennessee, initial survival of drought was very low (Schlarbaum, pers. comm,). Genys (1979) compared variability among 21 seedlings of blue pine originating from India, Pakistan, and Bhutan. Strains from Pakistan were hardier than those of other countries in Maryland. However, the performance of the species has not been satisfactory in Germany and Japan (Takahashi and others 1974, Stephan 1985). From these studies, it appears that blue pine as a hybrid parent has a potential for better growth and resistance against blister rust in the USA, but its performance in Europe and Asia has yet to be ascertained.

Conclusions and Recommendations

Seed origin of blue pine is critical to survival, growth, and perhaps blister rust resistance. Two general ecotypes are known and careful attention should be paid to which ecotype is represented in a seed collection. Selections and collection of blue pine seed in native stands from trees growing near glaciers or river banks as well as from buffer zone should be avoided as these sites may represent atypical habitat in blue pine. Efforts should be made to include meteorological data from actual sites to minimize biased estimates derived from the nearest weather stations. Because of dysgenic selection in several countries of the Himalayan region, including Pakistan, the establishment of *in situ* conservation stands in the xeric habitat could assist in mass-producing desirable individuals resistant to blister rust. These efforts should be supported by further research on genetic diversity in this widespread and phenotypically variable species.

Research has shown that blue pine often thrives in planting of mixtures with other coniferous species in certain environments. Additional research should be conducted to better delineate the effects of mixture composition, blue pine genetics, and site variation to maximize productivity.

Establishment of further blue pine provenance tests in the USA and Canada does not appear to be practical because of the demonstrated insufficiency of winter-hardiness in regions where the species might be useful. In contrast, cooperative international trials should be conducted in Pakistan and Romania to continue research and establish seed orchards and seed production areas from ecotypes showing higher degree of blister rust resistance. The prevalence of field races of the rust, as reported by Patton (1972), should also be explored in this species. Multiclonal hybrids, particularly of blue pine and *P. strobus*, could be developed for rust resistance as a cooperative effort of the USA, Canada, Romania, and Pakistan to achieve desirable objectives in breeding both species.

References

Ahsan, J.; Khan, M.I.R. 1972, *Pinus wallichiana* A.B. Jackson in Pakistan. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham and others USDA Forest Service Misc. Publ. 1221: 151-162.

Ashley, B; Fisk, T. 1980. Tree species trials in Nepal - some early results. Nepal/Australian Forest Tree Project; Canberra, Australia; 144 p.

Bakshi, B.K. 1972. Relative blister rust resistance of native and introduced white pines in Asia. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham and others USDA Forest Service Misc. Publ. 1221: 251-255.

Blada, I. 1994. Intraspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genetica 43:14-20.

Borlea, G.F. 1992. Preliminary trials into resistance of 5 pine (*Pinus*) species attacked by blister rust (*C. ribicola*). Revista - Padurilor 107: 3, 7-10.

Brandis, D. 1906. Indian trees. London, Archibald Constable; 767 pp.

Critchfield, W.B; Little, E.L. 1966. Geographic distribution of the pines of the world. USDA; Forest Service; Misc. Publ. 991, 97 pp.

Dallimore, W; Jackson, A.B. 1961. A hand book of Coniferae. London, Edward Arnold, 3rd ed. 686 p.

Dogra, P.D. 1972. Intrinsic qualities, growth and adaptation potential of *Pinus wallichiana*. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham *and others* USDA Forest Service Misc. Publ. 1221: 163-178.

Garrett, P.W. 1992. Performance of Himalayan blue pine in northeastern United States. Tree Planters Notes 43(3):76-80.

Genys, J.B. 1979. Intraspecific variation in Himalayan white pine, *Pinus griffthii*. USDA Forest Service; Technical Report No. NC-50, pp. 117-130.

Gremmen, J. 1972. Relative blister rust resistance of *Pinus strobus* in some parts of Europe. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham and others USDA Forest Service Misc. Publ. 1221: 241-249.

Grierson, A.J.C; Long, D.G; Page, C.N. 1980. Notes relating the flora of Bhutan (III). *Pinus bhutanica*: A new 5-needle pine from Bhutan and India. Notes from the Royal Botanical Garden Edinburgh 38: 2, 297-310.

Heimburger, C. 1972. Relative blister rust resistance of ntive and introduced white pines in eastern South America. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham, and others USDA Forest Service Misc. Publ. 1221: 257-269.

Kew Bulletin. 1938. Bulletin of miscellaneous information, No. 2. Royal Botanic Gardens, Kew, London. His Majesty's Stationary Office, 549 p.

Khan, A. 1979. Comments on the proposed intensive forest management in the temperate coniferous forests. Pak. Jour. For. 29: 90-92.

Khan, S.R. 1986. Ecotypic differentiation in *Pinus wallichiana*. Proc. IUFRO Conference. Williamsburg, Virginia. *Edited* by R. Weir and others, 325-333.

Khan, S.R. 1991. The establishment of seed transfer zones of blue pine (*Pinus wallichiana* A.B. Jacks) in Pakistan: A first attempt. Proc. 10th World Forestry Congress, Paris, vol. V:72-76.

Khan, S.R. 1994. Key structural determinants in enhancing infraspecific variability in *P. wallichiana*. *In:* Proc. Symp. Forest Tree Improvement in the Asia-Pacific region. *Edited* by X. Shen, Published by China Forestry Publishing House, Beijing, pp. 10-15.

Khan, S.R. 1995a. Genetic improvement of Himalayan white pine: ecotypic differentiation by phenological differences. *In*: Proc. IUFRO, XX World Congress, Tampere, Finland. Abstracts of Invited Papers, p. 147.

Khan, S.R. 1995b. Dryland forestry research vis-B-vis afforestation strategies in Pakistan. *In* Dryland forestry research. *Edited* by A. Shahzad and others Proc. IFS/IUFRO Workshop, Hyytyala, Finland, p. 146.

Khan, S.R. 1997. Genetic diversity and its role in afforestation in Pakistan. *In* Biodiversity of Pakistan. *Edited* by A. Shadzad and others eds. Publ. Pakistan Museum of Natural History and Florida Museum of Natural History, p. 107-113.

Khan, S.R. 2000. Intraspecific variation vis-B-vis management strategies for the natural stands of *Pinus wallichiana* A.B. Jackson. Pak. Jour. For. 50(1-2):17-23.

Khosla, P.K. Raina, K.K. 1995. Forest resources in the dry Himalaya region: Implications for long term sustainability. *In* Dryland forestry research. *Edited* by A. Shahzad and others Proc. IFS/IUFRO Workshop, Hyytyala, Finland, 147-148.

Kriebel, H.B. 1983. Breeding eastern white pine: a world-wide perspective. Forest Ecology and Management 6: 263-279.

Little, E.L., Jr. and Critchfield, W.B. 1969. Sub-divisions of the genus *Pinus* (Pines). U. S. Dept. Ag. Misc. Publ. 1144, Washington, D.C.

Osmaston, A.E. 1927. A forest flora of Kumaon. Allahabad, Indian Government Press; 605 pp.

Ouden, P.D; Boom, B.K. 1965. Manual of cultivated conifers. Martinus Nijhoff, The Hague. 526 pp.

Patschke, W. 1913. Uber die extrat ropischen ostarsiatischen coniferen und ihre Bedentung fur die pflanzengeog-raphische Gliederung Ostasiens. Bot. Jahrb. XLVIII:626-776. (In German).

Patton, R. F. 1972. Inoculation methods and problems in testing eastern white pine for resistance to *Cronartium ribicola*. *In* Biology of Rust Resistance in Forest Trees. *Edited* by R. T. Bingham, and others USDA Forest Service Misc. Publ. 1221: 373-385.

Paudel, K.C; Amatya, S.M; Harrison, AS. 1996. Provenance trial of *Pinus maximinoi* and comparison with *Pinus wallichiana* and *P. patula*. Working paper Llumle Regional Agr. Res. Centre, Nepal. No.96-38, 8 pp.

Rehder, A. 1940. Manual of cultivated trees and shrubs. 2nd Edition. MacMIllan, New York, 996 pp.

Shebbeare, E.O. 1934. The conifers of Sikkah, Himalaya and adjoining country. Indian Forester 60:710-713.

Siddiqui, K.M; Khan, J.A; Mehmood, I. 1989. Comparison of anatomical, physical and mechanical properties of *Abies pindrow, Cedrus deodara* and *Pinus wallichiana* from dry and wet temperate forests. Pak. Jour. For. 39:27-33.

Stephan, B.R. 1974. Geographic variation in *Pinus strobus* on the basis of preliminary results of field trials in Lower Saxony. Silvae Genet. 23:214-220.

Stephan, B.R. 1985. Resistance of 5-needled pines to blister rust. Allgemeine Forstzeitschrift 28:695-697.

Takahashi, N; Hamaya, T; Kurahashi, A. 1974. Records of the growth of young samples stands of introduced species in the Tokyo University Forests in Hokkaido. Tokyo University Forests 18:29-66.

Wallace, D. 1989. Report on provenance trial of *Pinus maximinoi / pseudostrobus*. PAC Tech. Paper, Pakhribas Agr. Centre No. 102, p. 8.

Wilson, E.H. 1916. The conifers and Taxads of Japan. Cambridge University Press; 85 pp.

Part II: Genetics, Genecology, and Breeding

Pinus lambertiana **(sugar pine)**

photo courtesy of B. Danchok

Phylogenetics, Genogeography and Hybridization of Five-Needle Pines in Russia and Neighboring Countries

Dmitri V. Politov
Konstantin V. Krutovsky

Abstract—Phylogenetic and population genetic studies of native five-needle pines growing in Russia and neighboring countries were reviewed. Four species, *Pinus cembra, P. sibirica, P. pumila* and *P. koraiensis*, together with North American species *P. albicaulis,* comprise the subsection *Cembrae* (stone pines) of the section *Strobus* (white pines). They share bird-dispersal related traits such as seed winglessness and cone indehiscence that differentiate them from most other white pines and related section *Parrya*. Phylogenetic analysis showed that *P. cembra, P. sibirica* and *P. albicaulis* represented a close group based on isozyme loci and other molecular genetic markers. *Pinus pumila* and *P. koraiensis* also clustered together in phylogenetic trees, but they were closer to *P. parviflora* and other East Asian pines of the subsection *Strobi* than to the *cembra-sibirica-albicaulis* group. Therefore, we hypothesized that seed winglessness and other bird-dispersal related traits could either arise independently in these lineages or could have been introduced via occasional hybridization. Natural hybridization between *P. sibirica* and *P. pumila* in their zone of sympatry in the Baikal region was confirmed by isozyme methods, but no significant introgression was revealed. The possibility of producing hybrids in artificial crosses between *P. sibirica* and *P. cembra*, and *P. sibirica* with *P. koraiensis* was also confirmed by isozyme analysis. Intrapopulation genetic variation, measured as expected heterozygosity of isozyme loci (H_e), was relatively high in *P. koraiensis* (H_e = 0.130) and *P. sibirica* (0.106) and similar to the average for other soft pines, but significantly higher in *P. pumila* (0.198) and lower in *P. cembra* (0.082). There was no obvious difference in the level of heterozygosity between bird-dispersed pines of the subsection *Cembrae* and wind-dispersed pines of the closely related subsection *Strobi*. Relatively low inbreeding was observed in embryos in all five-needle pines and was primarily caused by self-pollination. However, Hardy – Weinberg equilibrium or even a slight excess of heterozygosity was usually observed among mature trees, apparently as a result of selection against inbred progeny and in favor of heterozygotes. Two types of polyembryony, monozygotic and polyzygotic, were found in *P. sibirica*. Gene geography studies in *P. sibirica* and *P. pumila* populations based on multivariate analysis of isozyme data was also discussed.

Key words: *Cembrae,* genogeography, isozyme, phylogenetics, *Strobi*, stone pines, white pines

Introduction

Four species of five-needle pines, also known as haploxylon or soft pines (genus *Pinus* L., subgenus *Strobus* Lemm.), are native in Russia and neighboring countries (fig. 1). These four species - *P. cembra* L. (Swiss stone pine), *Pinus sibirica* Du Tour (Siberian stone pine), *P. pumila* (Pall.) Regel (Siberian dwarf pine) and *P. koraiensis* Sieb. et Zucc. (Korean pine) - are classified with section *Strobus* Loud. (white pines) and subsection *Cembrae* Loud. (stone pines). *Pinus sibirica* forms forests of great economical importance in Siberia and the Far East, while *P. pumila* occupies vast territory in East Siberia and along the Asian Pacific coast. *Pinus koraiensis* is distributed in the Russian Far East, including the Amur Region, Khabarovsk and Primorskii Territories, and the Island of Sakhalin. *Pinus cembra* is scattered in the East Carpathian Mountains in Ukraine.

Figure 1—Geographic distribution of five-needle pines in Russia and neighboring countries.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Dmitri V. Politov is Senior Research Associate, Laboratory of Population Genetics, N.I.Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 Gubkin St., GSP-1, Moscow 119991, Russia. Phone: 7 (095) 135-5067. Fax : 7 (095) 132-8962. E-mail: dvp@vigg.ru. Konstantin V. Krutovsky (author for correspondence) is Research Plant Molecular Geneticist, Institute of Forest Genetics, USDA Forest Service, Pacific Southwest Research Station, Environmental Horticulture Department, University of California, Davis, One Shields Avenue, Davis, CA 95616 USA. Phone: 530-752-8412. Fax: 530-754-9366. E-mail: kkroutovski@ucdavis.edu

The *Cembrae* species have large edible wingless seeds that are dispersed by birds and play a key role in Siberian taiga (*P. sibirica, P. koraiensis*) and subalpine (*P. cembra, P. pumila*) ecosystems. Although *P. parviflora* is a five-needle pine species that occurs in Russia, it is also endemic to Japanese islands and occurs in a few isolated populations on the Kuril Islands along the Russian Pacific coast.

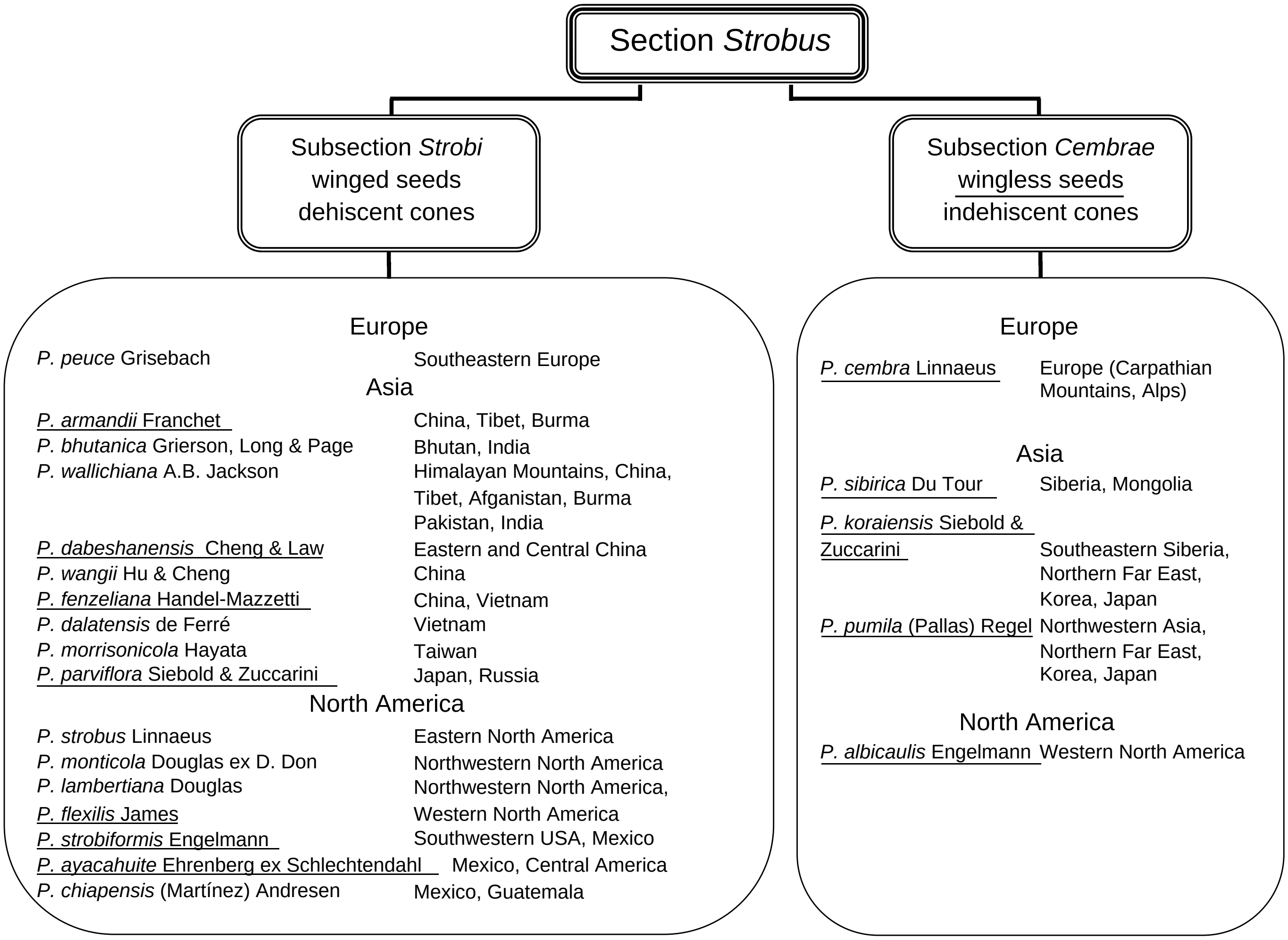
Evolutionary relationships and intraspecific population genetic structure of Eurasian *Cembrae* pines have been studied since early 1900s, primarily by traditional morphological methods (compare Bobrov 1978; Iroshnikov 1974; Lanner 1990, 1996, 1998; Smolonogov 1994). However, the levels of intrapopulation, intraspecific and interspecific genetic variation have been quantitatively estimated only after development of reliable molecular genetic markers.

This paper presents an overview of genetic studies of the above-mentioned five-needle pines. We have concentrated on results obtained using molecular genetic markers, but also included karyological and morphological data when needed.

Phylogenetics

All four *Cembrae* pine species produce functionally indehiscent cones and large wingless seeds. Based on those traits, these species and the North American whitebark pine (*P. albicaulis* Engelm.) are traditionally included in the subsection *Cembrae* (stone pines) within section *Strobus* (white pines). This section together with the species of the section *Parrya* Mayr. comprise the group of soft pines (subgenus *Strobus*, or Haploxylon) of the genus *Pinus*. We used the most widely accepted classification by Critchfield and Little (1966) with minor modifications and additions (Price and others 1998). According to this classification section *Strobus* is subdivided into two subsections *Cembrae* and *Strobi* Loud. that both have representatives in Eurasia and North America (fig. 2).

This subdivision is based on the occurrence of large wingless seeds and indehiscent (not opening upon ripening) macrostrobili (cones) in *Cembrae* pines. It has been widely accepted that both these traits evolved as a result of the



Europe

P. peuce Grisebach	Southeastern Europe

Asia

P. armandii Franchet	China, Tibet, Burma
P. bhutanica Grierson, Long & Page	Bhutan, India
P. wallichiana A.B. Jackson	Himalayan Mountains, China, Tibet, Afganistan, Burma Pakistan, India
P. dabeshanensis Cheng & Law	Eastern and Central China
P. wangii Hu & Cheng	China
P. fenzeliana Handel-Mazzetti	China, Vietnam
P. dalatensis de Ferré	Vietnam
P. morrisonicola Hayata	Taiwan
P. parviflora Siebold & Zuccarini	Japan, Russia

North America

P. strobus Linnaeus	Eastern North America
P. monticola Douglas ex D. Don	Northwestern North America
P. lambertiana Douglas	Northwestern North America,
P. flexilis James	Western North America
P. strobiformis Engelmann	Southwestern USA, Mexico
P. ayacahuite Ehrenberg ex Schlechtendahl	Mexico, Central America
P. chiapensis (Martínez) Andresen	Mexico, Guatemala

Europe

P. cembra Linnaeus	Europe (Carpathian Mountains, Alps)

Asia

P. sibirica Du Tour	Siberia, Mongolia
P. koraiensis Siebold & Zuccarini	Southeastern Siberia, Northern Far East, Korea, Japan
P. pumila (Pallas) Regel	Northwestern Asia, Northern Far East, Korea, Japan

North America

P. albicaulis Engelmann	Western North America

Figure 2—Taxonomic classification and generalized distribution of white pines (Critchfield and Little 1966; Price and others 1998). Underlined are species with wingless or almost wingless seeds (Lanner 1996, 1998).

adaptation to dispersal of their seeds by corvid birds (for example, Lanner 1996; Tomback and Linhart 1990), and particularly nutcrackers (*Nucifraga* spp.). The closely related subsection *Strobi* includes mainly typical wind-dispersed species with small winged seeds released from the cones upon ripening. However, seeds of some of *Strobi* species (underlined in fig. 2) are large and virtually maladapted to dispersal by wind. Moreover, traits that are normally characteristic of *Cembrae* pines (especially winglessness) sporadically occur also in *Strobi* pines as rare abnormalities or as within-population polymorphism. Reproductive barriers are incomplete between these two subsections, and there are numerous examples of artificial and natural interspecific hybridization both within and between subsections (Critchfield 1986; Blada 1994). The occurrence of intermediate forms and documented hybridization between species of different subsections make the hypothesis of monophyletic origin of *Cembrae* and *Strobi* subsections controversial. This problem stimulated repeated attempts to revise the taxonomy of the group and to rearrange the composition of the subsections. However, until recently, the problem was studied mainly using morphological comparisons (for example, Kupriyanova and Litvinceva 1974).

Muratova (1980) compared karyotypes of three Eurasian *Cembrae* pine species and found only slight differences in chromosome size and morphology, as well as in number and localization of nucleoli organizers. *Pinus cembra* and *P. sibirica* karyotypes were the most similar, although there were slight differences. The karyotype of *P. pumila* was closer to these species than to *P. koraiensis*, which partially contradicts molecular genetic data presented below.

The first molecular genetic evidence of great similarity between *P. cembra* and *P. sibirica* was provided with isozyme loci (Krutovsky and others 1990). This study also showed that *P. pumila* and *P. koraiensis* comprise the next closely related pair. These relationships among Eurasian *Cembrae* pines were confirmed later by other scientists (for example, Goncharenko and others 1991; Shurkhal and others 1992). *Pinus albicaulis* is the only North American *Cembrae* pine. This species together with all four Eurasian *Cembrae* pines were studied by Krutovsky and others (1994, 1995), which were the first studies where phylogenetic relationships were analyzed within the entire subsection *Cembrae* using molecular genetic markers. Two lineages were found again in *Cembrae* pines; *P. cembra* and *P. sibirica* formed a pair of the most closely related *Cembrae* species, while another lineage was represented by two Far Eastern species *P. pumila* and *P. koraiensis*. The position of *P. albicaulis* was not fully resolved at that time, but it was found to be closer to the *cembra–sibirica* group.

Krutovsky and others (1994, 1995) studied chloroplast DNA (cpDNA) restriction fragment length polymorphisms (RFLPs) in all five *Cembrae* pine species, as well as in four representatives of North American pines from subsection *Strobi* (*P. strobus* L., *P. lambertiana* Dougl., *P. monticola* Dougl., and *P. flexilis* James). Species of *Strobi* were close to *Cembrae* species, but they did not form a separate cluster in the dendrogram. A similar pattern was also observed in other studies based on cpDNA markers, although unfortunately neither of those studies included all representatives of *Cembrae* pines species (for example, Wang and Szmidt 1993; Wang and others 1999). For instance, Wang and Szmidt (1993) used cpDNA RFLP markers to study phylogenetic relationships in several Asian pine species including one *Cembrae* pine (*P. sibirica*) and five *Strobi* pines (*P. armandii* Franch., *P. griffithii* McClelland (syn. *P. wallichiana* A. B. Jacks.), *P. kwangtungensis* Chun & Tsiang, *P. parviflora* Sib. & Zucc., and *P. peuce* Griseb.). *Pinus sibirica* (*Cembrae*) clustered very closely with *P. parviflora* (*Strobi*) with 95 percent of the bootstrap support within the following topology in the maximum parsimony consensus tree: (*peuce* ((*parviflora*, *sibirica*) (*armandii* (*griffithii, kwangtungensis*)))). However, the absence of *Cembrae* species other than *P. sibirica* in the analysis made it impossible to draw any conclusions about phylogenetic relationships between *Cembrae* and *Strobi* pines. In a more recent study based on cpDNA markers (Wang and others 1999), the topology of the resulting consensus tree was different: (*peuce* (*strobus* (*monticola* (*parviflora* (*koraiensis*, *armandii*, *griffithii*, *cembra*, *kwangtungensis*, *pumila*))))). However, resolution within a group of six species belonging to both subsections *Strobi* (*P. armandii*, *P. griffithii* and *P. kwangtungensis*) and *Cembrae* (*P. cembra*, *P. koraiensis* and *P. pumila*) was insufficient to make any strong conclusions on the phylogeny of these species. The parsimony tree indicated that two North American (*P. strobus* and *P. monticola*) and one European (*P. peuce*) species of subsection *Strobi* appeared to be ancestral to all Asian white pine species including *P. cembra*. These results contradict our point of view that Asian pine species are ancestral to North American pines, which is supported by Belokon and others (1998) study based on more numerous nuclear markers and more representative set of white pine species. Liston and others (1999) used ribosomal internal transcribed spacer (ITS) DNA markers to study phylogeny of the genus *Pinus*. White pines of the section *Strobus* formed a separate cluster, but relationships within the section were unclear. The only clade well supported by bootstrap was comprised of *P. cembra* and *P. albicaulis*, which agreed with our earlier allozyme and cpDNA data (Krutovsky and others 1994, 1995). Belokon and others (1998) studied 13 white pine species using isozyme markers coupled with Principal Component Analysis (PCA) and also did not find a strong support for dividing section *Strobus* into the two traditional subsections *Cembrae* and *Strobi*. Instead, the study found even more evident differentiation between North American and Eurasian white pines. The updated results from the Belokon and others (1998) study with increased sample size and two additional species (*P. lambertiana* and *P. strobiformis*) are presented in figure 3 (Politov and others, unpublished). *Cembrae* pines were separated from *Strobi* pines along the first dimension on the two-dimensional (2D) plot obtained by multidimensional scaling of Nei's (1972) genetic distance matrix based on isozyme data, while the second dimension differentiated North American and Eurasian species. Among them, *P. peuce* of southern Europe was located (genetically) as the nearest species to North American *Strobi* pines (fig. 3). In general, Eurasian *Strobi* species were more differentiated, and *P. griffithii* (as *P. wallichiana* in the paper) was the nearest to the Asian *P. pumila* and North American *P. albicaulis* (*Cembrae* pines).

A representative of the related section *Parrya* (pinyons), *Pinus edulis* Engelm., was included as an outgroup in Belokon and others (1998) study. The dendrogram of white

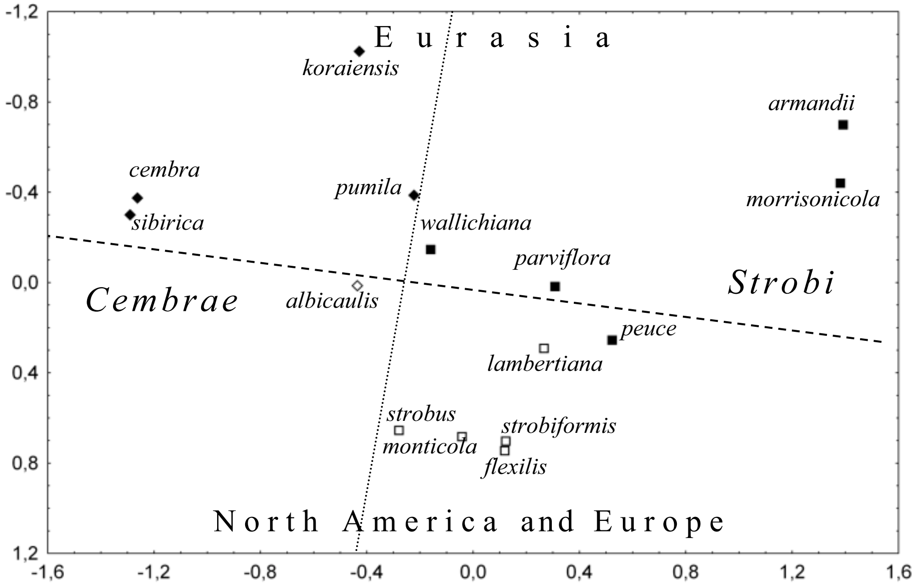


Figure 3—Two-dimensional (2D) plot of 13 white pine species (section *Strobus*) obtained by multidimensional scaling of Nei's (1972) genetic distance matrix based on isozyme loci. ◆ - Eurasian *Cembrae*; ◊ - North American *Cembrae*; ■, Eurasian *Strobi*; □ - North American *Strobi*.

pine species and *P. edulis* obtained in this study (fig. 1 in Belokon and others 1998) indicated that Asian *Strobi* pines are evolutionarily older than both American *Strobi* and all *Cembrae* pines. There is other evidence also supporting the hypothesis that subsection *Cembrae* is an evolutionarily younger taxonomic group. It is based on the relatively recent origin of nutcracker birds (*Nucifraga* spp.) that disperse seeds of *Cembrae* pines.

However, despite indicating evolutionary trends in section *Strobus,* none of the above mentioned phylogenetic studies was able to unambiguously confirm the validity of the subsection *Cembrae*. We believe that there can be several explanations for the controversial origin of *Cembrae* pines. Their wingless seeds and indehiscence could have been indeed inherited from a common ancestor (true monophyly), but sufficiently so long ago that relatedness is obscured by millions of years of evolution in diverse habitats. Allozyme variation is not necessarily neutral, at least in part, and may be shaped by selection of particular alleles or genotypes advantageous in particular environment reflecting local adaptation rather than phylogenetic relationships. The monophyly of subsection *Cembrae* assumed under this scenario does not necessarily mean monophyly of subsection *Strobi*. *Cembrae* pines could have arisen within wind-dispersed *Strobi*, but these two subsections are not necessarily two sister groups.

The second scenario assumes polyphyletic origin of *Cembrae* pines. Genetic divergence between *P. sibirica* – *P. cembra* (and perhaps *P. albicaulis*), from one side, and *P. pumila* – *P. koraiensis*, from another side, supports this hypothesis. It is relatively high and comparable to the level of divergence between species from different subsections, *Cembrae* and *Strobi*. This could mean an independent and multiple (polyphyletic) origins of wingless seeds and indehiscent cones in these two lineages. The wingless seeds occur also in several white pines of subsection *Strobi*. As to cone indehiscence, this trait is underlain by a simple mechanism; *Cembrae* cones lack specific tracheid fascicles (coarse fibers) in the scales. In other pine species, the fascicles contract, when a cone dries, causing scales to bend outward (Lanner 1990). The critical question is whether the absence of this tissue, a homologous trait, synapomorphic for all five *Cembrae* species. We think that this is not necessarily the case, since the lack of this tissue could arise independently in two evolutionary lineages *(P. sibirica - P. cembra - P. albicaulis* and *P. pumila - P. koraiensis)* as a result of nonhomologous mutations. Indehiscence then could be fixed via coevolution of pines and their dispersers, corvid birds (Tomback and Lindhart 1990), as this trait helps to hold the seeds in the cones making them more easily available for birds.

There is also a possibility of interspecific gene flow and multiple cases of gene exchange between different white pine species (Critchfield 1986). Interspecific hybridization and occurrence of natural interspecific hybrids were documented between *P. sibirica* and *P. pumila* within subsection *Cembrae* (Politov and others 1999), and between *P. pumila* and *P. parviflora* from different subsections *Cembrae* and *Strobi*, respectively (Watano and others 1995, 1996). Once indehiscence appeared, it could have crossed species borders as a result of sporadic hybridization events. Therefore, indehiscence could be a result of a "monophyletic" event (appearing once in the evolution). However, genes responsible for indehiscent cones could share the same ancestor gene or genes, while other genes in the genome may have a quite different evolutionary history. Past or present gene introgression may also be responsible for similarity of genetic markers in sympatric or nearly sympatric species, such as western North American white pines *P. monticola, P. flexilis* and *P. strobiformis* Engelm., or white pines from Taiwan, *P. morrisonicola* Hayata and *P. armandii* (which was represented by the Taiwanese variety in our material).

If introgressive hybridization indeed took place in the white pine evolutionary history, this could violate major principles of phylogenetic analysis, such as independence of compared operational taxonomic units (OTUs), and therefore, white pine evolution would be better described as reticulate evolution. Even a large number of molecular markers could be insufficient to resolve complicated reticulate patterns. Although molecular genetic markers have already provided valuable information for the understanding of genetic relationships in the section *Strobus*, their value for phylogeny is still not fully understood.

Genogeography

Pinus sibirica

Pinus sibirica was the first *Cembrae* pine in Russia that was studied by isozyme loci. Eleven isozyme systems coded by 19 loci were described more than a decade ago (Krutovsky and others 1987), and genetic differentiation among populations was estimated (Krutovsky and others 1989; Politov and others 1989; Politov and others 1992; Krutovsky and others 1994, 1995). The proportion of interpopulation variation in total species variation measured as F_{ST} value was relatively low (2.5 percent) in this species; nevertheless cluster analysis of 11 populations based on isozyme genetic distances showed good correspondence to their geographic origin. Populations from major regions that were substantially different in habitat types formed separate clusters: South Siberian Mountains, Western Siberian Plain, and Northern Siberia.

Using practically the same set of isozyme loci, Goncharenko and others (1993b) reported F_{ST} in *P. sibirica* to be 3.9 percent. Despite broader representation of samples (from northwestern Siberia to eastern Kazakhstan and to Lake Baikal Region in East Siberia), the concordance of UPGMA clustering to geographic origin was less pronounced in their study. We believe that this could have been caused by biased allele frequency estimations due to relatively low sample size, 12.9 trees on average per population as compared to 38.9 trees in Politov and others (1992) and Krutovsky and others (1994, 1995). Using seven isozyme loci analyzed in the needle tissue, 41 samples were studied by Podogas (1993), and the position of samples on the resulting PCA plot generally corresponded to the geographic location of populations while the F_{ST} value was also relatively low (3 percent).

Using 31 allozyme loci, Politov (1998) studied genetic differentiation among 15 populations in Lake Baikal region in East Siberia. A higher level of interpopulation diversity (F_{ST}=6.3 percent) was revealed in these populations, and genetic differentiation studied by PCA was in good concordance with geography (fig. 4). Comparative analysis of Baikalian populations together with earlier studied populations from West and Middle Siberia using 10 common loci showed that Baikalian populations were different from other major provinces (Politov 1998).

Pinus cembra

Allozyme variation in *P. cembra* populations was first studied by Szmidt (1982). The estimated population genetic differentiation (F_{ST} = 0.313) was unusually high compared to other pine species. In part, the high F_{ST} value could be explained by greater isolation among *P. cembra* populations. There are reasons, however, other than isolation that can explain that high F_{ST}. Based on the description of the geographic origin provided by the author, one of the samples appeared to be collected from a *P. sibirica* population in Chitinskaya Region in East Siberia. This area is sympatric for both *P. sibirica* and *P. pumila*. We compared genetic data obtained for East Siberian populations of *P. sibirica* and *P. pumila*, which revealed that this sample represented neither *P. cembra* nor *P. sibirica*. Several alleles in this sample are species specific for *P. pumila* and were never found in either *P. cembra* or *P. sibirica*. Correspondingly, we believe that this sample actually represented *P. pumila,* which is highly diverged from *P. sibirica* in isozyme loci. Subsequently, only a few single populations of *P. cembra* have been studied by isozymes (Krutovsky and others 1990, 1994, 1995; Goncharenko and others 1991; Politov and others 1992; Pirko 2001). However, recently Belokon, Belokon and Politov (in preparation) estimated genetic diversity in five *P. cembra* samples collected from the Alps (Switzerland and Austria) and the Carpathian Mountains populations in western Ukraine. The F_{ST} value was not exceptionally high (0.047). Therefore either stand isolation did not drastically affect population differentiation of this species or this isolation is possibly a recent event, and the stands were much more dense and widespread in the recent past. We conducted Correspondence Analysis (StatSoft 1998) on these five populations, and its results are shown in figure 5. Samples from the Alps and the Carpathian Mountains were well separated along the first dimension, while the second dimension differentiated northern and southern macroslopes of the eastern Carpathian Mountains. This differentiation showed that Alpine and Carpathian populations as well as populations from northern and southern macroslopes of the eastern Carpathian Mountains have different genetic constitutions, although the southern macroslopes were represented by only a single population in our study.

Gugerli and others (2001) studied 15 populations in *P. cembra,* one in *P. sibirica* and two in *P. pumila* using a few DNA markers of several different types, but no data on intraspecific variation were reported. The authors confirmed allozyme data that *P. pumila* was the most divergent species among these three species. However, they failed to differentiate *P. cembra* from *P. sibirica*, which clustered among *P. cembra* populations.

Pinus pumila

Genetic structure of *P. pumila* was first analyzed in studies on three populations from the north region of Kamchatka Peninsula (Krutovsky and others 1990; Politov and others 1992; Krutovsky and others 1994, 1995). F_{ST} value based on 22 allozyme loci was relatively low (2.1 percent), but dendrograms based on genetic distances corresponded to the geographic localization of the populations.

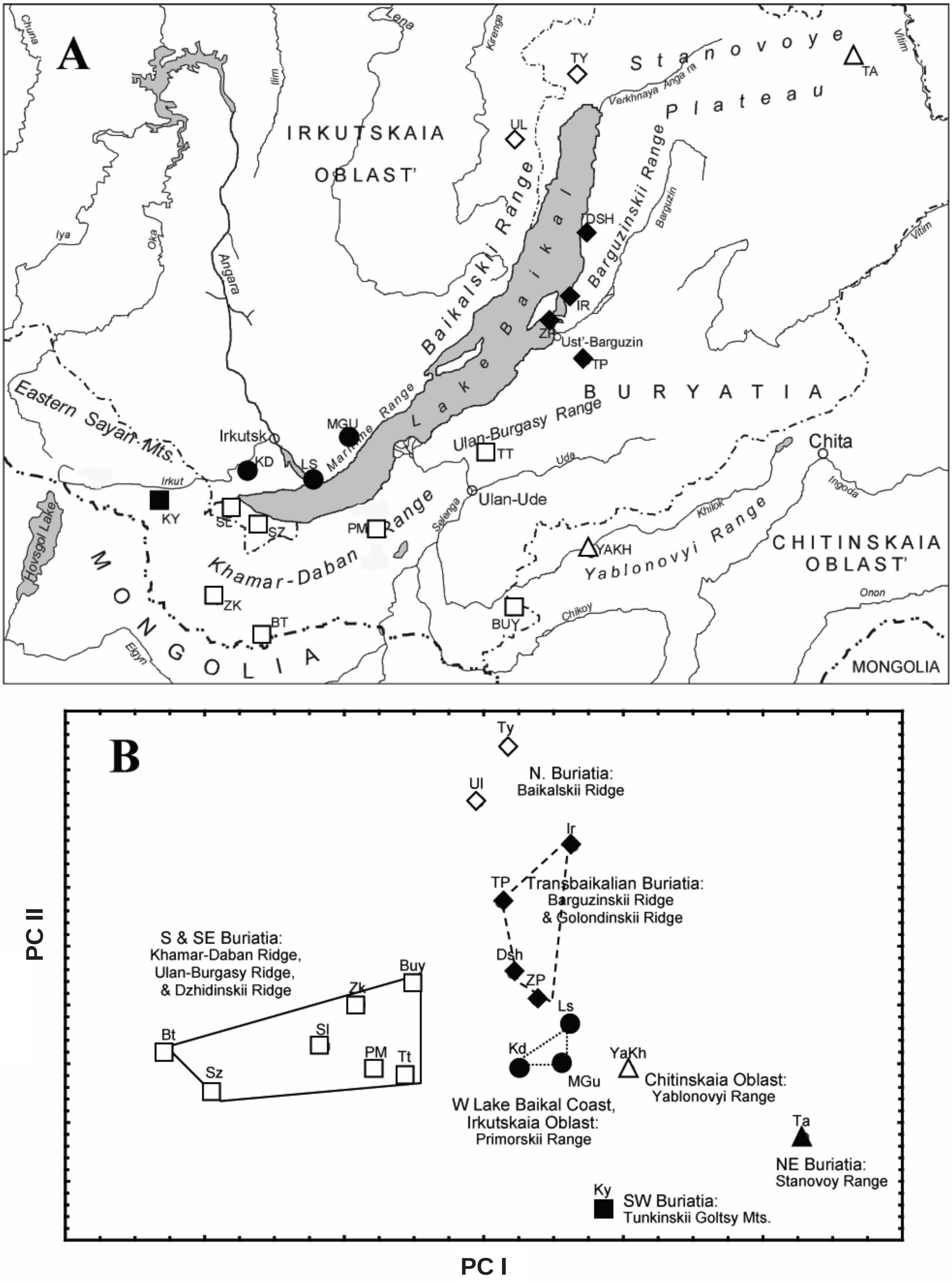


Figure 4—Sampling site locations (A) and results of Principal Component Analysis of 19 populations of *P. sibirica* based on allelic variation in 31 isozyme loci (B) studied in Baikal Lake region (Politov 1998).

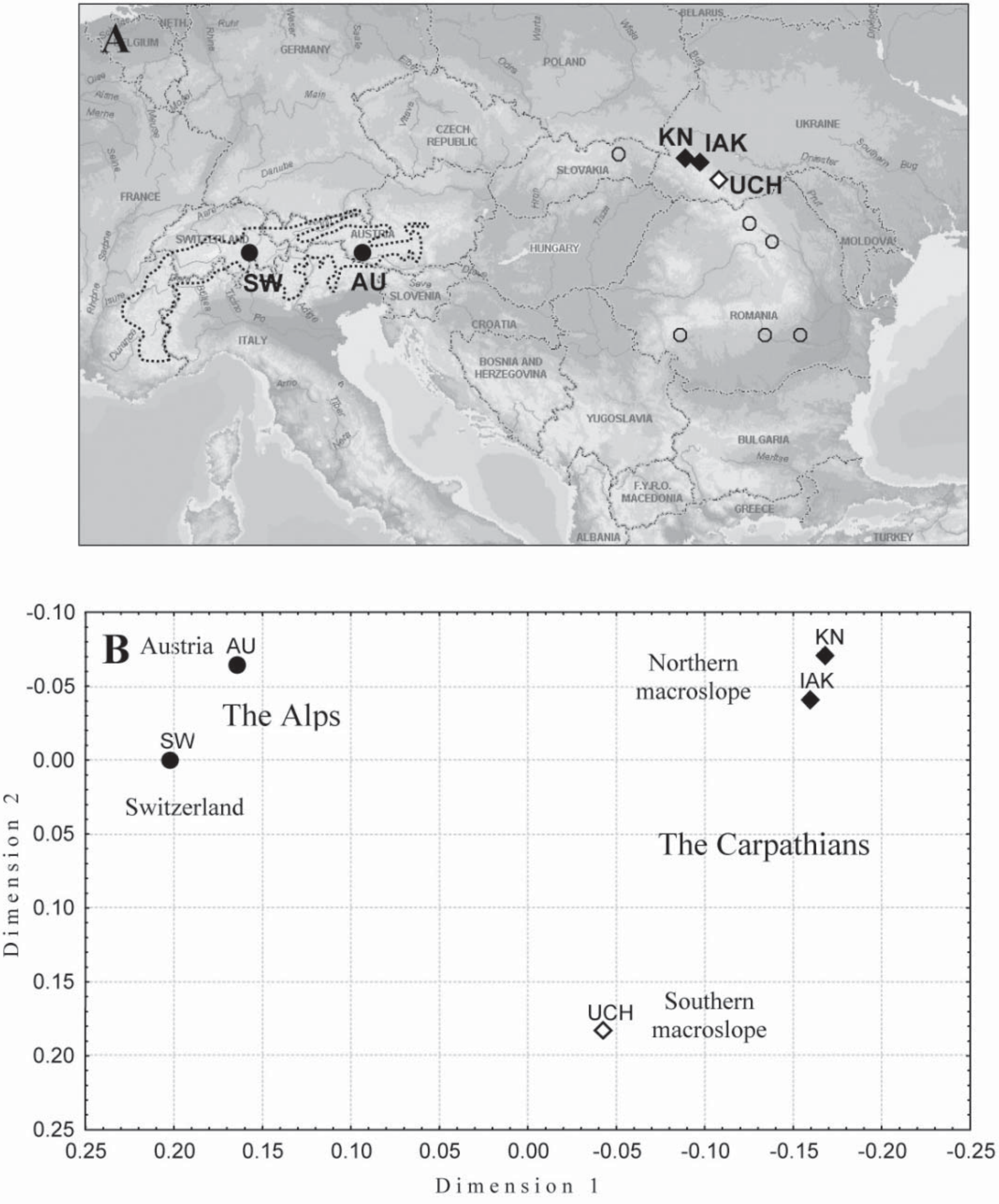


Figure 5—Sampling site locations (A) and results of Correspondence Analysis (B) of five populations of *P. cembra* based on allelic variation in 31 isozyme loci (Belokon, Belokon and Politov, unpublished). Eigenvalue 1 = 0.02466 (54.46 percent of inertia), eigenvalue 2 = 0.00891 (19,68 percent of inertia). Empty circles represent isolated populations or relatively small stands of *P. cembra* in the Carpathian Mountains that were not sampled.

Goncharenko and others (1993a) reported slightly higher differentiation (F_{ST}=4.3 percent) in five populations from two regions, the Chukotka Peninsula and Island of Sakhalin. However, the authors failed to find clear pattern in differentiation among populations. Similar to their abovementioned study of *P. sibirica*, this study has the same problem of the limited sample sizes (12.6 trees per population on average), which could bias estimation of allele frequencies in populations. Substantial genetic differentiation (F_{ST}=7.3 percent) was found in recent, more representative and wide ranging geographic studies of 22 populations of *P. pumila* sampled from the Lake Baikal region through the inland mountain ridges to the Pacific coast uncluding the Sakhalin Island (Maluchenko and others 1998; Politov 1998). The distribution of populations on a PCA plot based on 26 allozyme loci was in good correspondence to their geographic origin (fig. 6).

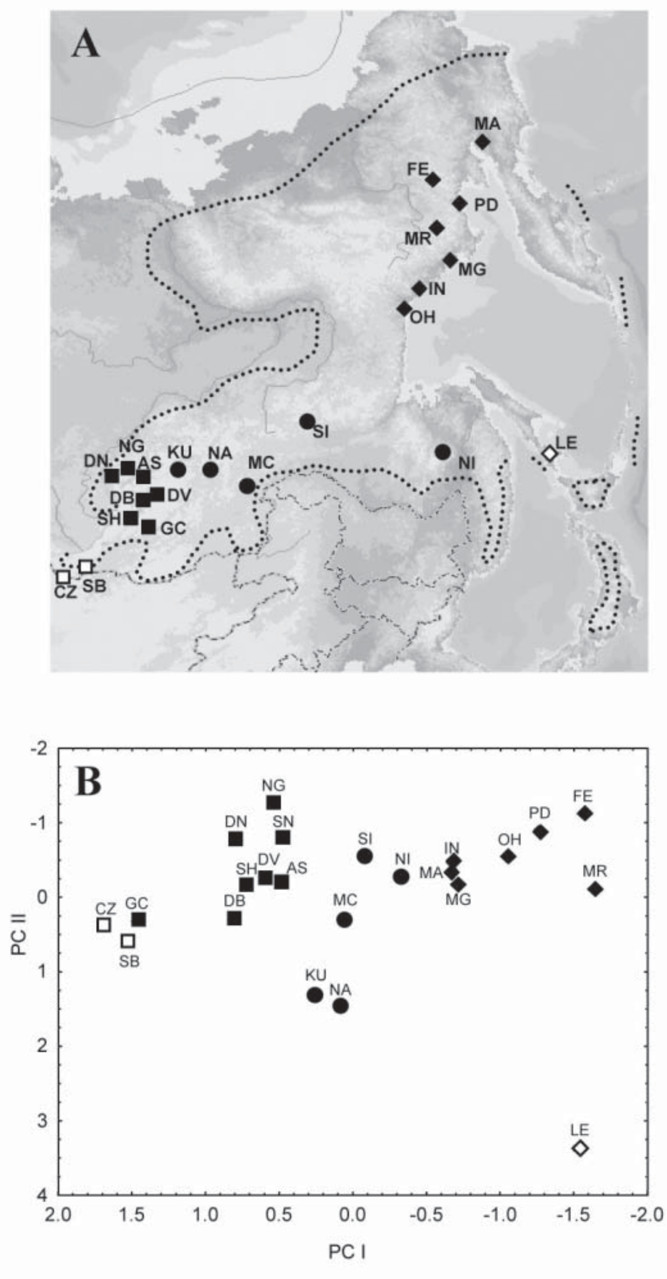


Figure 6—Sampling site locations (A) and results of Correspondence Analysis (B) of 23 populations of *P. pumila* based on allelic variation in 31 isozyme loci (Maluchenko and others 1998; Politov 1998). □ - Southeastern Baikal (Hamar-Daban Range), ■ - Northern Baikal, ● Inland Ridges, ◊ Sakhalin Island, ◆ - Northeastern coast of Sea of Okhotsk.

Pinus koraiensis

Early studies of three populations of P. koraiensis (Politov and others 1992; Krutovsky and others 1994, 1995) revealed a relatively low level of genetic differentiation (F_{ST}=4.0 percent). The F_{ST} value was even lower (1.5 percent) in a more representative study of 19 populations collected from most of *P. koraiensis*' range in Russia (Potenko and Velikov 1998), but the authors did not use any clustering technique to analyze genogeography. Belokon and Politov (2000) analyzed nine populations that included seven populations from three regions of Russian Far East (Primorski Territory, Amurskaya Oblast and Khabarovsk Territory) and two samples from northeastern China. However, populations did not cluster according to their geographic origin on the PCA plots. It appears that studies on genetic differentiation in *P. koraiensis* will require combined efforts of different scientists and more samples from the entire range (see also accompanying paper by V. V. Potenko).

Intra- and Interpopulation Genetic Variation in *Cembrae* Pines

The different number and type of isozyme loci or other genetic markers and the different size and origin of samples used in various studies make comparison among studies of levels of genetic variation between species and even within species problematic if not impossible. Estimates of genetic variation greatly varied in different studies of *Cembrae* pines. Expected heterozygosity (H_e) calculated from allele frequencies by assuming Hardy - Weinberg equilibrium in population and F_{ST} or G_{ST} parameters are the most common estimates of intrapopulation genetic variation and interpopulation genetic differentiation, respectively. We have summarized these estimates obtained in Russian populations of *Cembrae* pines (table 1). To make comparisons more accurate we presented only those H_e values that were based on the same set of loci. The F_{ST}/G_{ST} values based on the same or almost the same loci and obtained within a geographic range of the same or similar scale were also calculated and presented. Although these adjustments do not necessarily ensure correct comparisons, we believe that such comparisons are more objective than direct comparison of estimates based on unbalanced data. The H_e values increased in our study in the following order: *P. cembra* > *P. sibirica* > *P. koraiensis* > *P. pumila*. F_{ST} was also the greatest in *P. pumila* followed by *P. cembra*, *P. koraiensis*, and *P. sibirica* (fig. 7). The latter two species had similar F_{ST} values. We also calculated H_e values for 15 white pine species based on the

Table 1—Genetic diversity in Eurasian stone pine species (subsection *Cembrae*) estimated as expected heterozygosity (**H_e**) and interpopulation genetic differentiation (**F_{ST}** or **G_{ST}**) parameters using isozyme loci.

Species	Populations	Loci (mean)	F_{ST}/G_{ST}	F_{ST} [a]	H_e	Reference
P. cembra	5	30	0.047	0.065	0.093	Belokon, Belokon and Politov, unpublished
P. sibirica	32	19-31 (25)	0.025-0.063 [b]	0.035	0.124	Politov 1998
P. koraiensis	5	30	0.015-0.040	0.035	0.128	Belokon and Politov 2000
	19	26	-	-	0.182	Potenko and Velikov 1998
P. pumila	29	22-32 (30)	0.021-0.073	0.070	0.234	Politov 1998; Maluchenko and others 1998

[a] Calculated for populations within the same scale of geographic range and using the same set of loci for comparison.
[b] The range of estimates from different studies.

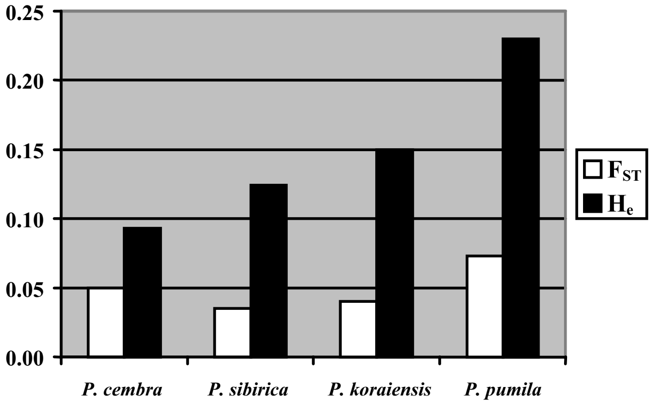


Figure 7—Comparison of intrapopulation (H_e) and interpopulation (F_{ST}) genetic diversity between four *Cembrae* pine species.

same set of 16 isozyme loci (table 2). There was considerable variation in H_e values among species, but differences were associated neither with subsections nor with subdivision of species into bird-dispersed versus wind-dispersed species (table 2). Birds are known as efficient dispersers that ensure substantial gene flow within *Cembrae* pine populations (Furnier and others 1987). It is commonly believed that nutcrackers may also be responsible for extensive migration among populations of pine species, with which they are associated (Bruederle and others 2001). However, although table 3 shows that F_{ST}/G_{ST} values were lower in bird-dispersed than in wind-dispersed pines, the variance of estimates was very high. Difference between mean values (0.057 ± 0.045 vs. 0.103 ± 0.082) for the two groups did not generally exceed the differences that often observed between different species within each group or between several independent studies conducted for the same species, but using different loci sets and/or different population range. Therefore, additional studies that are based on the same set of genetic markers and the same geographic and ecological range are needed to calculate F_{ST}/G_{ST} values that would allow a strong conclusion as to whether migration is higher in bird-dispersed pines.

In general, such factors as high gene flow (due to efficient mechanisms of seed and pollen dispersal) along with a high effective population size, a high stand density, and a predominantly outcrossing mating system should lead to a higher intrapopulation component of genetic variation and a lower differentiation among populations. This is a typical pattern of genetic structure observed in widely distributed coniferous species (Ledig 1998). However, the fact that F_{ST} values significantly vary among loci contradicts the common opinion that migration plays a major role in differentiation and possibly indicates a nonneutral nature of at least some of loci. Loci with low F_{ST} values can be subjected to balancing selection that equalize gene frequencies, while loci with higher than average values can be affected by diversifying selection, and loci with intermediate F_{ST} values can be neutral (Altukhov 1991). The introduction of new genetic markers that are more neutral than isozyme loci will provide an opportunity for testing this hypothesis and estimating the role of different factors in maintaining of genetic structure of *Cembrae* pines.

Heterozygosity Dynamics and Mating System

The combination of a haploid endosperm (megagametophyte) that represents a segregating maternal gamete with a diploid embryo in conifer seeds allows researchers to genotype two consecutive generations in seeds collected from individual trees. Genotypes of maternal trees can be inferred from segregation of isozyme alleles in megagametophytes. At the same time embryos of these seeds represent open-pollinated progenies (half- or full-siblings) that can be considered as next generation of individuals in a particular tree stand. Comparisons of observed genotype distributions in two generations with those that are expected assuming Hardy - Weinberg equilibrium revealed interesting and important trends of temporal changes in the level of heterozygosity in all Eurasian *Cembrae* pines. Embryos typically demonstrate slight to moderate deficiency of homozy-

Table 2—Genetic diversity in white pines (section *Strobus*) estimated as expected heterozygosity (**H_e**) parameter using the same 16 isozyme loci.

Species [a]	**H_e**
Subsection *Cembrae*	
P. cembra	0.082
P. sibirica	0.106
P. albicaulis	0.113
P. koraiensis	0.130
P. pumila	0.198
Mean	**0.126 ± 0.020**
Subsection *Strobi*	
P. armandii	0.080
P. wallichiana	0.091
P. strobus	0.093
P. parviflora	0.109
P. monticola	0.119
P. strobiformis	0.122
P. peuce	0.125
P. flexilis	0.128
P. morrisonicola	0.132
P. lambertiana	0.142
Mean	**0.114 ± 0.006**
Mean for bird-dispersed	**0.123 ± 0.012**
Mean for wind-dispersed	**0.112 ± 0.009**

[a] Underlined are species for which there is some evidence of bird-dispersal of seeds (Lanner 1996, 1998).

Table 3—Level of genetic differentiation between populations in bird- versus wind-dispersed white pines estimated as **F_{ST}** or **G_{ST}** parameters using isozyme loci.

Species	**Populations**	**Loci**	**Geographic scale**	**F_{ST} or G_{ST}**	**Reference**
			Bird-dispersed		
P. albicaulis	3	21	Local	0.004	Rogers and others 1999
	9	19	Local	0.025	Bruederle and others 1998
	14		Mediumwide	0.088	Yandell 1992
	30	17	Rangewide	0.034	Jorgensen and Hamrick 1997
P. sibirica	8	20	Mediumwide	0.039	Goncharenko and others 1993b
	11	17	Mediumwide	0.025	Krutovskii and others 1994, 1995
P. pumila	3	18	Local	0.021	Krutovskii and others 1994, 1995
	5	22	Mediumwide	0.043	Goncharenko and others 1993a
	18	19	Mediumwide	0.170	Tani and others 1996
	12	32	Rangewide	0.073	Maluchenko and others 1998
P. koraiensis	3	16	Mediumwide	0.040	Krutovskii and others 1995
	8	23	Mediumwide	0.059	Kim and others 1994
	10	26	Rangewide	0.063	Belokon and others unpublished
P. flexilis	8	10	Mediumwide	0.022	Schuster and others 1989
	30	24	Rangewide	0.101	Jorgensen and Hamrick 1997
	16	27	Rangewide	0.149	Hamrick and others 1994
	7	10	Rangewide	0.016	Latta and Mitton 1997
P. strobiformis	8	11	Rangewide	0.047	Ledig, pers. comm.
			Mean	**0.057 ± 0.045**	
			Wind-dispersed		
P. ayacahuite	2	23	Local	0.047	Ledig 1998
	14	23	Rangewide	0.222	Ledig 1998
P. monticola	28	12	Rangewide	0.148	Steinhoff and others 1983
P. strobus	27	12	Rangewide	0.080	Ryu and Eckert 1983
	10	18	Mediumwide	0.019	Beaulieu and Simon 1994
			Mean	**0.103 ± 0.082**	

gotes (Politov and others 1992; Politov and Krutovsky 1994; Krutovsky and others 1994, 1995; Politov 1998). Estimates of mating system parameters have shown this deficiency is caused by partial self-pollination that can occur with up to 15 percent in *P. sibirica* populations, 8 percent in *P. koraiensis,* and can be as high as 31 percent in *P. cembra.* The latter species exists in small isolated stands that can have a limited pollen flow distance, which often promotes self-pollination.

In contrast to embryos, heterozygote deficiency was not observed in mature trees (Politov and Krutovsky 1994; Krutovsky and others 1995). They typically demonstrate either Hardy – Weinberg equilibrium or slight heterozygote excess that likely indicates the selective elimination of progeny originated from self-pollination during early stages of life and probably, also the balancing selection in favor of heterozygotes in some loci (Politov and Krutovsky 1994; Krutovsky and others 1995). Assortative mating of parents with different genotypes and/or preferential fertilization of gametes with different haplotypes are mating system mechanisms that may potentially also contribute to the excess of heterozygosity, but it also would cause an excess of heterozygosity in both embryos and mature trees and would not explain the increase of heterozygosity with age. We are also unaware of any evidence or data that would prove that assortative mating of such kind occurs in pines or other conifers.

Interspecific Hybridization

Critchfield (1986) summarized data on interspecific crosses among white pines of the section *Strobus.* Successful crosses between pines within the subsection *Cembrae* such as *P. sibirica* x *P. cembra* were also mentioned in that review, although this successful hybridization is not surprising due to the phylogenetic proximity of the parental species. Titov (1988) obtained sound seeds from the cross between *P. sibirica* x *P. koraiensis*; however, these were never tested by genetic markers to confirm hybridity. Successful crosses between *Cembrae* and *Strobi* pines, such as *P. koraiensis* x *P. lambertiana* were also reported earlier (Bingham and others 1972).

Interspecific crosses of *P. sibirica* x *P. koraiensis* were conducted in Ivanteevka Arboretum (Moscow Region, Russia) in the late 1960s. Needles and buds from 27 putative "hybrid" mature trees were tested by 18 allozyme loci (Politov, Belokon and Belokon, unpublished). Only two trees were unambiguously identified as hybrids based on species specific alleles in loci *Gdh, Adh-1,* and *Lap-3*, while the other trees were apparently *P. sibirica.* It is worth noting that these hybrids were superior in growth and showed resistance to the pest *Pineus cembrae* Cholodkovsky 1888 (Adelgidae: Chermes).

Natural hybridization between *P. sibirica* and *P. pumila* in Lake Baikal region was confirmed genetically using isozyme analysis (Politov and others 1999). Both species were thoroughly studied in their allopatric areas and across a wide zone of their sympatric distribution. Putative natural hybrids were identified using 28 allozyme loci controlling 14 enzyme systems. The *Adh-1, Fe-2,* and *Lap-3* loci in the hybrids had genotypes that were typical for *P. sibirica*, but did not occur or were unlikely in *P. pumila*, while five other loci carried alleles and genotypes that are unknown in *P. sibirica*, but common in *P. pumila*. The *Skdh-2* locus was heterozygous for alleles, one of which was specific for *P. sibirica*, but another for *P. pumila*. Some embryos from the seeds of the hybrid were likely resulted from self-pollination while others from backcrosses with parental species. This was the first genetic evidence of natural hybridization and potential gene exchange between *P. sibirica* and *P. pumila*.

Hypothetically, gene exchange between *P. sibirica* and *P. pumila* may play a significant adaptive role. The zone of sympatry in the Baikal Region and Southern Yakutia is not optimal for both species and is intrinsically occupied by marginal populations. *P. sibirica* and *P. pumila* are adapted to different environments, and survival outside of their respective optimal environments may be promoted by genes from related species coming from another side of the sympatry zone with different environmental gradients. The frequency and distribution of hybrids in the sympatry zone of *P. sibirica* and *P. pumila* and their possible role in the species adaptation and evolution are still largely unknown. It is still questionable whether observed hybridization leads to extensive gene introgression, but if it is so the gene exchange could be a mechanism of increasing of total population fitness. Despite intercrossing among *Cembrae* species, we do not consider this fact alone as an evidence of their closer relationships to each other than to other white pines. Crossability and closeness of relationship can be correlated, but the lack of crossability is not convincing of a more distant relationship. Blada (1994) reported a number of successful artificial crosses between *P. cembra* and pines of subsection *Strobi.* Watano and others (1995, 1996) studied trees with morphological traits that were "intermediate" when compared to traits in "pure" *P. pumila* and *P. parviflora* using DNA markers, and proved the trees to be interspecific hybrids. High conservatism in the number and morphology of chromosomes probably facilitates hybridization of white pines as well as other *Pinaceae*, and interspecific gene exchange might be an important (although usually underestimated) factor of population genetic structure dynamics.

Acknowledgments

We thank Yuri Altukhov, Yuri Belokon, Maryana Belokon, Oleg Maluchenko, Alexey Podogas, and Natalia Gordon (Vavilov Institute of General Genetics, Moscow), Ekaterina Titorenko and German Ross (Moscow State University), and Le Thi Kinh (Cantho University) for their help in the study. This study was supported in part by grants from Russian Academy of Sciences (Cooperative Program "Plant Genome"), Russian Foundation for Basic Research (Program for Support of Scientific Schools), Russian Ministry of Science (Programs "Genetic Monitoring of Population Genetic Diversity" and "Development of Databases for Genetic Variability in Plants and Animals") and by Federal Program "State Support for Integration of Higher Education and Fundamental Research" (Educational and Scientific Center for Genetics).

References

Altukhov, Y.P. 1991. The role of balancing selection and overdominance in maintaining allozyme polymorphism. Genetica 85:79-90.

Beaulieu, J. and Simon, J.-P. 1994. Genetic structure and variability in *Pinus strobus* in Quebec. Can. J. For. Res. 24:1726-1733.

Belokon, M.M. and Politov, D.V. 2000. Evaluation of genetic diversity in Korean stone pine (*Pinus koraiensis* Sieb. et Zucc.) populations. *In* Proc. of the first conference on Conservation of biodiversity and sustainable management of natural resources, Moscow, p. 13.

Belokon, M.M., Politov, D.V., Belokon, Y.S., Krutovsky, K.V., Maluchenko, O.P. and Altukhov, Y.P. 1998. Genetic differentiation in white pines of section *Strobus*: Isozyme analysis data. Doklady Akademii Nauk. 358(5):699-702. Translated in English as Doklady Biological Sciences 358:81-84.

Bingham, R.T., Hoff, R.J. and Steinhoff, R.J. 1972. Genetics of western white pine. USDA Forest Service, Research Paper WO-12. Washington, DC. 28 p.

Blada, I. 1994. Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genet. 43:15-20.

Bobrov E. G. Forest-forming conifers of the USSR. Nauka, Moscow, 1978. 190 p.

Bruederle, L.P., Rogers, D.L., Krutovsky, K.V. and Politov, D.V. 2001. Population Genetics and Evolutionary Implications. *In* Tomback, D. F., Arno, S. F., Keane, R. E., editors. Whitebark Communities: Ecology and Restoration. Island Press, Washington, Covelo, London, pp. 137-153.

Critchfield, W.B. 1986. Hybridization and classification of the white pines (*Pinus* section *Strobus*). Taxon 35(4):647-656.

Critchfield, W.B. and Little, E.L. 1966. Geographic distribution of the pines of the world. USDA Forest Service, Miscellaneous Publication No. 991, Washington, D.C., USA. 98 p.

Furnier, G.R., Knowles, P., Clyde, M.A. and Dancik, B.P. 1987. Effects of avian seed dispersal on the genetic structure of whitebark pine populations. Evolution 41(3):607-612.

Goncharenko, G.G., Padutov, V.E. and Silin, A.E. 1991. Population structure, gene diversity and differentiation in natural populations of Cedar pines (*Pinus,* subsect. *Cembrae*, Pinaceae) in the USSR. Plant Syst. Evol. 182:121-134.

Goncharenko, G.G., Padutov, V.E. and Silin, A.E. 1993a. Allozyme variation in natural populations of Eurasian pines. I. Population structure genetic variation and differentiation in *Pinus pumila* (Pall.) Regel from Chukotsk and Sakhalin. Silvae Genet. 42(4-5):237-253.

Goncharenko, G.G., Padutov, V.E. and Silin, A.E. 1993b. Allozyme variation in natural populations of Eurasian pines. II. Genetic variation, diversity, differentiation, and gene flow in *Pinus sibirica* Du Tour in some lowland and mountain populations. Silvae Genet. 42:246-253.

Gugerli, F., Senn, J., Anzidei, M., Madaghiele, A., Buechler, C., Sperisen, C. and Vendramin, G.G. 2001. Chloroplast microsatellites and mitochondrial *nad*1 intron 2 sequences indicate congruent phylogenetic relationships among Swiss stone pine (*Pinus cembra*), Siberian stone pine (*Pinus sibirica*), and Siberian dwarf pine (*Pinus pumila*). Mol. Ecol. 10:1489-1497.

Hamrick, J.L., Schnabel, A.F. and Wells, P.V. 1994. Distribution of genetic diversity within and among populations of Great Basin conifers. *In*: Harper, K.T., St.Clair, L.L., Thorne, K.H. and Hess, W.W., editors. Natural history of the Colorado Plateau and Great Basin. University Press of Colorado, Niwot, Colorado, USA, pp. 147-161.

Iroshnikov A. I. 1974. Polimorphism in populations of Siberian stone pine [Russian]. Variability in conifers of Siberia. Institute of Forest, Siberian Branch of Russian Academy of Sciences, Krasoyarsk, Russia: pp. 77-103.

Jorgensen, S.M. and Hamrick, J.L. 1997. Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. Can. J. For. Res. 27:1574–1585.

Kim Z.-S., Lee S.-W., Lim J.-H., Hwang J.-W., Kwon K.-W. 1994. Genetic diversity and structure of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Korea. Forest Genetics 1 (1): 41-49.

Krutovsky, K.V., Politov, D.V. and Altukhov, Y.P. 1987. Genetic variability in Siberian stone pine *Pinus sibirica* Du Tour. I. Genetic control of isozyme system. Genetika (Russian) 23(12):2216-2228. Translated in English as Soviet Genetics (1988) 23:1568-1576.

Krutovsky, K.V., Politov, D.V. and Altukhov, Y.P. 1989. Genetic variability in Siberian stone pine Pinus sibirica Du Tour. IV. Genetic diversity and amount of genetic differentiation between natural populations. Genetika (Russian) 25(11):2009-2032. Translated in English as Soviet Genetics (1990) 25:1343-1362.

Krutovsky, K.V., Politov, D.V. and Altukhov, Y.P. 1990. Interspecific genetic differnetiation of pines of Eurasian stone pines for isozyme loci. Genetika (Russian) 26(4):694-707. Translated in English as Soviet Genetics (1990) 26:440-450.

Krutovsky, K.V., Politov, D.V. and Altukhov, Y.P. 1994. Study of genetic differentiation and phylogeny of stone pine species using isozyme loci. *In*: Schmidt, W.C. and Holtmeier, F.-K., compilers. Proceedings - International workshop on subalpine stone pines and their environment: The status of our knowledge. September 5-11, 1992, St. Mortiz, Switzerland. USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GRT-309, Ogden, Utah, pp. 19-30.

Krutovsky, K.V., Politov, D.V. and Altukhov, Y.P. 1995. Isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pine species (subsection *Cembrae*, section *Strobi*, subgenus *Strobus*). In: Baradat, P., Adams, W.T. and Mueller-Starck, G., editors. Population genetics and genetic conservation of forest trees. SPB Academic Publishing, Amsterdam, the Netherlands, pp. 279-304.

Kuprianova, L.A. and Litvintsetva, M.V. 1974. The group *Cembra* of the genus *Pinus*, its volume and relationships according to palynological data. Botanicheskii Zhurnal 59(5):630-644.

Lanner, R.M. 1990. Biology, taxonomy, evolution and geography of stone pines of the world. *In*: Schmidt, W. C. and McDonald, K. J., compilers. Proceedings of the Symposium on Whitebark Pine Ecosystems: Ecology and Management of a High-Mountain Resource, Bozeman, Montana, March 29-31, 1989. USDA Forest Service General Technical Report INT-270, Ogden, Utah, USA, pp. 14-24.

Lanner, R.M. 1996. Made for each other. A symbiosis of birds and pines. Oxford University Press, New York, New York, USA.

Lanner, R.M. 1998. Seed dispersal in *Pinus*. *In* Richardson, D.M., editor. Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge, UK, pp. 281-295.

Latta, R.G. and Mitton. J.B. 1997. A comparison of population differentiation across four classes of gene marker in limber pine (*Pinus flexilis* James). Genetics 146:1153-1163.

Ledig, F.T. 1998. Genetic variation in *Pinus*. *In*: D. M. Richardson, editor. Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge, UK, pp. 251-280.

Liston, A., Robinson, W.A., Pinero, D. and Alvarez-Buylla, E.R. 1999. Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Mol. Phylogenet. Evol. 11(1):95-109.

Maluchenko, O.P., Politov, D.V., Belokon, Y.S. and Belokon, M.M. 1998. Genetic differentiation of Siberian dwarf pine *Pinus pumila* (Pall.) Regel in Lake Baikal region. *In* Zhukova, L.A., Glotov, N.V., Zhivotovski, L A., editors. Life of populations in heterogeneous environment. Periodika Mariy El, Yoshkar-Ola, Russia, pp. 38-45.

Muratova, E.A. 1980. Caryotypes of pines of the *Cembra* group. Botanicheskii Zhurnal (Russian) 65(8):1130-1138.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Pirko, Y.V. 2001. Population genetic variation in three indigenous species of the genus *Pinus* in the Ukrainian Carpathians and Roztochiye. Ph.D. Dissertation. Nat. Acad. Sci. of Ukraine, Institute of Cell Biology and Genetic Engeneering, Kiyv. 187 p.

Podogas, A.V. 1993. Genetic differentiation in the genus *Pinus* by allozyme loci. Ph.D. Dissertation. Russian Academy of Sciences, Vavilov Institute of General Genetics, Moscow. 154 p.

Politov, D.V. 1998. Coniferous Forests of Baikal Lake Region: Native Population Genetic Structure and Human Impact. UNESCO Programme on Man and the Biosphere (MAB), MAB Young Scientist Research Award Scheme - Final Scientific Report. http://www.unesco.org/mab/capacity/mys/97/Politov/Politov.htm.

Politov, D.V., Belokon, M.M., Maluchenko, O.P., Belokon, Y.S., Molozhnikov, V.N., Mejnartowicz, L.E. and Krutovsky, K.V. 1999. Genetic evidence of natural hybridization between Siberian stone pine, *Pinus sibirica* Du Tour, and dwarf Siberian pine, *P. pumila* (Pall.) Regel. For. Genet. 6(1):41-48.

Politov, D.V., Krutovsky, K.V. and Altukhov, Y.P. 1989. Genetic variability in Siberian stone pine *Pinus sibirica* Du Tour. III. Linkage among isozyme loci. Genetika (Russian). 25(9):1606-1618. Translated in English as *Soviet Genetics*, 1990, March: 1053-1063.

Politov, D.V., Krutovsky, K.V. and Altukhov, Y.P. 1992. Characterization of gene pools of *Cembrae* pines on the set of isozyme loci. Genetika (Russian) 27(1):93-114. Translated in English as *Soviet Genetics*, 1992, July: 76-95.

Potenko, V.V. and Velikov, A.V. 1998. Genetic diversity and differentiation of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Russia. Silvae Genet. 47(4):202-208.

Price, R.A., Liston, A. and Strauss, S.H. 1998. Phylogeny and systematics of *Pinus*. *In*: Richardson, D.M. (ed.), Ecology and Biogeography of Pinus. Cambridge University Press, Cambridge, UK, pp. 49-68.

Rogers, D.L., Millar, C.I. and Westfall, R.D. 1999. Fine-scale genetic structure of whitebark pine (*Pinus albicaulis*): associations with watershed and growth form. Evolution 53:74-90.

Ryu, J.B. and Eckert, R.T. 1983. Foliar isozyme variation in twenty seven provenances of *Pinus strobus* L: genetic diversity and population structure. *In*: R.T. Eckert, editor. Proceedings of the Twenty-eighth Northeastern Forest Tree Improvement Conference. University of New Hampshire, Durham, New Hampshire, pp. 249-261.

Schuster, W.S., Alles, D.L. and Mitton, J.B. 1989. Gene flow in limber pine: Evidence from pollination phenology and genetic differentiation along an elevational transect. Am. J. Bot. 76 (9):1395–1403.

Shurkhal, A., Podogas, A. and Zhivotovsky, L. 1992. Allozyme differentiation in the genus *Pinus*. Silvae Genet. 41(2):105-109.

Smolonogov, E.P. 1994. Geographical differentiation and dynamics of Siberian stone pine forests in Eurasia. *In*: Schmidt, W.C. and Holtmeier, F.-K., compilers. Proceedings - International workshop on subalpine stone pines and their environment: The status of our knowledge. September 5-11, 1992, St. Mortiz, Switzerland. USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GRT-309, Ogden, Utah, pp. 275-279.

StatSoft, Inc. 1998. STATISTICA for Windows [Computer program manual]. Tulsa, OK: StatSoft, Inc.

Steinhoff, R.J., Joyce, D.G. and Fins, L. 1983. Isozyme variation in *Pinus monticola*. Can. J. For. Res. 13:1122-1132.

Szmidt, A.E. 1982. Genetic variation in isolated populations of stone pine (*Pinus cembra*). Silva Fenn. 16(2):196-200.

Tani, N., Tomaru, N., Araki, M. and Ohba, K. 1996. Genetic diversity and differentiation in populations of Japanese stone pine (*Pinus pumila*) in Japan. Can. J. For. Res. 26(8):1454-1462.

Titov, E.V. 1977. Opyt skreschivanija kedra sibirskogo s drugimi sosnami v uslovijakh severo-vostochnogo Altaja. Lesovedenie (Russian) 4:81-87.

Titov, E.V. 1988. Interspecific hybridization of stone pines (Russian). In: Czarev, A.P., editor. Hybridization of forest trees. Gosgkomles, CNIILGiS,Voronezh, pp. 76-84.

Tomback, D.F. and Linhart, Y.B. 1990. The evolution of bird-dispersed pines. Evol. Ecol. 4:185-219.

Wang, X.-R. and Szmidt, A.E. 1993. Chloroplast DNA-based phylogeny of Asian *Pinus* species (*Pinaceae)*. Plant Syst. Evol. 188:197-211.

Wang, X.-R., Tsumura, Y., Yoshimaru, H., Nagasaka, K. and Szmidt, A.E. 1999. Phylogenetic relationships of Eurasian pines (*Pinus*, Pinaceae) based on chloroplast *rbcL, MATK, RPL20-RPS18* spacer, and *TRNV* intron sequences. Am. J. Bot. 86(12):1742-1753.

Watano, Y., Imazu, M. and Shimizu, T. 1995. Chloroplast DNA typing by PCR-SSCP in the *Pinus pumila - P. parviflora* var. pentaphylla complex (Pinaceae). J. Plant Res. 108: 493-499.

Watano, Y., Imazu, M. and Shimizu, T. 1996. Spatial distribution of cpDNA and mtDNA haplotypes in a hybrid zone between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae). J. Plant Res. 109:403-408.

Yandell, U.G. 1992. An allozyme analysis of whitebark pine (*Pinus albicaulis* Engl.). M.Sc. Thesis, University of Nevada, Reno, 1992, 64 pp.

Yang Y., Wang S., Yin R. 1989. Genetic variation of isoenzymes within and among populations of Korean pine (*Pinus koraiensis* Seib. et Zucc.). Scientia Silvae Sinicae 25 (3): 201-208.

Studies of Genetic Variation with Five-Needle Pines in Germany

Bruno Richard Stephan

Abstract—After 30 years, a field trial with 65 seed samples of eastern white pine (*Pinus strobus*) showed that the best growing provenances have their origin in the Appalachians south of latitude 39°, and provenances with the slowest growth in regions north of latitude 45°. Provenances from regions between 39° and 45° latitude varied greatly in their growth, even when their origins were from adjacent locations. The great interspecific and intraspecific differences of five-needle pines in resistance to the blister rust fungus *Cronartium ribicola* was demonstrated by resistance tests. Obvious racial variation in the blister rust fungus was found by a joint inoculation experiment with alternate hosts (*Ribes* and *Pedicularis*).

Key words: Eastern white pine, *Pinus strobus*, provenance trial, blister rust, growth performance, *Pinus cembra*, *P. wallichiana*, *P. peuce*, *P. parviflora*

Introduction

Europe has in contrast to North America only two native five-needle pine species. Only Swiss stone pine (*Pinus cembra* L.) is native in Germany. This species occurs in small populations in the Alpine regions of southern Germany at elevations up to 1867 m.

The second European species is the Macedonian pine (*Pinus peuce* Griseb.) of the mountainous Balkans. This species is rather slow-growing and in general of less interest for forestry practice. However, because of a relatively high tolerance against air pollutants, it is suitable for afforestation in southeastern Germany (Lattke and others 1987, Lattke 1998), where other tree species (for example Norway spruce) were severely damaged or even eliminated during recent decades.

In addition to these native species, several other five-needle pine species were introduced, mainly for ornamental purposes: The Himalayan white pine (*Pinus wallichiana* A. B. Jacks.) from Pakistan and India is grown in parks and larger gardens in the warmer climate of southwestern Germany. In other regions the trees are subject to damage from late frost, as shown in some provenance trials (for example Stephan 1974).

The Japanese white pine (*Pinus parviflora* Sieb. et Zucc.) is a common ornamental tree species in parks, arboreta, gardens and cemeteries, where it is of interest because of its slow growth and attractive blue needle colour.

Pinus strobus L., the eastern white pine from North America, is the only five-needle pine with extensive silvicultural use in Germany. The species was introduced in Europe in 1605. It can be grown successfully under various environmental site conditions and shows good natural regeneration. The main disadvantage is the high susceptibility to blister rust disease caused by *Cronartium ribicola* J.C. Fischer.

In the following paper some results of a provenance trial with *P. strobus* will be presented. In addtion, results are summarized from resistance tests with several five-needle pine species and studies of variation in *Cronartium ribicola*.

Materials and Methods

Provenance Trials

Provenance trials with 65 seed samples from the natural range of *P. strobus* were started in 1963. Geographical data for the provenances are given in table 1. Field trials were established with 3-year-old plants on two sites in northern Germany in 1966 and 1967. Measurements and assessments of growth performance, mortality (rust and non-rust related) and the presence of stem infections as well as branch infections by blister rust were conducted in the following years. Detailed information is given in earlier papers (Stephan 1974, 1986a). The last evaluations were carried out in 1994 when the trees were 32 years old, and these data are presented here. Because of the design of the trial and the narrow space within the plots, further exact evaluations are not possible.

Resistance Test with Five-Needle Pines

The Institute for Forest Genetics at Grosshansdorf participated in the international IUFRO experiment testing resistance of white pines to *Cronartium ribicola*. This joint experiment was initiated by Bingham and Gremmen (1971). A total of 17 five-needle pine species including 76 provenances and progenies were tested by artificial inoculation (Stephan 1986b). About 10,000 pine plants were grown in containers under greenhouse conditions at temperatures above 0 °C to avoid frost damage of the frost sensitive species. For artificial inoculations, rust-infected leaves of the alternate host *Ribes nigrum* L. were used. The pine seedlings were inoculated at an age of two years. Each plant was assessed annually for the rust symptoms, beginning with needle lesions or spots, appearance of spermogonia, normal cankers and/or bark reactions, and blisters with

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The author is with the Institute for Forest Genetics and Forest Tree Breeding, Siekerlandstrasse 2, D-22927 Grosshansdorf, Germany. Telephone and fax no.: + 49 - 4102 – 63555. E-mail: r-c.stephan@t-online.de

Table 1—Provenance trial with *Pinus strobus* at Forest District Nordhorn (Wielen Ki 26) in northwestern Germany. Provenances are listed in order of their volume under bark per ha at age 27.

Seed-book no.	County, state	Latitude N	Longitude W	Altitude (m)	Height (m) age 27	Dbh (cm) age 32	m^3/ha age 27
3862	Newaygo, MI	43°43'	85°55'	262	13.30	26.00	321.8
3851	South Carolina	34°39'	82°55'	533	13.50	30.35	319.7
3874	Clearfield, PA	41°00'	78°27'	—	12.80	—	307.7
3800	Henderson, NC	35°20'	82°30'	671	13.03	22.00	304.9
3850	Kentucky	37°00'	87°00'	—	13.25	29.00	285.9
3812	Garrett, MD	39°30'	79°25'	707	12.95	21.40	279.7
3871	Garrett, MD	39°30'	79°25'	707	12.70	27.67	281.2
3829	Carroll, VA	36°42'	80°52'	780	13.13	29.00	275.7
3803	Schoharie, NY	42°45'	74°25'	274	12.60	—	272.8
3839	Buncombe, NC	35°28'	82°32'	655	13.00	28.50	274.4
3861	Manistee, MI	44°16'	86°03'	213	13.05	26.70	269.9
3857	McKean, PA	41°42'	78°55'	457	12.78	22.35	272.0
3818	Washington, MD	39°41'	78°14'	194	12.78	27.25	269.0
3875	Warren, NY	43°37'	73°44'	305	13.35	21.85	267.2
3825	Saratoga, NY	43°00'	73°43'	152	12.95	28.50	260.8
3827	Dunn/Polk, WI	45°00'	91°19'	366	12.35	26.10	260.1
3804	Berkshire, MA	42°30'	73°14'	274	12.80	24.40	258.0
3872	Pike, PA	41°10'	75°00'	335	12.38	27.50	251.5
3837	Oconee, SC	34°50'	83°10'	457	12.95	21.75	246.5
3876	Warren, NY	43°41'	73°41'	396	12.55	26.25	244.4
3810	Middlesex, CT	41°38'	72°30'	—	13.03	26.00	240.8
3842	Quebec, QC	46°55'	71°31'	168	12.95	24.50	239.9
3823	Litchfield, CT	41°58'	73°13'	390	12.58	26.25	238.8
3809	Hillsborough, NH	43°06'	71°55'	262	12.05	23.30	241.3
3838	Wytha, VA	37°00'	81°15'	762	12.65	19.25	237.5
3801	Greenbrier,WV	38°59'	80°09'	686	12.50	25.15	231.8
3808	Chittenden, VT	44°27'	73°12'	91	12.28	25.50	236.1
3802	Strafford, NH	43°08'	70°57'	31	12.53	25.07	232.5
3821	Somerset, PA	39°47'	79°02'	640	12.95	24.00	228.9
3820	Somerset, PA	39°47'	79°02'	640	12.08	26.00	234.2
3824	Litchfield, CT	41°58'	73°13'	408	12.40	25.75	234.2
3815	Allegany, MD	39°40'	78°28'	239	12.50	23.40	226.9
3853	Schoharie, NY	42°45'	74°25'	305	12.63	24.00	226.8
3870	Strafford, NH	43°08'	70°56'	18	12.10	23.05	227.7
3822	Tucker, WV	39°10'	79°35'	503	12.38	26.57	227.6
3819	Garrett, MD	39°42'	79°08'	678	13.23	24.93	228.5
3830	Carroll, VA	36°37'	80°53'	780	12.75	22.90	233.0
3835	Sauk, WI	43°30'	89°55'	305	12.18	25.15	227.4
3805	Juneau, WI	43°35'	90°00'	210	12.00	23.00	217.7
3806	Chittenden, VT	44°28'	73°09'	290	12.15	24.67	216.6
3814	Preston, WV	39°33'	79°29'	777	11.63	22.00	208.9
3833	Garrett, MD	39°33'	79°21'	756	12.48	16.00	213.3
3828	Coerthier, QC	46°17'	73°25'	213	12.30	25.00	207.4
3855	Addison, VT	44°07'	73°13'	122	12.30	24.03	206.4
3834	Garrett, MD	39°25'	79°24'	701	12.00	23.50	201.8
3811	Garrett, MD	39°30'	79°25'	707	12.38	24.20	201.7
3836	Sauk, WI	43°30'	89°55'	305	12.25	22.15	199.0
3843	Sawyer, WI	46°00'	91°25'	—	12.95	22.25	194.3
3844	Renfrew, ON	45°57'	77°27'	160	11.50	23.00	194.2
3807	Chittenden, VT	44°28'	73°09'	122	12.23	23.85	195.0
3841	Lake, MN	48°02'	91°36'	402	12.15	22.50	188.4
3877	Essex, NY	44°20'	73°46'	229	12.05	21.70	181.7
3845	Sunbury, N.B.	46°22'	66°11'	122	11.50	26.75	175.5
3848	LaSalle, IL	41°19'	88°59'	155	11.90	23.73	173.4
3840	York, ME	43°22'	70°53'	122	11.83	20.33	169.8
3854	Todd, MN	46°21'	94°12'	405	12.58	21.70	170.1
3826	Itasca, MN	47°19'	93°34'	397	12.13	21.10	166.0
3846	Quebec, QC	46°57'	71°31'	305	12.25	24.00	147.5
3813	Preston, WV	39°33'	79°29'	786	11.75	21.50	139.7
3847	Manitoba, MN	54°00'	100°00'	—	11.40	20.90	130.1
3856	Ogle, IL	41°57'	89°23'	221	11.00	20.00	119.5
3831	North Carolina	—	—	—	12.75	27.00	—
3852	Wisconsin	—	—	—	12.73	26.90	—
3849	Michigan	—	—	—	12.50	26.33	—
3832	North Carolina	—	—	—	11.70	21.37	—
Average					12.47	24.23	228.4

aeciospores. Rust related and non-rust related mortality was recorded. During the experiment all plants were kept in greenhouses until they reached the age of eight to nine years. The white pine plants were not exposed to further natural infections.

Studies on Race Differences of *C. ribicola*

The objective of these studies was to investigate the extent of pathogenic variation in *C. ribicola* in ability to infect alternate host species. Therefore, joint inoculation experiments were carried out with *Ribes nigrum* L. (*Saxifragaceae*) and *Pedicularis resupinata* L. (*Scrophulariaceae*) in Germany and South Korea. Various cultivars of *R. nigrum* are grown in Germany and are the main alternate hosts of the white pine blister rust fungus. *Pedicularis resupinata* is native in eastern Asia and is also an alternate host plant of *C. ribicola*. Both alternate host species or cultivars were grown in Germany as well as in South Korea, and inoculated with the respective *C. ribicola* aeciospores in both countries. Further details of the materials and methods are given in the paper of Stephan and Hyun (1983).

Results

Provenance Trial with *Pinus strobus*

Height growth (age 27), stem diameter at breast height (1.3 m) (age 32) and calculated total volume under bark (m^3/ha) (age 27) are shown as an example for the trial at Wielen (Ki 26), northwestern Germany, in table 1. The averages for height growth at age 27 varied between 11 m and 13.5 m, for diameter at age 32 from 16 cm to about 30 cm, and for volume at age 27 from 119.5 m^3/ha to 321.8 m^3/ha.

The performance of the provenances at the two test sites was very similar. The provenances differed significantly in their growth performance at different ages. Traits are correlated negatively with provenance latitude (for correlation coefficients see Stephan 1974). Trees from provenances in the southern Appalachians south of 39° latitude grew well under the conditions in northern Germany (fig. 1). Provenance samples from the regions north of latitude 45° grew poorly. Provenance samples from areas between 39° and 45° latitude showed great variation in growth rate.

Differences between provenances in blister rust infection could be observed. As the trees were mostly infected at the lower part of the stems or branches, it could be assumed that they were obviously infected already as young plants in the nursery. The range in rust infection was relatively low and varied from 2 percent and 7 percent between the two test sites, but from 0 percent to 25 percent between provenances. There was a weak correlation ($r = 0.32$) among provenances over the two test sites. A correlation between origin of the provenances and infection could not be found. One provenance from Maryland and one from Quebec had no rust infected trees after seven years in the field. On the contrary, 25 percent trees of a provenance from Wisconsin were rust infected.

Wood density of the provenances was investigated separately and varied only slightly, ranging from 0.257 to 0.290 g/cm^3 with an average of 0.271 g/cm^3.

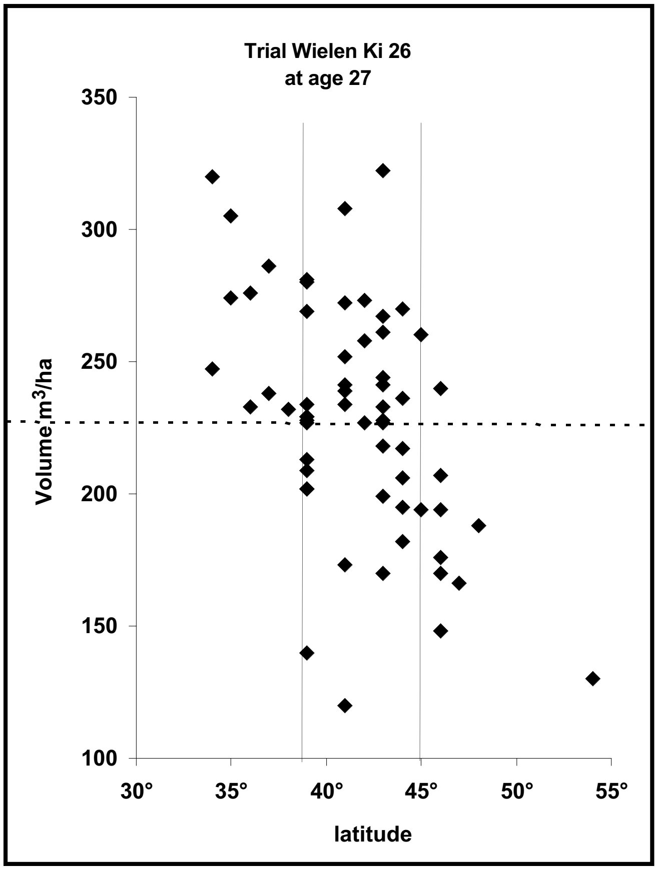


Figure 1—Volume under bark (m^3/ha) at 27 years of 61 provenances of eastern white pine in relation to geographic latitude at one provenance trial (Ki 26) site location at Wielen, northwestern Germany.

Differences Among Five-Needle Pines in Blister Rust Resistance

There was wide genetic variation among pine species, provenances and progenies in the reaction after artificial inoculation with basidiospores of *C. ribicola* (table 2). Generally, European and Asian pines remained uninfected or were less infected by the rust fungus on the basis of needle lesions, percent of trees with stem symptoms and mortality rate, than the extremely susceptible North American pines. Particularly *P. cembra*, *P. armandii* and *P. pumila* showed neither needle symptoms nor stem symptoms. Large differences of percent rust infected trees existed among seed samples within the Asian species *P. parviflora* and *P. wallichiana* (table 2). Among the North American pine species, four *P. aristata* provenance samples had the lowest percentage of tree with stem cankers (mean of 66 percent).

Progenies from crosses between selected rust-free parents from two of the North American species had some trees with heavy infection, but in general they had fewer infected trees than did trees in provenances of their respective pine species six and a half years after inoculation (see *P. lambertiana* and *P. monticola* in table 2). A few years later, however, these canker-free progenies were also heavily infected by blister

Table 2—Blister rust infection (percent of trees with cankers) of 8- and 9-year-old white pines about 6 years after artificial inoculation with *Cronartium ribicola*.

White pine species	No. of provenances/progenies	Blister rust attack (%) Mean (provenances)	Mean (species)
Europe			
Pinus cembra	1	0	0
P. peuce	6	0-30	22
Asia			
P. armandii	2	0	0
P. pumila	1	0	0
P. sibirica	1	—	17
P. parviflora	3	0-67	22
P. koraiensis	3	18-29	23
P. wallichiana	5	17-60	40
P. morrisonicola	1	—	40
North America			
P. aristata	4	50-94	66
P. strobiformis	4	75-95	88
P. balfouriana	4	74-100	90
P. lambertiana	6	88-100	97
— R-progeny [a)]	1	—	76
P. albicaulis	2	94-100	97
P. flexilis	4	97-100	98
P. monticola	6	93-100	99
— R-progenies [a)]	10	85-100	97
P. strobus	8	98-100	100
P. strobus (Germany)	4	60-88	71

[a)] R-progenies = F_1 and F_2 progenies from controlled crosses between parent trees resistant in North America

rust and subsequently died. Canker development in these progenies seemed to require more time. Natural infection was excluded as all trees were grown in greenhouses far away from alternate host plants.

Interestingly, the four seed samples of *P. strobus* populations grown in Germany and used in the inoculation experiment were obviously more tolerant to the German blister rust race than were autochthonous samples of North American *P. strobus* (table 2).

Genetic Variation Within *Cronartium ribicola*

Alternate hosts of the white pine blister rust fungus were inoculated simultaneously with aeciospores of the fungus in a joint experiment in Germany and South Korea. In the German trial only *Ribes nigrum* and in the Korean trial only *Pedicularis resupinata* were infected, although in both countries the respective other alternate host was also inoculated. Urediniospores and teliospores were formed after infection only on the leaves of *R. nigrum* in Germany and *P. resupinata* in South Korea. The respective other host plant species remained uninfected. Therefore, one can assume that the *C. ribicola* types used in both countries differed in their pathogenicity. Differences between various *C. ribicola* samples regarding the size of aeciospores and urediniospores could not be found.

Discussion

Pinus strobus is the most important species among the white pines of interest for forestry uses in Germany (Ritter 1978, Stratmann 1988, Waldherr 2000). The first plantation was established in southwestern Germany around 1770 (Stratmann 1988). Growth performance and natural regeneration are superior compared to the native Scots pine (*P. sylvestris* L.). The main problem is its high susceptibility to blister rust, presenting an obstacle to its otherwise desirable use as a main tree species for silviculture. First observations of the blister rust disease are known from Estonia (northeastern Europe) around 1854. Thirty years later the fungus had reached the Atlantic Ocean in western Europe and had caused tremendous losses of *P. strobus* afforestations. Therefore, around 1930 growing of eastern white pine was prohibited. Later the prohibition was again canceled and instructions for the afforestation of *P. strobus* were given. To avoid most severe losses it is recommended that eastern white pines be planted in mixture with other tree species and at a greater distance than at present from villages and plantations, where the alternate host *Ribes nigrum* is cultivated. As evidenced by the lower blister rust infection of the German land race (compared to the North America provenances) some natural selection for *C. ribicola* resistance may be occurring. Further investigation of the potential for developing more resistance in the German land race may be warranted.

There is a wide intraspecific variation in *P. strobus*, as shown by provenance trials. The results of the German trials agreed very well with those in the United States of America, Australia and New Zealand (Genys and others 1978). In Germany, southern provenances from the Appalachians are of particular interest. They seem to be very well adapted to climatic and other site conditions, but, unfortunately, resistant progenies of *P. strobus* are not available yet.

The resistance tests clearly showed that the German blister rust race used for artificial inoculations was more aggressive than the race used in western North America (Idaho), since progenies of rust resistant parents of *P. lambertiana* and *P. monticola* were also heavily infected (table 2). Our results were in a generally good agreement with French results, but differed from the American test results (Delatour and Birot 1982, Stephan 1986a). This may demonstrate similarity within the European blister rust fungus, but differences from the North American fungal type.

Race differences have also been found between the German and South Korean blister rust fungus, and a wider pathogenic variation of *C. ribicola* can be assumed in eastern Asia, for example in Korea and Japan (Stephan and Hyun 1983). These areas can be considered as the main gene centers, where host and pathogen coexisted during long periods. Because of the common coevolution tolerance of the host as well as virulence of the parasite are there in a dynamic equilibrium (Leppik 1970).

Acknowledgments

The author is indebted to Professor Howard B. Kriebel and an anonymous reviewer for help with the manuscript and valuable comments.

References

Bingham, R.T. and Gremmen, J. 1971. A proposed international program for testing white pine blister rust resistance. Eur. J. For. Path. 1: 93-100.

Delatour, C. and Birot, Y. 1982. The international IUFRO experiment on resistance of white pines to blister rust. The French trial. *In* Heybroek, H.M., Stephan, B.R. and von Weissenberg, K. (Eds.). Resistance to diseases and pests in forest trees. PUDOC, Wageningen, pp. 412-414.

Genys, J.B., Canavera, D., Gerhold, H.D., Jokela, J.J., Stephan, B.R., Thulin, I.J., Westfall, R. and Wright, J.W. 1978. Intraspecific variation of eastern white pine studied in U.S.A., Germany, Australia and New Zealand. Center for Environmental and Estuarine Studies, Univ. of Maryland, College Park. Special Report No. 8: 27 pp.

Lattke, H. 1998. Kiefern für die Immissionsschadgebiete der Mittelgebirge - züchterische Ergebnisse und Perspektiven. Schriftenreihe der Sächsischen Landesanstalt für Forsten, Graupa, Germany, Heft 13/98: 24-35.

Lattke, H., Braun, H. and Richter, G. 1987. *Pinus peuce* Griseb., eine erfolgversprechende Alternativbaumart für die Schadgebiete des oberen Erzgebirges. Sozial. Forstwirtschaft 37: 279-282.

Leppik, E.E. 1970. Gene centers of plants as sources of disease resistance. Ann. Rev. Phytopath. 8: 323-344.

Ritter, H. 1978. Die Weymouthskiefer – eine verlorene Holzart? Holz-Zentralblatt 104: 1287-1288.

Stephan, B.R. 1974. Zur geographischen Variation von *Pinus strobus* aufgrund erster Ergebnisse von Versuchsflächen in Niedersachsen. Silvae Genetica 23: 214-220.

Stephan, B.R. 1985. Zur Blasenrostresistenz von fünfnadeligen Kiefernarten. Allgemeine Forstzeitschrift 40: 695-697.

Stephan, B.R. 1986 a. Results of a 22-year old provenance trial with eastern white pines in northern Germany. *In* Proc. 18th IUFRO World Congress, Ljubljana, September 1986. Division II, Vol. II, p. 859 (Abstract).

Stephan, B.R. 1986 b. The IUFRO experiment on resistance of white pines to blister rust (Cronartium ribicola) in northern Germany. *In* Proc. 18th IUFRO World Congress, Ljubljana, September 1986. Division II, Vol. I, p. 80-89.

Stephan, B.R. and Hyun, S.K. 1983. Studies on the specialization of *Cronartium ribicola* and its differentiation on the alternate hosts *Ribes* and *Pedicularis*. Zeitschr. f. Pflanzenkrankh. und Pflanzensch. 90: 670-678.

Stratmann J. 1988. Ausländeranbau in Niedersachsen und den angrenzenden Gebieten. Schriften aus der Forstl. Fak. der Univ. Göttingen und der Niedersächs. Forstl. Versuchsanst., Band 91: 131 pp.

Waldherr, M. 2000. Die Strobe in Ostbayern (Niederbayern - Oberpfalz). Wachstum und waldbauliche Erfahrungen. Forst und Holz 55: 35-39.

Adaptive Genetic Variation in Sugar Pine

Jay H. Kitzmiller

Abstract—Sugar pines from range-wide provenances displayed a complex genetic structure in adaptive traits in four contrasting common gardens. Strong, rank-changing G-x-E interactions for 10- to 17-year survival and growth require restrictions in seed transfer across altitudes and coastal-interior transects. Geographic and altitudinal subdivisions on a regional scale correspond with major changes in climate, soil, and vegetation. Altitude of origin was the primary geographic variable influencing growth potential; coastal proximity also affected it. Genetic sources accounted for about 40 percent of the variability in growth. Central Sierra Nevada tests at contrasting low and high altitudes exhibited better growth for seed sources from altitudes similar to the test sites. However, distant sources from high elevation, southern and eastern origins had higher survival at the low (840 m) altitude site. Best survival (and growth) at the high (1860 m) altitude test was attained by sources from matching altitudes in the Sierra Nevada, peaking for 1850 m origins, and diminishing rapidly below 1530 m and above 2030 m. In Oregon tests, the local region had best growth, southern regions had least, and sources within region were interactive between the two contrasting coastal and interior tests. Seed transfer from southern Sierra Nevada northward is desirable to boost rust resistance (also growth and genetic diversity) in northern populations, where natural resistance is rare. Seed transfer up to 220 km northward along the Sierra Nevada west-slope may be advantageous and safe, if temperature regimes of seed source and planting site are matched, and if proven-superior sources are used.

Key words: sugar pine, tree genetics

Introduction

Sugar pine (*Pinus lambertiana* Dougl.) is often described as the largest, noblest, and most beautiful of pines in the world. With long life and a remarkable capacity to sustain growth into old age, not reaching culmination of mean annual increment until 400 years, individual trees may reach enormous size, some over 76 m tall, 305 cm dbh (Larson and Woodbury 1916, Oliver 1996). Excellent wood qualities that include uniformity, stability, workability, and finish ability, elevate the commercial value of sugar pine to most preferred species for paneling, cabinetry, and moulding (Willits and Fahey 1991).

Sugar pine ranges widely in latitude (over 14°) and in altitude (2750 m) from Baja California to northern Oregon (Critchfield and Little 1966). Sugar pine possesses wide ecological amplitude, having adapted to different temperature, humidity, and soil environments from coastal to inland montane forests. This late seral species is adapted to high elevations because of its short period of height growth, and to low elevations due to its tolerance of hot, dry sites. Distribution is nearly continuous through the Siskiyou and Klamath Mountains and along the western slopes of the Cascade and Sierra Nevada Ranges (Oliver 1996).

Rarely forming pure stands, sugar pine is typically a minor (less than 25 percent of canopy cover) but highly consistent (more than 70 percent constancy) component with high ecological value in mixed-conifer forests (Fites 1996). These majestic pines protect watersheds, provide large, edible seeds for wildlife forage and large snags for cavity nesting birds, enhance ecological diversity, and improve recreation. Best development has been observed in virgin stands on deep soils in the Stanislaus-Toulumne Experimental Forest, and Yosemite, Kings Canyon, and Sequoia National Parks.

Unfortunately, the high resource values and future of sugar pine are seriously threatened by its extreme susceptibility to an introduced disease, white pine blister rust (WPBR) caused by *Cronartium ribicola* Fisch. Natural regeneration is further impeded by poor seed dispersal, heavy seed predation, low tolerance of competition, and the lack of recurrent, non-stand-replacing fires (Oliver 1996). Needed are proper pest management, genetic conservation, and silviculture, using group selection harvest and artificial regeneration with resistant stock.

Genetic conservation programs in both California and Oregon aim to improve genetic resistance and to restore sugar pine in its native habitats (Samman and Kitzmiller 1996, Sniezko 1996). A major (dominant) gene (R) conveys resistance in natural populations. Natural resistance is rare (R frequency is less than 0.01) in northern populations, where the disease entered 75 years ago and spread southward in sporadic “wave years”. Resistance is highest, but still very low, in southern Sierra populations ($R<0.09$). WPBR is absent below 35° N. “Slow rusting” and ontogenetic resistance mechanisms also exist (Kinloch and Davis 1996).

Management policy seeks to maintain the viability of sugar pine in mixed-conifer forests. Restoration will be needed most in the north, because of the greater impact of WPBR on local occurrence. Likewise, an adequate genetic base of durable resistant genotypes for restoration will be extremely difficult to find in northern populations. Moving resistant genotypes from southern populations to northern planting sites could help solve this problem.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The author is Regional Geneticist, USDA Forest Service, Pacific Southwest Region, Genetic Resource and Conservation Center, 2741 Cramer Lane, Chico, CA 95928. Telephone: (530) 879-6607. Fax: (530) 879-6610. E-mail: jkitzmiller@fs.fed.us.

If sugar pine is closely adapted to regional or local environments, long-distance seed transfer could have adverse effects, *e.g*. maladaptation to “foreign” restoration sites or genetic “contamination” of local gene pools. If sugar pine is not closely adapted, then long-distance transfers would infuse vitally needed genetic diversity to maintain species viability and restore it towards pre-WPBR distribution.

Management policy in California aims to protect the natural genetic structure of sugar pine and all woody plant species (Kitzmiller 1976, 1990). The “default” policy restricts movement of seed (genes) to relatively short distances, defined by standard seed zones (fig. 1) and transfer guides (Buck and others 1970) that apply to all species (“one size fits all”). Transfer is allowed within the local seed zone (152 m elevation band and ca 80 km), and, in certain cases, across climatically similar seed zones (305 m elevation band and ca 200 km). Developed 30 years ago, this general forest seed policy assumes highly structured sub-populations within species, which theoretically form as a result of high environmental heterogeneity, strong natural selection, and limited gene flow.

Natural selection promotes adaptation of populations to local environments. Climatic, edaphic, and biotic factors affect survival, health, and growth. These traits measure adaptability of seed sources to their transplant environment. Vigorous, healthy trees are expected when natural selection at test and seed origin environments sufficiently

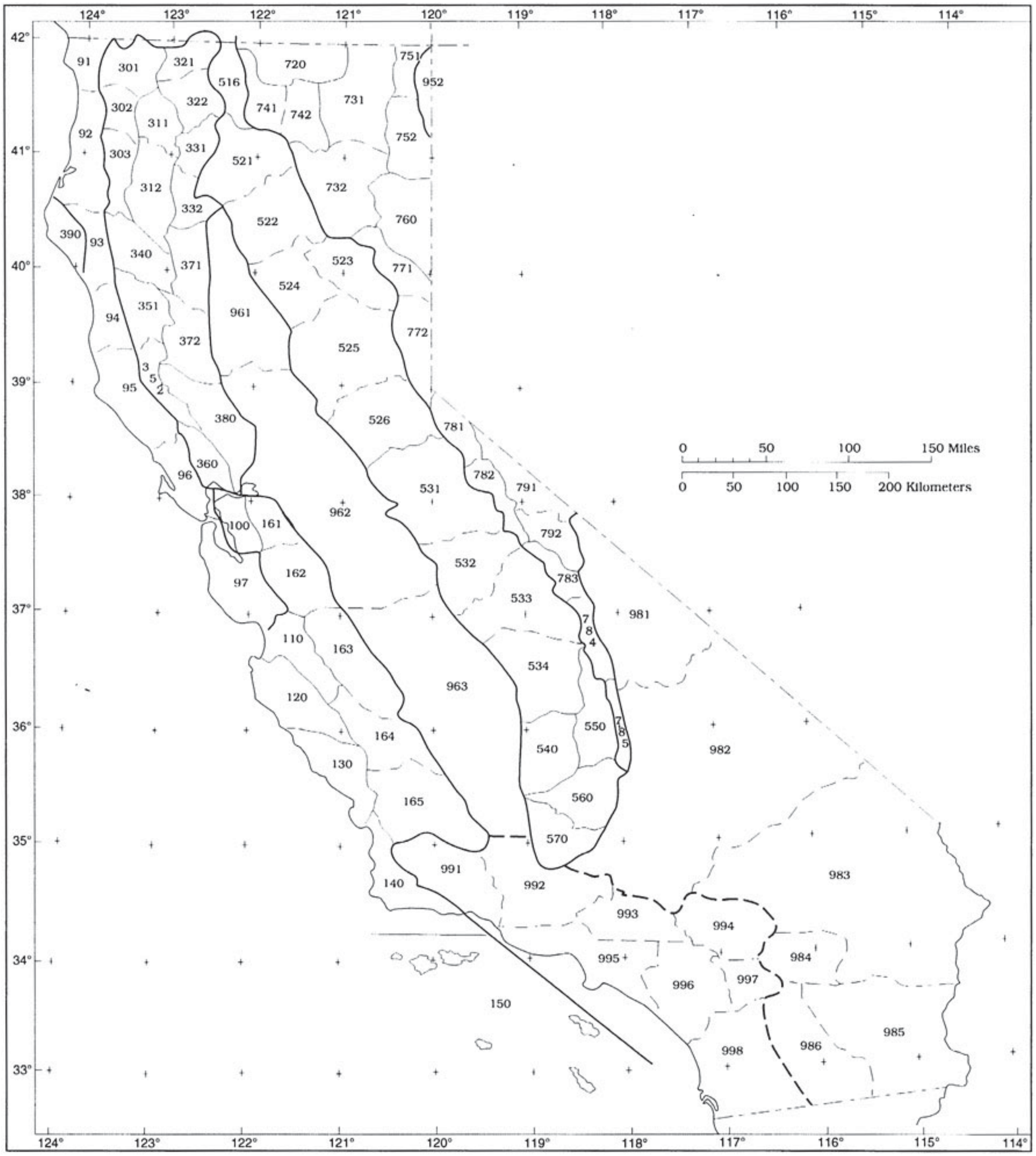


Figure 1—California tree seed zone map.

corresponds to favor similar genotypes. When test and seed origin environments differ widely, maladaptation is expected. Seed zones restrict seed transfer to similar origin and planting environments.

Genetic Studies of Sugar Pine

Both conservation goals, maintaining species viability and protecting adaptive genetic structure, require knowledge of the nature and pattern of adaptive genetic variation. Together, allozyme and common garden studies provide this knowledge. Conkle (1996) reported strong allozyme differentiation into at least four groups (southern California-Baja, southern Sierra Nevada, northern Sierra Nevada, northern California-Oregon) with a north-south cline in gene frequency. Seed zones with steeper genetic gradients (more complex topography and climate) reflect higher genetic heterogeneity and transfer risk. Heterogeneity of allozyme multi-locus genotypes is a transfer risk parameter that estimates the proportion of genotypes that do not "match" the group (zone) and presumably are maladapted (Westfall 1991). Within Sierra Nevada seed zones, heterogeneity varied in a north-south clinal pattern (Kitzmiller, data filed 1995). The most heterogeneous was 24 percent within the southern-most seed zone 540 (fig. 1), and the least was 13 percent within the northern-most zone 524. Mendocino interior coast zones ranged between 8 to 13 percent, while California North Coast, Klamath, and Southern Cascade zones were 2 to 8 percent. Thus, heterogeneity and transfer risk within geographic seed zones was lower in northwestern California than in southern Sierra Nevada."

In three separate nursery common gardens, sugar pine showed marked geographic variation in early growth and phenology. Altitudinal provenances (or the original geographic source of seed) from a central Sierra Nevada transect were highly differentiated (Harry and others 1983). Regional patterns were evident for 1185 families representing 27 California seed zones (Kitzmiller and Stover 1996). Campbell and Sugano (1987) found about 50 percent of the adaptive genetic variability in Oregon occurred among local populations. They developed larger and very different zones from the standard Oregon seed zones delineated in 1966 without the aid of genecological data.

Ideally, seed zones should be defined from long-term field trials. Such studies with associated species, ponderosa pine (*Pinus ponderosa* Dougl.) (Conkle 1973) and white fir (*Abies concolor* (Gordon & Glend.) Lind.) (Jenkinson data filed, 1985) have shown strong adaptation to altitudinal climatic gradients. In a 9-year California field study (Kitzmiller and Stover 1996), 37 sugar pine families at seven common garden sites expressed Genotype x Environment (G-x-E) interaction for height. Families originating in coastal influence belts, North Coast-Klamath Mountains (NC-KM), grew faster at three NC-KM sites than families from the northern Sierra Nevada (NSN). NSN families grew faster at the two coldest NSN sites, but no regional differences were expressed at the two NSN sites with long, warm growing seasons. Close inspection revealed that about 30 percent of the families were stable, with half ranking consistently high across all sites. Liberal transfer of seed was suggested for stable families if trends continued.

This paper presents recent results of the most comprehensive study of adaptation and G-x-E interaction in sugar pine established by the Institute of Forest Genetics, PSW Research Station, and the Siskiyou and Eldorado National Forests. This range-wide provenance trial was planted in 1984 in paired low and high elevation Sierra Nevada sites in northern California, and in 1988 in paired coastal and inland sites in southwest Oregon. Jenkinson (1996) reported early results (4- to 8-year). Seed source elevation was significant for 8-year growth of Sierra Nevada sources at the low elevation Sierra Nevada site. There, height was reduced by 10 percent and volume by 26 percent per 300 m rise in source elevation. At the high elevation Sierra Nevada site, where annual winter damage from wet, wind-driven snows became evident at 7-years, high sources were beginning to excel. Source elevation was less important for northern sources at both 4-year-old Oregon sites. Best early growth in all plantations was attained by sources from the middle to upper elevations of the most productive center of the species range.

My objectives are to further assess adaptation and G-x-E interactions in sugar pine for changes in early trends, and to determine the current results and their implications for seed transfer. This paper reports for the first time: (1) comparisons among all four tests for 10-year survival and growth, (2) paired comparisons for survival and growth after 13-years (Oregon) and 17-years (California), and (3) rust infection and health of rust-free trees.

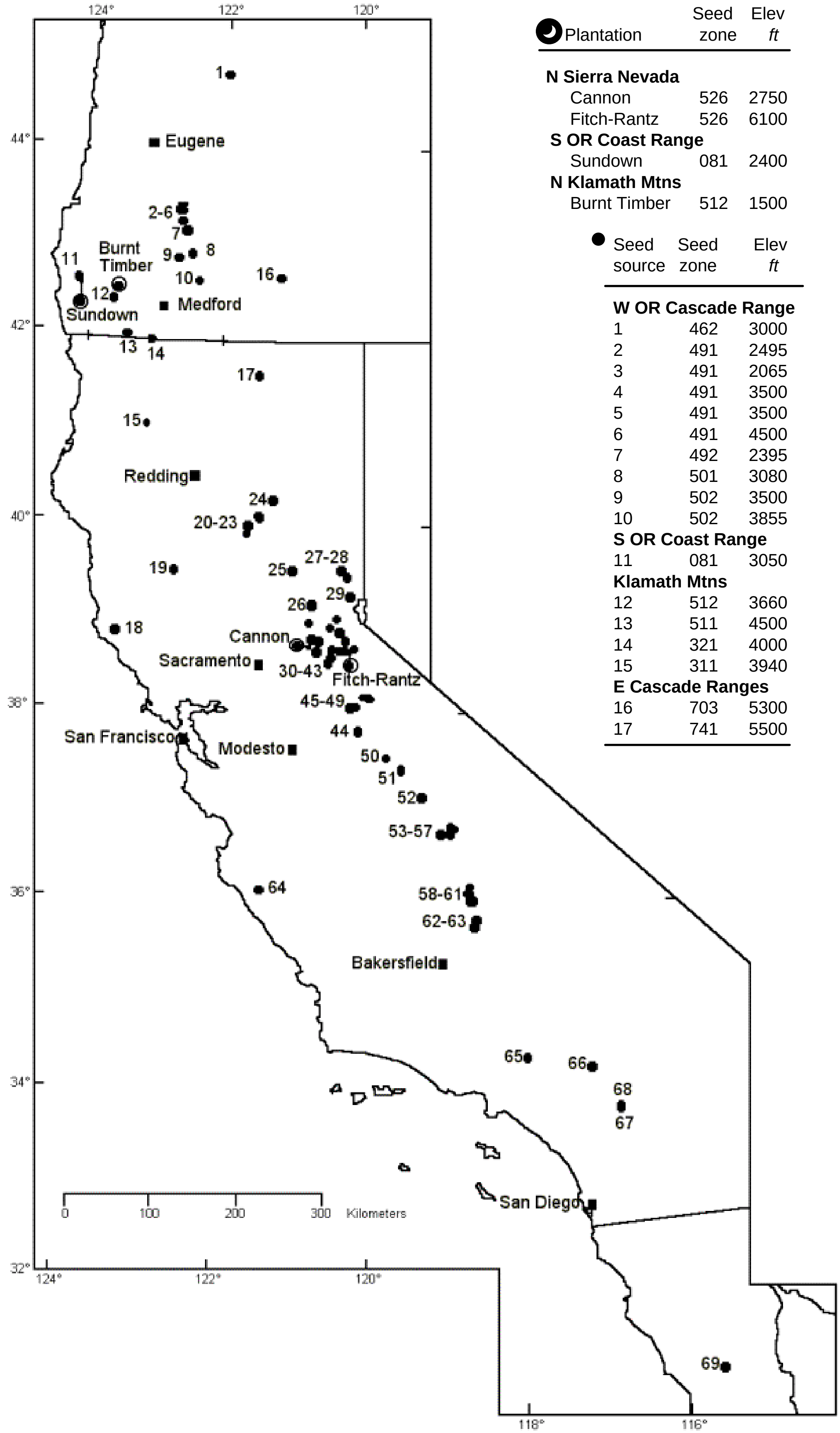
Materials and Methods

Study Design and Establishment

Jenkinson (1996) detailed selection of seed sources, nursery and planting procedures, and early results. Seed sources represent 69 wild stands extending across environmental gradients associated with elevation, latitude, and distance from the Pacific Ocean (fig. 2, table 1). Provenance (seed source) samples were well distributed, except in California for the southern Cascades and interior north coast. Each was represented by offspring from 4 to 20 (predominantly 10) healthy, vigorous natural seed parents occurring 0.8 km to 3.2 km apart. The 69 sources were grouped by region (physiographic province) of origin.

Four contrasting common garden test locations were selected to represent and compare two main areas within the ecologically-variable species range: (1) compare range-wide provenance performance in coastal (Sundown) *versus* inland (Burnt Timber) forest environments within southwestern Oregon, and (2) compare range-wide provenance performance at low (Cannon) *vs* high elevation (Fitch-Rantz) forest environments within the Sierra Nevada.

Bayleton systemic fungicide was sprayed annually in autumn for 5-years to reduce blister rust infection. California tests were lightly irrigated the first two summers to ensure initial survival. Herbaceous vegetation was controlled intensively for 2-years using chemical and mechanical methods. Woody vegetation (including *Ribes*) was controlled every 3- to 5-years.



◐ Plantation	Seed zone	Elev ft
N Sierra Nevada		
Cannon	526	2750
Fitch-Rantz	526	6100
S OR Coast Range		
Sundown	081	2400
N Klamath Mtns		
Burnt Timber	512	1500

● Seed source	Seed zone	Elev ft
W OR Cascade Range		
1	462	3000
2	491	2495
3	491	2065
4	491	3500
5	491	3500
6	491	4500
7	492	2395
8	501	3080
9	502	3500
10	502	3855
S OR Coast Range		
11	081	3050
Klamath Mtns		
12	512	3660
13	511	4500
14	321	4000
15	311	3940
E Cascade Ranges		
16	703	5300
17	741	5500

● Seed source	Seed zone	Elev ft
North Coast Range		
18	094	1500
19	371	4500
N Sierra Nevada		
20	524	2330
21	524	3775
22	524	5250
23	524	5500
24	523	6100
25	525	4380
26	525	3965
27	772	5880
28	772	6475
29	772	6400
30	526	2530
31	526	2800
32	525	3000
33	526	3400
34	526	4000
35	526	4500
36	526	4650
37	525	5000
38	526	5100
39	526	5265
40	526	5600
41	526	5925
42	526	6600
43	525	7000
44	531	3160
45	531	4000
46	531	4720
47	531	5450
48	531	6250
49	531	6950
S Sierra Nevada		
50	532	6070
51	532	6055
52	533	5860
53	534	5565
54	534	5710
55	534	6565
56	534	6660
57	534	7615
58	534	6725
59	540	6265
60	540	5915
61	540	6100
62	540	6265
63	540	6300
South Coast Range		
64	120	5800
Transverse-Peninsular Ranges		
65	993	6950
66	994	6720
67	997	5950
68	997	7500
Sa San Pedro Martir		
69	—	8200

Figure 2—Sugar pine seed sources and locations of common garden tests.

Table 1—Seed sources of sugar pine evaluated in common gardens in northern California and southwest Oregon. Sources are listed by latitude and elevation.

			Seed	Elevation		Lat	Lon	Tested	No.	Map
Region [a]	Locale	County	Zone [b]	(ft)	(m)	°N	°W	In [c]	Fam	Code
WOC	Breitenbush R	Marion	462	3000	915	44.80	122.03	C,F,_,_	4	1
WOC	Steelhead Ck	Douglas	491	2495	761	43.40	122.66	C,F,S,B	7	2
WOC	Grass Ck	Douglas	491	2065	595	43.35	122.70	C,F,S,B	10	3
WOC	Limpy Rock NE	Douglas	491	3500	1067	43.33	122.62	C,F,_,_	7	4
WOC	Limpy Rock SW	Douglas	491	3500	1067	43.33	122.62	C,F,S,B	10	5
WOC	OK Butte	Douglas	491	4500	1372	43.22	122.65	C,_,S,B	10	6
WOC	Camp Comfort	Douglas	492	2395	730	43.12	122.58	C,F,S,B	10	7
WOC	Woodruff Flat	Jackson	501	3080	939	42.87	122.49	C,F,S,B	10	8
WOC	Elk Ck	Jackson	502	3500	1067	42.85	122.68	C,_,S,B	10	9
WOC	Camp Ck	Jackson	502	3855	1175	42.60	122.38	C,F,S,B	10	10
SOC	Gold Beach	Curry	81	3050	930	42.60	124.15	C,_,S,B	10	11
KM	Pea Soup	Josephine	512	3660	1116	42.40	123.63	C,_,S,B	10	12
KM	Bolan	Josephine	511	4500	1372	42.05	123.43	C,F,_,_	10	13
KM	Dutch Ck	Siskiyou	321	4000	1220	41.98	123.07	C,F,S,B	10	14
KM	Salmon R	Siskiyou	311	3940	1201	41.08	123.10	C,F,S,B	10	15
EC	Black Hills	Klamath	703	5300	1616	42.64	121.20	C,F,_,_	10	16
EC	Glass Mtn	Siskiyou	741	5500	1677	41.60	121.50	C,F,_,_	20	17
NC	Fish Rock Rd	Mendocino	94	1500	457	38.87	123.46	C,F,S,B	10	18
NC	S Fk Elk Ck	Glenn	371	4500	1372	39.56	122.65	C,F,S,B	10	19
NSN	Forest Ranch	Butte	524	2330	710	39.93	121.65	C,F,S,B	10	20
NSN	Diamond I	Butte	524	3775	1151	40.02	121.63	C,F,S,B	10	21
NSN	Jonesville	Butte	524	5250	1601	40.12	121.48	C,_,S,B	10	22
NSN	Colby Mtn	Tehama	524	5500	1677	40.13	121.52	C,F,S,B	10	23
NSN	Stover Mtn	Plumas	523	6100	1860	40.28	121.30	C,F,S,B	10	24
NSN	Cal-Ida	Sierra	525	4380	1335	39.54	121.01	C,F,_,_	6	25
NSN	N Shirttail Cyn	Placer	525	3965	1209	39.18	120.75	C,F,_,_	6	26
NSN	Sierraville	Sierra	772	5880	1793	39.55	120.34	C,F,_,_	6	27
NSN	Little Truckee	Sierra	772	6475	1974	39.48	120.24	C,F,_,_	5	28
NSN	Upper Truckee	Placer	772	6400	1951	39.27	120.21	C,F,_,_	5	29
NSN	Pleasant Valley	Eldorado	526	2530	771	38.68	120.67	C,F,S,B	5	30
NSN	Crozier Loop	Eldorado	526	2800	854	38.82	120.72	C,F,S,B	10	31
NSN	Breedlove	Eldorado	525	3000	915	38.98	120.78	C,F,S,B	10	32
NSN	Big X Mtn	Eldorado	526	3400	1037	38.79	120.63	C,F,S,B	10	33
NSN	Big Mtn	Eldorado	526	4000	1220	38.57	120.52	C,F,S,B	10	34
NSN	Snow Mill Rd	Eldorado	526	4500	1372	38.71	120.46	C,F,S,B	10	35
NSN	Caldor Rd	Eldorado	526	4650	1418	38.62	120.47	C,F,S,B	10	36
NSN	Uncle Tom’s	Eldorado	525	5000	1524	38.93	120.48	C,F,S,B	10	37
NSN	Tells Creek	Eldorado	526	5100	1555	38.89	120.37	C,F,S,B	10	38
NSN	Alder Ck	Eldorado	526	5265	1605	38.69	120.30	C,F,S,B	10	39
NSN	Pilliken	Eldorado	526	5600	1707	38.69	120.35	C,F,S,B	10	40
NSN	Sugarloaf	Eldorado	526	5925	1806	38.79	120.28	C,F,S,B	10	41
NSN	Mule Cyn	Eldorado	526	6600	2012	38.72	120.18	C,F,S,B	10	42
NSN	Bunker Hill	Placer	525	7000	2134	39.04	120.39	C,F,S,B	10	43
NSN	Groveland Stn	Tuolumne	531	3160	963	37.82	120.11	C,F,_,_	10	44
NSN	Sugar Pine	Tuolumne	531	4000	1220	38.08	120.20	C,F,S,B	10	45
NSN	Lyons Reservoir	Tuolumne	531	4720	1439	38.10	120.17	C,F,S,B	10	46
NSN	Summit Stn	Tuolumne	531	5450	1662	38.20	120.03	C,F,S,B	10	47
NSN	Pinecrest	Tuolumne	531	6250	1905	38.19	119.98	C,F,S,B	10	48
NSN	Dodge Ridge	Tuolumne	531	6950	2119	38.18	119.97	C,F,S,B	10	49
SSN	Chowchilla Mtns	Mariposa	532	6070	1851	37.53	119.72	C,F,S,B	10	50
SSN	N Fk Willow Ck	Madera	532	6055	1846	37.41	119.53	C,F,S,B	7	51
SSN	Shaver Lake	Fresno	533	5860	1787	37.12	119.26	C,F,S,B	8	52
SSN	Landslide	Fresno	534	5565	1697	36.77	118.88	C,F,S,B	10	53
SSN	Happy Gap	Fresno	534	5710	1741	36.73	119.00	C,F,S,B	10	54
SSN	Lockwood Grove	Fresno	534	6565	2002	36.80	118.87	C,F,S,B	10	55
SSN	Hume Lake Rd	Tulare	534	6660	2030	36.73	118.88	C,F,S,B	10	56
SSN	Burton Mdws	Fresno	534	7615	2322	36.78	118.83	C,_,S,B	10	57
SSN	Hossack Mdw	Tulare	534	6725	2050	36.17	118.62	C,F,_,_	5	58
SSN	Black Mtn Grove	Tulare	540	6265	1910	36.11	118.65	C,F,_,_	9	59
SSN	Peyrone Camp	Tulare	540	5915	1803	36.03	118.62	C,F,_,_	6	60
SSN	Cunningham Grove	Tulare	540	6100	1860	36.02	118.58	C,F,_,_	10	61
SSN	Bull Run Basin	Tulare	540	6265	1910	35.81	118.54	C,F,_,_	6	62
SSN	Greenhorn Sum	Kern	540	6300	1921	35.74	118.56	C,F,_,_	9	63
SC	Junipero Serra	Monterey	120	5800	1768	36.15	121.42	C,F,S,B	10	64
T-P	San Gabriel	Los Angeles	993	6950	2119	34.35	117.92	C,F,_,_	10	65
T-P	San Bernardino	San Bernardino	994	6720	2049	34.24	117.10	C,F,_,_	10	66
T-P	San Jacinto	Riverside	997	5950	1814	33.80	116.75	C,F,S,B	10	67
T-P	San Jacinto	Riverside	997	7500	2287	33.83	116.75	C,F,S,B	10	68
SSPM	Parque Nacional	Baja CA		8200	2500	31.00	115.57	C,F,_,_	10	69

[a] WOC: Western Oregon Cascades; SOC: South Oregon Coast; KM: Klamath Mt; NC: North Coast; NSN: Northern Sierra Nevada; SSN: Southern Sierra Nevada; SC: South Coast; T-P: Transverse- Peninsular.

[b] Buck et al 1970, Kitzmiller 1976, 1990.

[c] C=Cannon, F=Fitch-Rantz, S=Sundown, B=Burnt Timber.

Measurements

Survival, height, and basal diameter at ground level (dg) through age 10 were taken in all four tests. In fall of 2000, at age 13, these same measurements were repeated at Sundown and Burnt Timber. At Cannon and Fitch-Rantz, survival, height, and diameter at breast height (db) were recorded in the fall of 2000 at age 17. Stem volume (dm ^{3}) of individual trees was calculated using the formula: $v = 0.1 \pi d^2 h$, where d=db or dg and h=height, in dm. At Sundown and Burnt Timber, 13-year volumes were based on dg. In contrast, 17-year volumes at Cannon and Fitch-Rantz were based on db. At California sites, tree injuries caused by blister rust, insects, weather, and stem defects (including forking) were recorded. A different crew measured Oregon tests; damage codes consisted mostly of blister rust (infected or not), which became readily apparent after 10-years. Other causes of injuries were difficult to evaluate on rust-infected trees. So, rust-free trees formed the base for assessing freedom from other injuries. For seed sources, rust infection and mortality were expressed as percent of surviving trees and of total planted trees, respectively.

Design Differences between California and Oregon Tests

The study is really composed of two pairs of different experiments in terms of: year of establishment, provenance and family representation, number and size of block replications and of trees per source-plot, and measurement schedule (table 2). Cannon (CAN) and Fitch-Rantz (FIT) had 63 sources (provenances) in common. Sundown (SUN) and Burnt Timber (BUR) had 46 common sources. All four tests shared 42 sources and 10-year data in common for a combined analysis. An important difference in experimental design was family representation within source-plots. CAN/FIT source-plots consisted of 10 families each with one tree. SUN/BUR source-plots had 8 families with two contiguous trees each. The effect of families within source could be evaluated accurately only for SUN/BUR tests due to missing family-plots at CAN/FIT.

Statistical Analysis

Statistical Analysis System (SAS) software (v. 8.1) was used exclusively. PROC SUMMARY computed simple means, within family plot variances, and correlations. Means were built sequentially starting at the family-plot level (that is, "mean of means" method). ANOVAs were based on family-plot (SUN/BUR pair) or source-plot means (CAN/FIT pair). INSIGHT computed simple and canonical correlations, simple and polynomial regressions, and contour-plot graphs to relate adaptability traits to two geographic variables using test-source means. PROC MIXED (method=REML, option=satterth) estimated variance components, least square means, and standard errors of means and of differences between two means using appropriate degrees of freedom for unbalanced subclasses. PROC GLM (option=test) generated accurate expected mean squares and synthesized the proper F-tests.

The analysis consisted of three parts: (1) 42 common sources for all four tests, (2) 63 common sources for the CAN/FIT pair, and (3) 46 common sources for the SUN/BUR pair. Analyses were made both across plantations and by individual test plantation. Models were developed using two sets of assumptions regarding fixed and random effects. One model assumed all factors random (REM) to estimate variance components for all factors studied (table 3a). Since test plantations and regions of seed origins were not actually selected at random, and since least square means are computed only for fixed effects, a more realistic mixed effects model (MEM) was also used. For this MEM, Plantations, Regions, and P-x-R interaction were assumed fixed, while Sources within Region and P-x-S(R) interaction were random (table 3b). Lastly, to compute least square means for S(R) and P-x-S(R) interaction, a third model was used where only Blocks and Error were assumed random. Least squares means were considered most useful for unbalanced data (*e.g.* missing source plots, unequal replication among tests, unequal sources within regions). G-x-E interactions were examined for cause attributable to scale effects or true rank changes according to Surles and others (1995).

The general run sequence was: (1) PROC MIXED for the REM to estimate variance components; (2) PROC GLM for

Table 2—General description contrasting the four common garden tests.[a]

Description	Cannon	Fitch-Rantz	Sundown	Burnt Timber
State	**California**	**California**	**Oregon**	**Oregon**
Year Planted	1984	1984	1988	1988
Blocks	6	6	5	5
Sources	68 (14OR53CA1M)	64 (10OR53CA1M)	46 (8OR38CA)	46 (8OR38CA)
Trees/Plot	10	10	16	16
Spacing	2.25 m	1.83 m	1.83 m	1.83 m
Elevation	838 m	1860 m	732 m	457 m
Latitude	38.73	38.68	42.55	42.52
Longitude	120.75	120.21	124.13	123.58
Aspect	south	east	south	south
Climate	warm, dry	cool, moist	cool, moist	warm, dry
Soil PM	volcanic basalt	granite	schist	metavolcanic
Forest Type	Lower M-C	Upper M-C	Coastal M-C	Inland M-C
Irrigate 2-Yrs	3 week interval	3 week interval	none	none
Measure Age	2,3,4,6,8,9,10,17	2,3,4,6,8,9,10,17	3,4,5,6,8,10,13	3,4,5,6,8,10,13

[a](14OR53CA1M) indicates 14 Oregon, 53 California, and 1 Mexico seed sources in test.

Table 3a—ANOVA structure for the random model based on family plot means.[a]

Source of variation	DF	Expected mean square (EMS)
Plantations	p-1	$\sigma^2 + 4\,\sigma_{PF}^2 + 32\,\sigma_{PS}^2 + 100\,\sigma_{PR}^2 + 148\,\sigma_B^2 + 740\,\sigma_P^2$
Blocks (P)	p(b-1)	$\sigma^2 + 313\,\sigma_B^2$
Regions	r-1	$\sigma^2 + 4\,\sigma_{PF}^2 + 8\,\sigma_F^2 + 33\,\sigma_{PS}^2 + 66\,\sigma_S^2 + 147\,\sigma_{PR}^2 + 293\,\sigma_R^2$
PxR	(p-1)(r-1)	$\sigma^2 + 4\,\sigma_{PF}^2 + 33\,\sigma_{PS}^2 + 146\,\sigma_{PR}^2$
Sources (R)	r(s-1)	$\sigma^2 + 4\,\sigma_{PF}^2 + 9\,\sigma_F^2 + 34\,\sigma_{PS}^2 + 68\,\sigma_S^2$
PxS(R)	(p-1)r(s-1)	$\sigma^2 + 4\,\sigma_{PF}^2 + 34\,\sigma_{PS}^2$
Families (SxR)	rs(f-1)	$\sigma^2 + 4\,\sigma_{PF}^2 + 9\,\sigma_F^2$
PxF(SxR)	(p-1)rs(f-1)	$\sigma^2 + 4\,\sigma_{PF}^2$
Error	[b]	σ^2

[a] Includes family effect; coefficients are rounded to nearest whole number; p=number of plantations, b=number of blocks or replications within plantation, r=number of regions, s=number of sources within region, and f=number of families within source.

[b] Error degrees of freedom = p(b-1)(r-1) + p(b-1)r(s-1) + p(b-1) rs(f-1).

Synthesized Error Terms (computed by GLM):

Plantation: 0.47*MS Blk(P) + 0.68*MS PxR + 0.29*MS PxS(R) + 0.01*MS PxF(SxR) – 0.46*MS Error;
Region: MS PxR + 0.97*MS S(R) – 0.96*MS PxS(R) + MS F(SxR) – 0.01*MS PxF(SxR) – MS Error;
PxR: 0.96*MS PxS(R) + 0.02*MS PxF(SxR) + 0.02*MS Error;
Sources (R): MS PxS(R) + 0.99*MS F(SxR) – 0.99*MS PxF(SxR) + MS Error;
PxS(R): 0.98*MS PxF(SxR) + 0.02*MS Error.

Table 3b – ANOVA structure for a mixed model based on source plot means.[a]

Source of variation	DF	Expected mean square (EMS)
Plantations	p-1	$\sigma^2 + \mathbf{5}\,\sigma_{PS}^2 + \_ + 21\,\sigma_B^2 + Q_P$
Blocks (P)	p(b-1)	$\sigma^2 + 46\,\sigma_B^2$
Regions	r-1	$\sigma^2 + \mathbf{5}\,\sigma_{PS}^2 + \mathbf{10}\,\sigma_S^2 + \_ + Q_R$
PxR	(p-1)(r-1)	$\sigma^2 + \mathbf{5}\,\sigma_{PS}^2 + Q_{PR}$
Sources (R)	r(s-1)	$\sigma^2 + \mathbf{5}\,\sigma_{PS}^2 + 10\,\sigma_S^2$
PxS(R)	(p-1)r(s-1)	$\sigma^2 + \mathbf{5}\,\sigma_{PS}^2$
Error	p(b-1)(r-1) +p(b-1) r(s-1)	σ^2

[a] P, R, and P-x-R are assumed fixed effects; "_" denotes their absence relative to random model; **bold** components would be absent from a mixed model with S(R) and PxS(R) also assumed fixed; coefficients are rounded to nearest whole number; p=number of plantations, b=number of blocks or replications within plantation, r=number of regions, s=number of sources within region, and f=number of families within source.

Synthesized Error Terms (computed by GLM):

Plantation: 0.47*MS Blk(P) + 0.96*MS PxS(R) – 0.42*MS Error;
Region: 0.97*MS S(R) + 0.03*MS Error

the REM to make F-tests; (3) PROC GLM for a MEM to make F-tests for Plantations, Regions, and P-x-R, (4) PROC MIXED on the MEM to estimate and test differences between least square means of significant factors; (5) repeat 3 and 4 above for the Sources(R) level. The random *vs* fixed assumption affected the F-test outcome for the main effects: Plantations and Regions, because P-x-R was part of the error term in the REM but was not in the MEM. Similarly, the assumption for S(R) and P-x-S(R) affected the F-test outcome for P-x-R and for S(R) (table 3b).

Trees shorter than breast-height (BUR:132, FIT: 62) were discarded from growth analyses. All other trees were used, even rusted and injured trees. Results were similar whether rusted/injured trees were included or not. Rust on limbs had no effect on current height or diameter. Rust on stem reduced height by 4 to 5 percent, but had no effect on diameter. Since this effect was rather small, and preservation of plot sample size was needed to prevent missing plots, all except severely stunted trees were analyzed. I also excluded trees that died after 10 years in my 10-year analysis across all tests.

Results

Survival, Rust Infection, and General Health

Survival was highest at SUN and BUR (73.3 and 72.5 percent, respectively) followed closely by FIT (69.3 percent). Survival was lowest at CAN (38.3 percent) due to progressive mortality from charcoal root rot (*Macrophomina* phaseolina [Tassi] Goid). During the last nine years, mortality was 29 percent at CAN, 9 percent at FIT, 15 percent at SUN, and 8 percent at BUR. *Note*: Mortality the first 3-years was 23 (CAN), 16 (FIT), 2 (SUN), and 18 (BUR) percent.

Trees living with rust infection were very low at CAN (0.1 percent), much higher at FIT (19.3 percent) and BUR (20 percent), and highest at SUN (44 percent). No trees were recorded as dead from rust at CAN, SUN, or BUR, but 40 trees were rust-killed at FIT. The high post-establishment mortality at SUN was probably rust-related, though it was not so recorded. Of rust-free trees, 95 percent were healthy and without major stem defects at CAN, 65 percent at FIT, 98 percent at SUN, and 99 percent at BUR. Of surviving trees, 52 percent (in other words, 0.65*(1-0.19)) were healthy at FIT and 95 percent were healthy at CAN. Only 36 percent of total trees planted at both California tests were healthy at 17-years (table 4).

The widest range in survival among sources (table 4) was at FIT (18 to 95 percent). At FIT, survival (and health) varied in a curvilinear pattern with elevation of origin (R^2=0.57, n=64, (R^2=0.40, n=64, respectively)), with highest survival (and health) occurring for sources that match the test site elevation (fig. 3). Survival and health of living trees was directly correlated at FIT (r=0.70, n=63); sources with higher survival were also healthier. Among tests, current survival is significantly correlated only for CAN x FIT (r=0.44, n=63). At CAN, survival based on regional means was highly correlated with elevation of origin (R^2=0.88, n=10); Trans-

Table 4—Survival, health, and rust infection by seed source and test plantation.[a]

Seed Origin:[b]					Cannon		Fitch-Rantz				Sundown			Burnt Timber		
Region	Locale	MC	Sdz	Elev	Surv	Heal	Surv	Heal	LR	DR	Surv	Heal	LR	Surv	Heal	LR
				m	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - % - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -	- - - - - -
WOC	Breitenbush R	1	462	915	24	92	48	20	13	2	*np*	*np*	*np*	*np*	*np*	*np*
WOC	Steelhead Ck	2	491	761	38	91	45	50	11		83	96	20	84	100	18
WOC	Grass Ck	3	491	595	30	89	58	48	17	2	74	98	29	79	96	13
WOC	Limpy Rock NE	4	491	1067	10	83	72	55	28		*np*	*np*	*np*	*np*	*np*	*np*
WOC	Limpy Roc SW	5	491	1067	20	100	50	33	20		78	100	37	75	100	20
WOC	OK Butte	6	491	1372	40	100	*np*	*np*	*np*		*np*	*np*	*np*	*np*	*np*	*np*
WOC	Camp Comfort	7	492	730	45	96	43	42	8		86	98	36	73	100	31
WOC	Woodruff Flat	8	501	939	48	90	43	65	23	2	56	100	38	75	100	17
WOC	Elk Ckn	9	502	1067	27	94	*np*	*np*	*np*		*np*	*np*	*np*	*np*	*np*	*np*
WOC	Camp Ck	10	502	1175	32	100	65	70	15	3	80	90	52	60	100	35
SOC	Gold Beach	11	81	930	22	100	*np*	*np*	*np*		74	97	36	59	100	23
KM	Pea Soup	12	512	1116	27	94	*np*	*np*	*np*		70	97	39	79	98	19
KM	Bolan	13	511	1372	45	89	50	40	7	4	*np*	*np*	*np*	*np*	*np*	*np*
KM	Dutch Ck	14	321	1220	12	86	43	40	23	2	68	100	59	81	100	18
KM	Salmon R	15	311	1201	53	91	88	65	13		65	100	63	56	100	22
EC	Black Hills	16	703	1616	43	100	92	69	18		*np*	*np*	*np*	*np*	*np*	*np*
EC	Glass Mtn	17	741	1677	43	100	55	72	24		*np*	*np*	*np*	*np*	*np*	*np*
NC	Fish Rock Rd	18	94	457	50	90	18	0	0		84	100	33	64	100	14
NC	S Fk Elk Ck	19	371	1372	12	86	55	48	12		74	100	66	61	100	8
NSN	Forest Ranch	20	524	710	38	96	27	46	19		86	98	28	78	100	16
NSN	Diamond I	21	524	1151	38	96	67	54	30		83	100	27	79	100	8
NSN	Jonesville	22	524	1601	43	96	*np*	*np*	*np*		75	100	65	63	100	44
NSN	Colby Mtn	23	524	1677	12	86	78	95	21	3	55	100	50	70	100	34
NSN	Stover Mtn	24	523	1860	32	100	85	86	14	3	75	100	43	79	100	17
NSN	Cal-Ida	25	525	1335	32	95	60	46	28	2	*np*	*np*	*np*	*np*	*np*	*np*
NSN	N Shirttail Cyn	26	525	1209	23	93	73	64	25		*np*	*np*	*np*	*np*	*np*	*np*
NSN	Sierraville	27	772	1793	48	100	93	84	20		*np*	*np*	*np*	*np*	*np*	*np*
NSN	Little Truckee	28	772	1974	47	100	83	90	16		*np*	*np*	*np*	*np*	*np*	*np*
NSN	Upper Truckee	29	772	1951	50	100	83	76	16	3	*np*	*np*	*np*	*np*	*np*	*np*
NSN	Pleasant Valley	30	526	771	45	93	47	71	14		81	100	35	75	98	18
NSN	Crozier Loop	31	526	854	42	84	70	63	17		73	97	50	83	98	20
NSN	Breedlove	32	525	915	33	90	48	48	14		90	100	39	86	100	14
NSN	Big X Mtn	33	526	1037	20	100	53	52	22		88	100	43	64	100	25
NSN	Big Mtn	34	526	1220	25	100	77	77	15		75	100	32	90	100	28
NSN	Snow Mill Rd	35	526	1372	25	93	68	63	15		68	100	48	79	100	24
NSN	Caldor Rd	36	526	1418	35	90	62	78	38		78	91	45	58	100	15
NSN	Uncle Tom's	37	525	1524	37	91	77	62	15		73	100	52	79	100	22
NSN	Tells Creek	38	526	1555	30	100	92	65	22		78	100	53	80	100	23
NSN	Alder Ck	39	526	1605	42	100	92	80	16		83	100	48	85	100	19
NSN	Pilliken	40	526	1707	47	96	68	93	27		73	97	38	74	100	37

(con.)

Table 4 (Con.)

Seed Origin:[b]					Cannon		Fitch-Rantz				Sundown			Burnt Timber		
Region	**Locale**	**MC**	**Sdz**	**Elev**	**Surv**	**Heal**	**Surv**	**Heal**	**LR**	**DR**	**Surv**	**Heal**	**LR**	**Surv**	**Heal**	**LR**
				m	%											
NSN	Sugarloaf	41	526	1806	48	93	66	82	24	2	80	100	55	76	100	15
NSN	Mule Cyn	42	526	2012	50	97	85	81	29	3	60	86	54	68	97	30
NSN	Bunker Hill	43	525	2134	33	90	70	87	10	2	74	100	36	69	100	13
NSN	Groveland Stn	44	531	963	23	100	58	68	3		*np*	*np*	*np*	*np*	*np*	*np*
NSN	Sugar Pine	45	531	1220	37	100	68	70	27		81	97	45	71	100	21
NSN	Lyons Reservoi	46	531	1439	43	96	75	68	18		66	100	49	68	100	22
NSN	Summit Stn	47	531	1662	28	82	75	82	24	5	78	100	55	75	100	28
NSN	Pinecrest	48	531	1905	33	95	83	76	24	3	69	100	55	66	100	15
NSN	Dodge Ridge	49	531	2119	33	100	93	76	13		76	100	43	79	98	13
SSN	Chowchilla Mtn	50	532	1851	33	90	77	77	33	2	71	100	35	64	100	20
SSN	N Fk Willow Ck	51	532	1846	53	97	83	77	30		59	100	40	74	100	12
SSN	Shaver Lake	52	533	1787	65	97	87	80	23		88	100	44	86	100	10
SSN	Landslide	53	534	1697	43	100	95	61	23	3	63	95	56	68	100	33
SSN	Happy Gap	54	534	1741	42	100	77	76	28	2	65	95	58	80	100	27
SSN	Lockwood Grov	55	534	2002	42	100	83	69	28	2	64	100	61	74	100	20
SSN	Hume Lake Rd	56	534	2030	53	100	83	69	28	2	48	100	34	76	100	21
SSN	Burton Mdws	57	534	2322	*np*	*np*	73	63	14	2	56	94	64	56	100	18
SSN	Hossack Mdw	58	534	2050	32	95	75	59	36		*np*	*np*	*np*	*np*	*np*	*np*
SSN	Black Mtn Grov	59	540	1910	65	92	77	71	26		*np*	*np*	*np*	*np*	*np*	*np*
SSN	Peyrone Camp	60	540	1803	42	96	83	83	20	3	*np*	*np*	*np*	*np*	*np*	*np*
SSN	Cunningham G	61	540	1860	52	100	87	72	31		*np*	*np*	*np*	*np*	*np*	*np*
SSN	Bull Run Basin	62	540	1910	45	93	92	86	24		*np*	*np*	*np*	*np*	*np*	*np*
SSN	Greenhorn Su	63	540	1921	38	91	83	91	12	3	*np*	*np*	*np*	*np*	*np*	*np*
SC	Junipero Serra	64	120	1768	37	95	58	32	20		71	100	42	61	100	6
T-P	San Gabriels	65	993	2119	67	95	62	65	8	3	*np*	*np*	*np*	*np*	*np*	*np*
T-P	San Bernardino	66	994	2049	42	100	67	65	15	3	*np*	*np*	*np*	*np*	*np*	*np*
T-P	San Jacinto	67	997	1814	45	89	67	45	18		*np*	*np*	*np*	*np*	*np*	*np*
T-P	San Jacinto	68	997	2287	63	100	68	45	2		85	100	29	64	100	8
SSPM	Parque Nacion	69		2500	63	95	52	59	6	3	*np*	*np*	*np*	*np*	*np*	*np*

[a] *np* signifies not planted. Map Code 54 at Cannon had 4% LR.
Surv=Survival as % of trees planted. Heal= Healthy trees as percent of living rust-free trees.
LR= Trees living with rust infection as % of living trees. DR= Trees dead from rust as % of total trees planted

[b] WOC: Western Oregon Cascades; SOC: South Oregon Coast; KM: Klamath Mts; NC: North Coast; NSN: Northern Sierra Nevada; SSN: Southern Sierra Nevada; SC: South Coast; T-P: Transverse- Peninsular.

verse-Peninsular and Sierra San Pedro Martir sources had highest survival (fig. 4a). In contrast, based on regional means at FIT, survival varied in a curvilinear pattern with elevation of origin ((R^2=0.68, n=9); southern Sierra Nevada sources had highest survival (fig. 4b). Contour plots for survival pattern by latitude and elevation of origin likewise revealed distinctions between test sites.

Generally, sources with higher survival and health at SUN had lower survival and health at FIT (r=-0.40, r=-0.35, n=42). At SUN, highest survival was associated with lower elevation of source origin (r=0.44, n=46). At BUR, survival was not associated with source origin. Rust infection of sources was not consistent (not correlated) across test sites. Survival and rust infection of sources at FIT were weakly correlated (r=0.35, quadratic R^2=0.18, n=63); sources with 11 percent or lower infection had less than average survival.

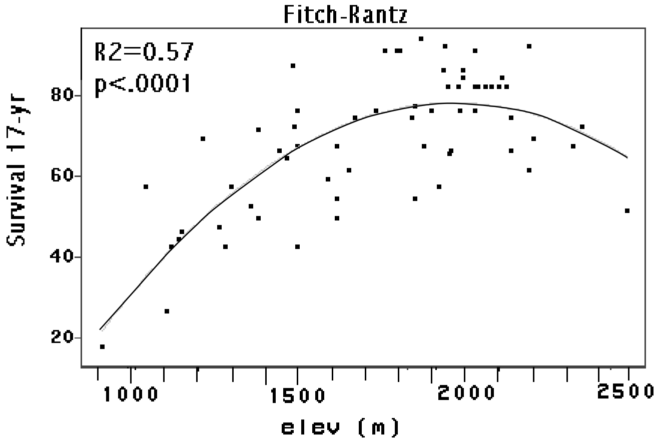


Figure 3—Association between 17-year survival and origin elevation for all provenances at Fitch-Rantz.

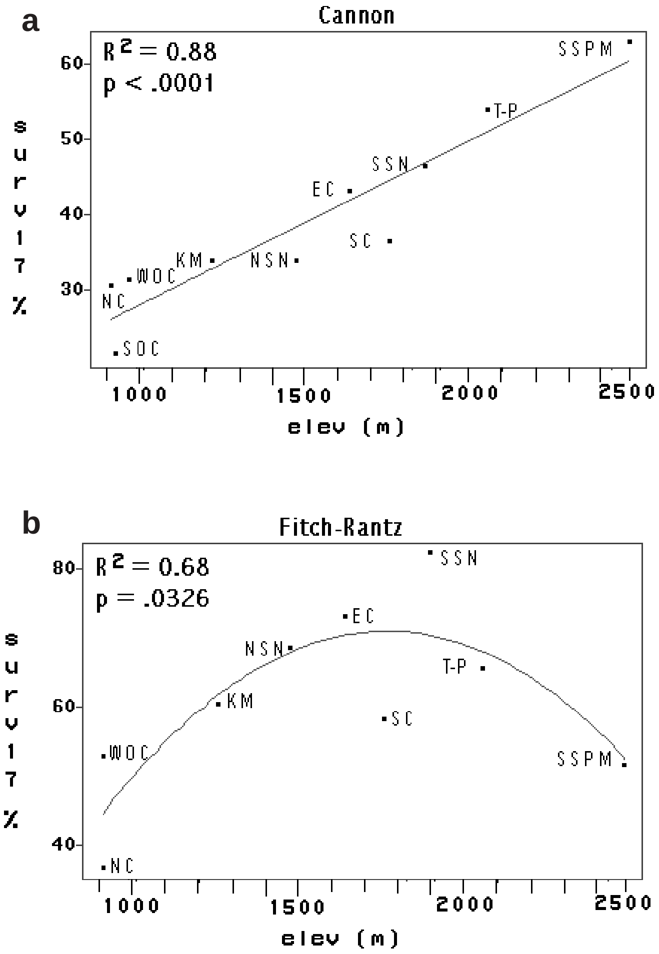


Figure 4—Association between 17-year survival and origin elevation for regions at: a. Cannon and b. Fitch-Rantz.

All 4 Test Plantations: 42 Common Sources

Genetic Structure in Adaptive Traits: ANOVA: 10-Year Growth—All factors except Regions reflected significant differences for 10-year height across plantations (table 5). Plantations accounted for most of the variability in growth; trees at CAN and SUN grew much faster than trees at FIT and BUR (table 6). All pair-wise differences between test means were significant (p<0.05), except 10-year height at CAN and SUN were similar. The G-x-E interactions: P-x-R and P-x-S(R) were about twice the magnitude of their genetic main effects (Regions and Sources within Region) (table 5). Thus, height means for regions and sources within region changed ranking across test plantations. Together, genetic effects and their interactions accounted for about 11 percent of the total variability in height and volume (8 percent for diameter). Comparing across all four plantations, Regions and Sources(R) were about equal for height and diameter, but for volume, Sources(R) far exceeded Regions.

Analysis by plantation revealed that BUR, a xeric site, was a relatively poor site for expression of genetic differences in growth (table 5). At BUR, diameter and volume did not vary significantly by genetic source, and differences were relatively weak for height. CAN was a good site for genetic expression, but only at the Sources(R) level. Genetic differences for all traits were expressed best at FIT, even with its short growing season; Regions were 2.5 to 4 times more variable than Sources(R). Together, genetic effects accounted for: 39, 65, and 30 percent in height, diameter, and volume, respectively. SUN also was excellent for total genetic expression for height (65 percent of total variability), and, at the Sources(R) level, for diameter and volume (35 percent).

The region effect was similar and small for growth at CAN and BUR, but it was very different at FIT and SUN, where Regions were about twice the magnitude of Sources(R) for height. Both sites expressing strong regional differences have low moisture stress and productive soils. But SUN has a strong maritime influence and long growing season without snow pack, while FIT has a short growing season with deep, persistent snow pack. Trees at SUN grew twice as much in height and three times as much in volume as trees at FIT (table 6). At SUN the region effect was due most notably to superiority of the western Oregon Cascade and North Coast sources, with Klamath Mts. and Sierra Nevada sources intermediate, and South Coast and Transverse-peninsular sources were distinctly inferior in growth (table 7). In contrast, at FIT, Sierra Nevada trees grew most, and North Coast and Transverse-Peninsular trees grew least. The Plantation-x-Region interaction was caused by region height differences between SUN and FIT, the two most climatically distinct tests.

Seed Source Correlations Among All Test Sites (10, 13-, and 17-Year Data)—Simple correlations between tests were consistently strongest for height, and consistently lowest when FIT was involved. Simple correlations between test-source means for growth traits among low altitude tests (CAN, SUN, BUR) were positive and mostly significant (r = 0.21 to 0.65, n=42, p <0.05 when r > 0.31). But r-values are relatively low and show evidence for G-x-E interaction. Correlations between CAN and SUN source growth means were highest (0.48 to 0.65). CAN x BUR (0.34 to 0.48) followed by SUN x BUR (0.21 to 0.46) were lower still. Height at FIT was inversely related to height at SUN (r = 0.36), but all other growth trait correlations between FIT and other sites were non-significant and generally negative. Survival was significantly correlated only for CAN x FIT (r = 0.44, p<0.01).

Overall, source means were generally correlated between low altitude tests, but not impressively so. Ranking among sources at FIT were most different from ranking in other tests. These results imply relatively large genetic source x test environment interaction.

Geographic Trends in Seed Source Means—Simple correlations by test site between source mean growth traits and their geographic origin variables (elevation, latitude, longitude, and distance from test site to seed origin) were relatively high (table 8). Highest correlations were inverse associations between growth and source elevation, except at FIT, the high elevation test, where growth-source elevation correlations were always positive. Also, correlations at FIT were opposite in sign for source latitude and longitude from the other three tests. SUN exhibited the strongest correlations of all tests, with over 60 percent of the variation in height

Table 5—Variance component analyses combining all test plantations with 42 common seed sources for 10-yr height (h10), diameter (dg10), and volume (v10) across and by plantation.

Source of		h10			dg10			v10		
Variation	DF	VarC	%	PR > F	VarC	%	PR > F	VarC	%	PR > F
Plantations	3	7463	69	0.0001	680	69	0.0001	38	60	0.0001
Blocks(P)	18	444	4	0.0001	46	5	0.0001	3	5	0.0001
Regions	6	196	2	0.2325	32	3	0.0161	0	0	0.1891
Sources(R)	35	172	2	0.0061	17	2	0.0020	2	3	0.0028
P*R	18	376	3	0.0002	5	1	0.1995	0	1	0.2382
P*S(R)	105	395	4	0.0001	24	2	0.0001	5	7	0.0001
Error	680	1772	16		186	19		16	24	
	865	10818	100		990	100		64	100	
Cannon										
Regions	6	0	0	0.3607	3	1	0.2684	0	0	0.5206
Sources(R)	35	1012	20	0.0001	76	15	0.0021	16	24	0.0001
Blocks	5	756	15	0.0001	75	15	0.0001	8	12	0.0001
Error	157	3246	65		338	69		42	64	
	203	5014	100		492	100		66	100	
Fitch-Rantz										
Regions	6	507	28	0.0001	52	26	0.0001	1	23	0.0001
Sources(R)	35	200	11	0.0004	13	6	0.0205	0	6	0.0174
Blocks	5	124	7	0.0001	22	11	0.0001	1	13	0.0001
Error	196	969	54		112	57		3	57	
	242	1801	100		198	100		6	100	
Burnt Timber										
Regions	6	199	6	0.0569	15	5	0.0613	0	1	0.3639
Sources(R)	35	204	7	0.0344	6	2	0.2597	0	5	0.0963
Blocks	4	840	27	0.0001	72	26	0.0001	2	20	0.0001
Error	163	1877	60		190	67		6	74	
	208	3120	100		283	100		8	100	
Sundown										
Regions	6	1535	42	0.0008	17	7	0.0484	3	9	0.0677
Sources(R)	35	843	23	0.0001	82	35	0.0001	10	35	0.0001
Blocks	4	50	1	0.0298	14	6	0.0002	1	5	0.0006
Error	164	1197	33		121	52		14	51	
	209	3625	100		234			28	100	

Table 6—Least square means for test plantations by growth traits.[a]

Plantation	Trait - Least Square Mean			
	cm	*mm*	*dm^3*	*cm*
46 sources	h13	db13	v13	hgr3
Sundown	466	130	72.8	146
Burnt Timber	284	76	17.8	95
Std Error	17.1	4.4	5.2	5.4
63 sources	h17	db17	v17	hgr7
Cannon	714	154	52.4	378
Fitch-Rantz	458	90	11.1	234
Std Error	14.4	3.1	2.4	9.8
42 sources	h10	dg10	v10	
Sundown	320	95.6	11.07	
Burnt Timber	188	49.8	2.19	
Cannon	325	108.9	14.31	
Fitch-Rantz	158	74.1	3.36	
Std Error	10	3.3	0.9	

[a] h=height, db=diameter breast height, db=diameter ground level, v=volume, hgr=recent 3- or 7-yr height growth increment; Based on three analyses: 10-yr-all plantations-42 common seed sources; 13-yr-SUN/BUR-46 common sources; 17-yr-CAN/FIT-63 common sources.

and 50 percent in volume associated with source elevation. Weakest correlations between growth and geographic variables other than elevation were for CAN.

Distance was expressed both as simple kilometer distance irrespective of direction ("Dist") and as north vs south direction-dependent (sources north of test latitude were given positive kilometer values, and sources south of test were given negative values). All test plantations except CAN expressed an association for better growth for sources closer in distance to the test site,"*i.e.*"correlations between growth and "Dist" were negative (table 8). Close examination revealed one local source (Big X Mtn) with vastly superior growth at CAN, and some superior sources at FIT were transferred 250 km. For Oregon tests, nearly all sources originated southward, so both variables gave the same result. However, at FIT, better growth was associated with closer source distance, and southern sources tended to outgrow northern sources ("Dist1" negative r-values, table 8).

Canonical correlations (1st variates) between growth and geographic variables were higher for SUN (r=0.89), and FIT (r=0.86), and lower for CAN (r=0.80) and BUR (r=0.77). Height and elevation were weighted most. Sixty-three percent of the variance in the derived geographic origin variable was associated with the variance in the derived growth trait variable.

Table 7—Least square means and ranks by test, region and 10-yr height (h10), diameter (dg10), and volume (v10).

	Sundown		Burnt Timber		Cannon		Fitch-Rantz	
Region and Trait	**Rank**	**LSM**	**Rank**	**LSM**	**Rank**	**LSM**	**Rank**	**LSM**
h10								
W Oregon Cascade	1	375	1	224	3	338	4	153
Klamath Mts	3	348	2	197	1	369	3	158
North Coast	2	372	4	189	2	360	7	129
N Sierra Nevada	4	322	3	194	4	330	2	184
S Sierra Nevada	5	307	5	183	5	328	1	195
South Coast	6	258	6	170	6	277	5	151
Transverse-Peninsular	7	254	7	160	7	274	6	133
Plantation Mean		320		188		325		158
dg10								
W Oregon Cascade	1	106.4	1	58.4	5	109.4	5	73.0
Klamath Mts	4	100.1	4	49.9	3	116.0	4	73.5
North Coast	3	103.2	5	46.9	4	111.2	6	68.2
N Sierra Nevada	2	103.5	2	55.8	1	117.0	2	82.5
S Sierra Nevada	5	100.0	3	51.3	2	116.1	1	84.7
South Coast	6	79.1	6	46.3	6	100.7	3	76.5
Transverse-Peninsular	7	76.9	7	40.0	7	92.2	7	60.5
Plantation Mean		95.6		49.8		108.9		74.1
v10								
W Oregon Cascade	1	15.24	1	3.29	5	14.21	5	3.07
Klamath Mts	4	12.28	3	2.16	1	17.86	4	3.20
North Coast	2	14.61	5	1.91	3	16.66	6	2.19
N Sierra Nevada	3	12.47	2	3.01	2	16.67	2	4.69
S Sierra Nevada	5	11.33	4	2.16	4	15.88	1	5.27
South Coast	6	6.07	6	1.74	6	9.91	3	3.30
Transverse-Peninsular	7	5.47	7	1.10	7	8.96	7	1.80
Plantation Mean		11.07		2.19		14.31		3.36

Seed Source Rank Changes Across Test Plantations—Rank comparisons for the same 42 seed sources on the same four test sites for 10-year growth traits revealed clear genetic source interactions across tests (table 9). These interactions were not due to scale effects; rather they were determined to be true rank change interactions (Surles and others 1995). Major rank changes among tests for the same sources were evident between: Oregon sites, California sites, and states. Patterns of rank changes may reflect adaptive responses to different climates. Based on the subjective criterion of at least one quartile rank change (11+) in both height and volume, within the Oregon pair (coastal *vs* inland tests) 12 sources were interactive, five of which (Map Codes: 18, 23, 41, 45, 50) were highly so. Within the California pair (low *vs* high elevation tests), 18 sources were interactive, 12 of which (5, 7, 8, 14, 18, 24, 32, 33, 37, 39, 42, 51) were highly so. Some sources displayed specific preference for their "home" state (2, 3, 8, 50, 56). Among the four test sites, 18 sources (43 percent) were considered highly interactive. Some 13 sources were interactive only at FIT, being stable across all other tests; five low elevation sources were especially maladapted to FIT; eight mid to high elevation sources were especially well-adapted. Only 10 sources (24 percent) were relatively stable in rank across all tests (15, 19, 31, 34, 43, 46, 52, 55, 64, 68).

Growth Trends Over Time—Within-test simple correlations between 10-year total growth and subsequent 3- or 7-year growth increment were made to monitor potential trend changes among sources over time. Most recent 7-year height increment at FIT was highly correlated with height at age 10 (r=0.94, p<0.0001), but much less so at CAN (r=0.62, p<0.0001). Corresponding values for recent 3-year increment and 10-year total were identical at both SUN and BUR (r=0.87, p<0.0001). Thus, the pattern of growth differences among sources at CAN changed to a greater degree during recent years compared to the other tests, which remain very stable.

Cannon and Fitch-Rantz: 63 Common Seed Sources: 17-Year Data

Genetic Structure in Adaptive Traits: ANOVA Variance Components and Means—Analysis of 17-year data for CAN/FIT provided 21 additional sources to better represent regions and sources within regions. Forty-two of the 63 common sources were from the Sierra Nevada. Plantations accounted for the majority of variability in all traits (66 to 74 percent for growth, and 50 percent for form, Table 10). Trees at CAN grew 1.6 times more height and 4.7 times more volume as trees at FIT (table 6). Genetic sources accounted for 10 to 23 percent of total variability. Significant G-x-E interactions (P*R, P*S(R)) imply that growth trait differences among Regions and Sources(R) must be interpreted separately for each test plantation. G-x-E interactions (composed mainly of P*R for height, and mainly P*S(R) for volume) were 3 times the magnitude of genetic main effects (table 10).

Table 8—Simple correlations between seed source growth trait means and geographic origin variables at each test site.[a]

Test_Trait [b]	Elev	Lat	Lon	Dist	Dist1
Sun_h10	**–0.808**	**0.624**	***0.638***	**–0.637**	**–0.637**
Sun_dg10	**–0.565**	*0.330*	0.290	*–0.347*	*–0.347*
Sun_v10	**–0.698**	**0.424**	**0.423**	**–0.436**	**–0.436**
Sun_h13	**–0.792**	**0.638**	***0.653***	**–0.652**	**–0.652**
Sun_dg13	**–0.599**	*0.370*	*0.327*	*–0.378*	*–0.378*
Sun_v13	**–0.712**	**0.478**	**0.469**	**–0.484**	**–0.484**
Sun_hgr3	**–0.685**	**0.610**	**0.626**	**–0.624**	**–0.624**
Sun_dggr3	**–0.559**	0.396	*0.355*	*–0.379*	*–0.379*
Sun_vgr3	**–0.709**	**0.504**	**0.490**	**–0.507**	**–0.507**
Bur_h10	**–0.571**	**0.550**	**0.441**	**–0.517**	**–0.517**
Bur_dg10	**–0.413**	*0.362*	0.216	*–0.342*	*–0.342*
Bur_v10	**–0.398**	0.275	0.187	–0.264	–0.264
Bur_h13	**–0.580**	**0.590**	**0.495**	**–0.556**	**–0.556**
Bur_dg13	*–0.360*	*0.355*	0.207	*–0.339*	*–0.339*
Bur_v13	**–0.396**	*0.319*	0.224	–0.302	–0.302
Bur_hgr3	**–0.546**	**0.615**	**0.554**	**–0.582**	**–0.582**
Bur_dggr3	–0.155	0.256	0.138	–0.250	–0.250
Bur_vgr3	*–0.388*	*0.340*	0.242	*–0.320*	*–0.320*
Can_h10	**–0.633**	0.284	*0.367*	–0.045	*0.324*
Can_dg10	*–0.371*	0.017	0.050	*–0.378*	0.028
Can_v10	**–0.475**	0.064	0.134	–0.298	0.089
Can_h17	**–0.583**	0.302	*0.345*	–0.007	*0.333*
Can_db17	*–0.383*	0.052	0.094	*–0.315*	0.071
Can_v17	**–0.465**	0.101	0.161	–0.242	0.129
Can_hgr7	*–0.377*	0.256	0.233	0.048	0.268
Fit_h10	**0.551**	–0.292	**–0.460**	**–0.559**	*–0.360*
Fit_dg10	*0.389*	–0.276	*–0.351*	**–0.632**	*–0.314*
Fit_v10	**0.472**	*–0.323*	**–0.441**	**–0.603**	*–0.372*
Fit_h17	**0.556**	–0.268	**–0.463**	**–0.547**	*–0.339*
Fit_db17	**0.525**	–0.272	**–0.471**	**–0.609**	*–0.347*
Fit_v17	**0.475**	–0.279	**–0.426**	**–0.611**	*–0.331*
Fit_hgr7	**0.547**	–0.249	**–0.455**	**–0.529**	*–0.321*

[a] *Italics: p<.05, n=42* **Bold: p<.01, n=42**. Elev: elevation, Lat: latitude, Lon: longitude, Dist: seed origin distance from test site, Dist1: distance of northern (+) or southern (–) origins from test site.

[b] Sun,Bur,Can,Fit refers to test: Sundown, Burnt Timber, Cannon, Fitch-Rantz. h, db, dg, v, hgr, dggr, and vgr refers to trait: height, diameter breast height, diameter ground level, volume, recent 3- or 7-yr growth increment for height, diameter ground level, and volume respectively.

Analyses by test revealed large genetic source effects at both plantations, accounting for 38 to 40 percent for height and 32 to 40 percent for diameter and volume (table 10). Region of origin contributed most to growth variability at CAN/FIT, but Sources(R) was a highly significant contributor to growth in each test. Regions accounted for four times that of Sources(R) at FIT and 1.5 to two times Sources(R) at CAN. Regions were less interactive with plantations than Sources(R), perhaps because Regions were more highly buffered with broad genetic diversity. The P*R interaction involved regions with high elevation persistent snow-packs, Eastern Cascade and Sierra Nevada Ranges grew more at FIT than coastal and milder regions which grew more at CAN (fig. 5). Seed source means were highly variable between tests (tables 10, 12).

Growth and Geographic Origin Factors: Correlation and Regression—Region height means at CAN decreased with origin elevation (R^2=0.76, p=0.001, n=10). At the source level, height of Sierra Nevada sources decreased linearly with increase in elevation (R^2=0.37, p<0.0001, n=42, fig. 6a). At FIT, growth of Sierra Nevada sources increased with source elevation in a curvilinear pattern peaking at 1860 m and then decreasing (R^2=0.44, p<0.0001, n=42, fig. 6b).

For 63 sources at both test sites, volume (v17) and previous 7-year height growth (hgr7) varied in curvilinear patterns with distance in miles north (+) or south (-) from test to seed origin (dist1), with R^2=0.22, p=0.0006 at CAN (fig. 7a) and R^2=0.38, p<0.0001 at FIT (fig. 7b) for v17. Corresponding values for hgr7 were only slightly lower. Best hgr7 growth occurred for sources nearest CAN. At FIT best growth occurred for sources near the site and up to 240 km south. Recent height growth (hgr7) was correlated with 10-year height more closely at FIT (r=0.93) than at CAN (r=0.68), just as it was in the 10-year 42-source analysis.

Table 9—Ranking among 42 common sources within test for 10-yr height (h10), diameter (dg10), and volume (v10).

Regn	Seed Origin: Locale	Sdz	Elev m	Map Code	SUN h10	BUR h10	CAN h10	FIT h10	SUN dg10	BUR dg10	CAN dg10	FIT dg10	SUN v10	BUR v10	CAN v10	FIT v10
WOC	Steelhead Ck	491	761	2	5	8	29	38	18	19	36	40	11	19	30	38
WOC	Grass Ck	491	595	3	7	4	14	26	8	8	26	32	7	5	25	32
WOC	Limpy Rock SW	491	1067	5	17	2	13	40	34	2	33	36	32	2	29	39
WOC	Camp Comfort	492	730	7	1	14	8	36	3	22	23	33	2	24	18	35
WOC	Woodruff Flat	501	939	8	3	6	19	34	2	12	17	35	3	12	17	37
WOC	Camp Ck	502	1175	10	16	7	17	31	31	18	21	26	27	15	23	30
KM	Dutch Ck	321	1220	14	11	23	5	39	29	34	11	39	25	35	7	40
KM	Salmon R	311	1201	15	15	19	15	23	23	23	25	24	22	18	19	21
NC	Fish Rock Rd	94	457	18	2	32	4	42	9	41	15	41	4	39	9	42
NC	S Fk Elk Ck	371	1372	19	19	15	26	28	30	28	34	37	28	22	24	34
NSN	Forest Ranch	524	710	20	13	20	9	33	14	16	16	23	14	17	14	31
NSN	Diamond I	524	1151	21	12	3	3	25	12	3	3	16	10	3	3	18
NSN	Colby Mtn	524	1677	23	33	17	35	16	37	13	39	31	36	13	40	29
NSN	Stover Mtn	523	1860	24	14	24	31	3	20	25	40	11	20	27	39	5
NSN	Pleasant Valley	526	771	30	24	10	27	37	19	7	30	38	16	7	28	36
NSN	Crozier Loop	526	854	31	6	11	6	17	6	5	1	21	6	11	2	16
NSN	Breedlove	525	915	32	10	1	1	32	17	1	2	30	12	1	1	28
NSN	Big X Mtn	526	1037	33	8	21	2	30	4	14	5	25	5	16	4	22
NSN	Big Mtno	526	1220	34	9	16	10	12	5	9	4	4	8	8	5	9
NSN	Snow Mill Rd	526	1372	35	22	12	22	22	15	11	7	6	21	6	11	12
NSN	Caldor Rd	526	1418	36	23	28	16	29	16	27	12	19	18	29	12	26
NSN	Uncle Tom’s	525	1524	37	31	30	37	14	24	33	22	9	26	36	32	10
NSN	Tells Creek	526	1555	38	38	22	20	7	39	10	13	8	39	14	15	7
NSN	Alder Ck	526	1605	39	29	42	32	4	26	40	20	3	29	41	22	3
NSN	Pilliken	526	1707	40	28	27	23	11	22	24	18	14	24	25	20	13
NSN	Sugarloaf	526	1806	41	26	9	38	15	25	4	37	15	23	4	35	19
NSN	Mule Cyn	526	2012	42	37	39	39	1	35	31	32	2	35	26	38	4
NSN	Bunker Hill	525	2134	43	34	31	36	27	33	35	28	27	34	32	36	23
NSN	Sugar Pine	531	1220	45	4	33	12	24	1	32	8	28	1	34	10	25
NSN	Lyons Reservoi	531	1439	46	25	18	25	13	13	17	27	17	17	9	26	15
NSN	Summit Stn	531	1662	47	30	35	24	5	27	29	14	5	30	31	16	2
NSN	Pinecrest	531	1905	48	39	41	42	18	36	30	41	22	38	30	37	24
NSN	Dodge Ridge	531	2119	49	32	26	34	19	28	20	31	18	31	21	34	17
SSN	Chowchilla Mtn	532	1851	50	21	37	11	2	10	39	9	10	13	40	8	8
SSN	N Fk Willow Ck	532	1846	51	18	29	30	6	7	26	24	1	9	28	27	1
SSN	Shaver Lake	533	1787	52	20	5	7	10	11	6	6	12	15	10	6	11
SSN	Landslide	534	1697	53	40	25	28	20	40	21	29	20	40	20	33	20
SSN	Happy Gap	534	1741	54	27	13	21	8	21	15	19	7	19	23	21	6
SSN	Lockwood Grov	534	2002	55	35	38	33	21	38	38	35	34	37	38	31	27
SSN	Hume Lake Rd	534	2030	56	36	36	18	9	32	36	10	13	33	37	13	14
SC	Junipero Serra	120	1768	64	41	34	40	35	41	37	38	29	41	33	41	33
T-P	San Jacintos	997	2287	68	42	40	41	41	42	42	42	42	42	42	42	41

SUN and BUR: 46 Common Seed Sources: 13-Year Data

Genetic Structure in Adaptive Traits: Variance Components and Means—The family-plot structure at Oregon test plantations allowed estimation of variance components that included among-families and within-family plots (table 11). Plantations accounted for 42 to 51 percent of the total variability in growth traits. Trees at SUN grew 1.64 times taller and 4 times more volume than BUR at 13-years (table 6). Regions, Families, and Plantations-x-Sources(R) were highly significant for 13-year height. Applying a fixed assumption for P-x-R interaction (MEM) resulted in significance for Regions main effect for all traits (not shown). About 10 percent of all variability in 13-year height was attributable to genetic main effects and their interactions with plantations (table 11). The within-family plot variance is composed of confounded genetic and environmental factors. Within-family plot variance was similar but slightly smaller than the among-family plot (experimental error).

Genetic sources at SUN accounted for 25 percent of the total variability in 13-year height including the within-plot component (or 38 percent excluding it). Corresponding values at BUR were only 11 percent (or 16 percent) and about the same magnitude as Blocks. Although Regions and Sources(R) were significant for 13-year height, Regions was larger at SUN, while Sources(R) was larger at BUR. Effect of Regions was not significant for 13-year diameter and

Table 10—Variance component percent of total variability and F-tests for growth traits of 63 common seed sources at Cannon and Fitch-Rantz.[a]

Source of		h17		dbh17		v17		h17/dbh17		hgr7	
Variation	**DF**	**%**	**Pr > F**	**%**	**Pr > F**	**%**	**Pr > F**	**%**	**Pr > F**	**%**	**Pr > F**
Across Tests:											
Plantations	1	73	0.0001	74	0.0001	68	0.0001	48	0.0002	67	0.0001
Blocks(P)	10	2	0.0001	2	0.0001	2	0.0001	2	0.0001	3	0.0001
Regions	8	2	0.1630	3	0.0399	3	0.1088	2	0.2808	3	0.2038
Sources(R)	54	0	0.8324	0	0.5797	0	0.5967	2	0.0897	0	0.9388
P*R	8	5	0.0016	3	0.0051	3	0.0532	15	0.0001	6	0.0005
P*S(R)	54	3	0.0001	3	0.0001	6	0.0001	4	0.0024	3	0.0001
Error	537	14		15		20		28		18	
By Test: Cannon											
Regions	8	25	0.0003	21	0.0001	15	0.0031	12	0.0001	18	0.0009
Sources(R)	54	15	0.0001	11	0.0012	17	0.0001	0	0.5933	8	0.0040
Blocks	5	7	0.0001	3	0.0043	4	0.0020	7	0.0001	5	0.0001
Error	243	52		64		64		80		69	
By Test: Fitch-Rantz											
Regions	8	31	0.0001	33	0.0001	29	0.0001	35	0.0001	32	0.0001
Sources(R)	54	7	0.0007	7	0.0002	9	0.0001	12	0.0001	11	0.0001
Blocks	5	11	0.0001	11	0.0001	12	0.0001	3	0.0012	12	0.0001
Error	243	51		48		50		50		45	

[a] h, dbh, v, hgr: height, diameter breast height, volume, and recent periodic height increment. Random Effects Model was assumed to estimate variance components and F-tests.

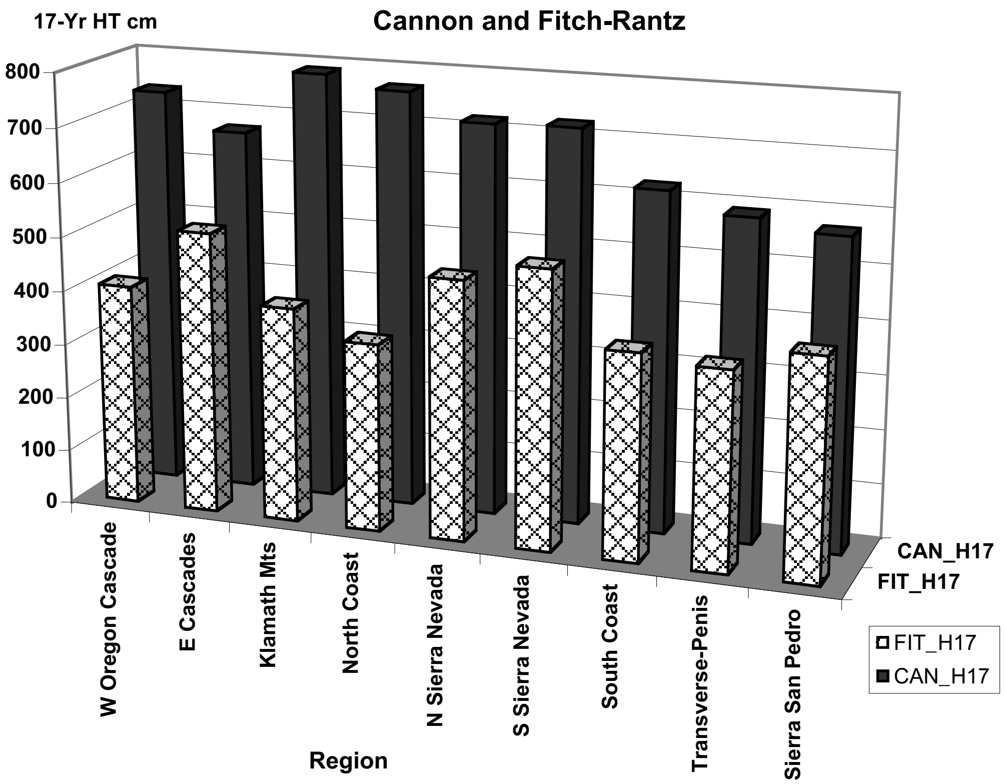


Figure 5—Mean 17-year height for regions at Cannon and Fitch-Rantz.

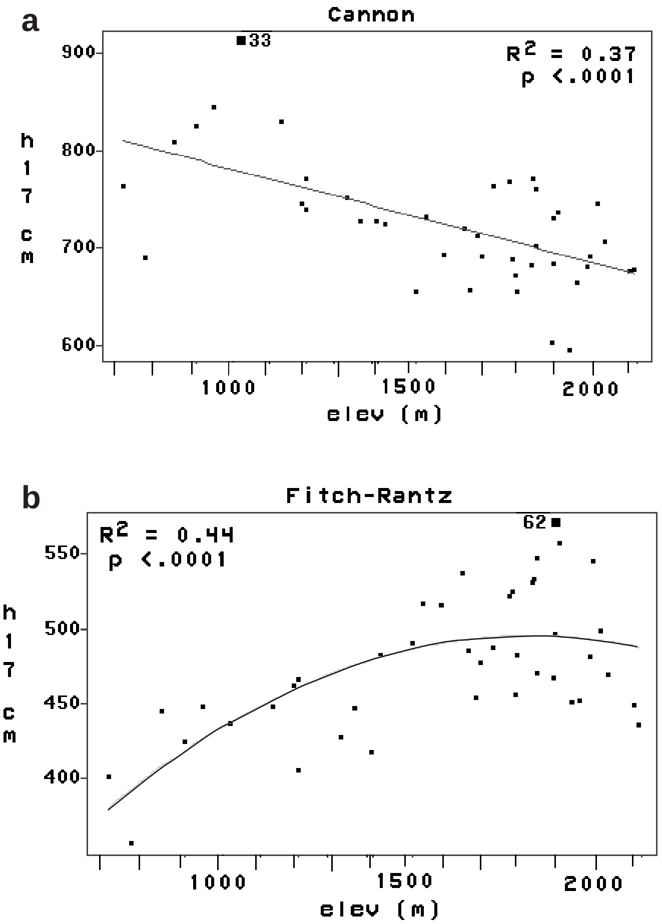


Figure 6—Association between 17-year height and origin elevation for Sierra Nevada provenances at: a. Cannon and b. Fitch-Rantz.

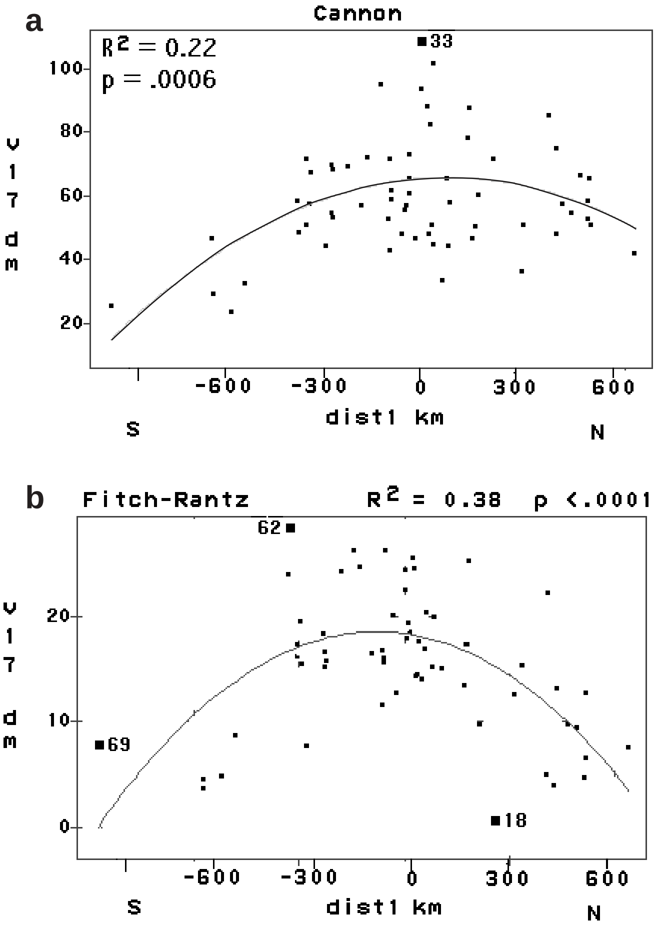


Figure 7—Association between 17-year volume and provenance distance north(+) or south(-) of test locations for: a. Cannon and b. Fitch-Rantz.

volume in either test. Effect of families was relatively small for all traits but was significant at SUN (table 11).

Ranking of regions for h13 was: WOC > NC > KM > SOC > NSN > SSN > SC > T-P. Significant ($p<.05$) differences between Region means across tests for h13 were: WOC > NSN, SSN, SC, T-P; SOC > T-P; KM > SSN, SC, T-P; NC > SSN, SC, T-P; NSN > T-P; where: WOC = Western Oregon Cascade, SOC = South Oregon Coast, KM = Klamath Mountains, NC = North Coast, NSN = Northern Sierra Nevada, SSN = Southern Sierra Nevada, SC = South Coast, and T-P = Transverse-Peninsular. Seed sources within region varied greatly between test sites (table12).

Stability in Performance Across Plantations: Correlations and Regressions—Simple correlations between test plantation-seed source means, a measure of G-x-E interaction, were significant only for height (r=0.53 for h10, r=0.51 for h13, r=0.37 for hgr3, n=46). This indicates substantial differences in performance ranking of sources between SUN and BUR that are increasing with age.

Growth and Geographic Origin Factors: Correlation and Regression—Growth was inversely correlated with elevation of origin for region means (fig. 8a, 8b) and for source means (fig. 8c, 8d). Among traits, height was most highly correlated with seed origin. Among seed origin variables, elevation was most highly correlated with growth. SUN provided the strongest pattern of growth with seed origin for source means: 64 percent of the variation in 13-year height was associated with elevation. The corresponding value at BUR was 40 percent. Based on regional means, 85-86 percent of the variation in height was associated with region mean elevation at both sites. The dominant pattern was that growth decreased linearly with increasing elevation and with decreasing latitude and longitude of seed origin. Using canonical correlation, the derived seed origin variable accounted for 79 percent of the variation in the derived growth variable for SUN, and 61 percent for BUR. Further, the derived growth variable for SUN accounted for 62 percent of the variation in the derived growth variable for BUR. So, the implied adaptive pattern of growth response was somewhat different at these Oregon sites.

Discussion

Sugar pine displayed a complex genetic structure in adaptive traits. Strong G-x-E interactions were expressed for

Table 11—Variance components, percent of total variability, and F-tests for 13-yr height diameter, and volume for 46 common seed sources at Sundown and Burnt Timber across and by plantation.

Source of		h13			dg13			v13		
Variation	**DF**	**VarC**	**%**	**PR > F**	**VarC**	**%**	**PR > F**	**VarC**	**%**	**PR > F**
Plantations	1	15964	49	0.0001	1464	51	0.0001	262	42	0.0001
Blocks(P)	8	975	3	0.0001	59	2	0.0001	9	1	0.0001
Regions	7	1548	5	0.0050	47	2	0.0590	11	2	0.1489
Sources(R)	38	337	1	0.1244	15	1	0.3925	2	0	0.3397
Family(R S)	321	151	0	0.0001	11	0	0.0004	3	1	0.0158
P*R	7	0	0	0.5367	0	0	0.9389	0	0	0.3053
P*S(R)	38	1092	3	0.0001	84	3	0.0001	33	5	0.0001
P*F(R S)	320	0	0	0.9996	0	0	0.9982	0	0	0.9031
Block * (R,S,F)	2495	6983	21		653	23		161	26	
Within Fam Plot	2094	5472	17		556	19		140	22	
		32522	100		2889	100		622	100	
Burnt Timber										
Regions	7	570	4	0.0180	21	2	0.1053	1	1	0.4006
Sources(R)	38	1031	7	0.0001	70	6	0.0001	10	7	0.0001
Families(R S)	320	0	0	1.0000	0	0	0.9999	0	0	1.0000
Blocks	4	1722	12	0.0001	107	9	0.0001	11	9	0.0001
Blocks*R,S,F	1219	6738	46		602	49		67	51	
Within Fam Plot	1062	4450	31		435	35		42	32	
		14510	100		1236	100		130	100	
Sundown										
Regions	7	2460	13	0.0013	15	1	0.1054	19	3	0.0602
Sources(R)	38	1852	10	0.0001	148	9	0.0001	62	10	0.0001
Families(R S)	320	359	2	0.0078	26	2	0.0224	11	2	0.0194
Blocks	4	235	1	0.0001	11	1	0.0002	6	1	0.0001
Blocks*R,S,F	1276	7166	39		699	44		249	42	
Within Fam Plot	1032	6528	35		682	43		240	41	
		18600	100		1581	100		587	100	

Table 12—Least square means for growth traits by test plantation and seed source.[a]

Seed Origin:				Cannon			Fitch-Rantz			Sundown			Burnt Timber		
Locale	**MC**	**Sdz**	**Elev**	**h17**	**db17**	**v17**	**h17**	**db17**	**v17**	**h13**	**dg13**	**v13**	**h13**	**dg13**	**v13**
			m	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*
Breitenbush R	1	462	915	637	133	42.1	364	68	7.9	*np*	*np*	*np*	*np*	*np*	*np*
Steelhead Ck	2	491	761	716	144	51.6	398	69	7.8	532	144	95.3	321	82	21.7
Grass Ck	3	491	595	740	163	66.0	444	85	13.1	548	149	106.3	359	94	32.2
Limpy Rock NE	4	491	1067	799	154	61.4	374	65	4.9	*np*	*np*	*np*	*np*	*np*	*np*
Limpy Rock SW	5	491	1067	736	142	53.0	388	70	6.6	485	125	69.2	386	99	38.4
OK Butte	6	491	1372	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*
Camp Comfort	7	492	730	822	157	68.2	420	81	9.7	609	157	127.8	316	80	19.6
Woodruff Flat	8	501	939	705	152	55.6	423	77	8.4	543	153	120.4	330	91	26.7
Elk Ckn	9	502	1067	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*	*np*
Camp Ck	10	502	1175	733	152	56.5	450	85	13.4	485	129	71.3	320	82	20.7
Gold Beach	11	81	930	*np*	*np*	*np*	*np*	*np*	*np*	487	129	69.9	299	75	16.5
Pea Soup	12	512	1116	*np*	*np*	*np*	*np*	*np*	*np*	495	131	73.7	318	81	23.2
Bolan	13	511	1372	793	171	75.3	374	66	4.3	*np*	*np*	*np*	*np*	*np*	*np*
Dutch Ck	14	321	1220	859	174	85.8	349	61	5.4	499	132	77.2	294	73	14.3
Salmon R	15	311	1201	714	141	51.4	464	87	15.6	516	144	92.6	304	80	20.5
Black Hills	16	703	1616	726	141	50.0	587	106	22.5	*np*	*np*	*np*	*np*	*np*	*np*
Glass Mtn	17	741	1677	616	123	37.2	452	86	12.8	*np*	*np*	*np*	*np*	*np*	*np*
Fish Rock Rd	18	94	457	828	161	72.4	263	33	0.0	587	148	116.5	278	71	14.6
S Fk Elk Ck	19	371	1372	707	158	63.8	429	83	9.9	486	133	76.0	306	77	19.9
Forest Ranch	20	524	710	762	170	78.0	469	103	13.9	504	143	90.0	284	83	19.7
Diamond I	21	524	1151	834	182	89.7	457	89	17.6	504	143	93.5	345	96	31.5

(con.)

Table 12 (Con.)

Seed Origin:				**Cannon**			**Fitch-Rantz**			**Sundown**			**Burnt Timber**		
Locale	**MC**	**Sdz**	**Elev**	**h17**	**db17**	**v17**	**h17**	**db17**	**v17**	**h13**	**dg13**	**v13**	**h13**	**dg13**	**v13**
			m	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*	*cm*	*mm*	*dm^3*
Jonesville	22	524	1601	*np*	*np*	*np*	*np*	*np*	*np*	475	132	70.9	269	72	15.9
Colby Mtn	23	524	1677	642	142	44.2	487	96	17.6	412	119	51.8	300	89	24.5
Stover Mtn	24	523	1860	706	151	52.6	548	109	25.6	492	140	82.8	287	80	19.4
Cal-Ida	25	525	1335	756	164	67.4	434	92	15.3	*np*	*np*	*np*	*np*	*np*	*np*
N Shirttail Cyn	26	525	1209	746	188	102.5	486	106	20.6	*np*	*np*	*np*	*np*	*np*	*np*
Sierraville	27	772	1793	686	158	59.0	526	102	20.3	*np*	*np*	*np*	*np*	*np*	*np*
Little Truckee	28	772	1974	664	144	45.3	462	91	15.5	*np*	*np*	*np*	*np*	*np*	*np*
Upper Truckee	29	772	1951	595	129	34.1	460	99	17.2	*np*	*np*	*np*	*np*	*np*	*np*
Pleasant Valley	30	526	771	690	140	47.3	424	78	9.6	454	136	78.1	306	89	27.2
Crozier Loop	31	526	854	812	185	95.7	455	92	17.9	538	157	111.4	307	91	25.6
Breedlove	32	525	915	815	179	87.3	457	93	14.3	516	145	98.1	383	105	56.1
Big X Mtn	33	526	1037	908	189	107.0	448	90	14.5	517	152	101.9	287	88	22.3
Big Mtn	34	526	1220	743	170	74.7	476	102	20.4	515	155	104.3	288	86	24.9
Snow Mill Rd	35	526	1372	712	165	66.2	461	98	18.8	465	147	85.2	301	88	27.2
Caldor Rd	36	526	1418	735	153	61.5	452	92	13.0	458	142	83.5	272	81	17.9
Uncle Tom’s	37	525	1524	640	148	47.1	502	113	24.8	437	139	75.5	246	72	12.8
Tells Creek	38	526	1555	723	176	81.4	518	109	25.8	406	121	52.3	296	95	26.7
Alder Ck	39	526	1605	685	150	56.2	512	105	22.9	443	136	70.3	228	69	10.7
Pilliken	40	526	1707	682	151	56.3	485	102	18.3	455	138	79.9	270	85	18.7
Sugarloaf	41	526	1806	655	146	51.6	483	106	19.6	464	136	77.8	316	97	30.7
Mule Cyn	42	526	2012	701	146	49.6	547	109	24.7	399	123	53.2	242	75	17.4
Bunker Hill	43	525	2134	678	143	45.4	456	92	14.8	422	127	60.0	258	75	17.0
Groveland Stn	44	531	963	829	187	95.6	448	91	16.8	*np*	*np*	*np*	*np*	*np*	*np*
Sugar Pine	45	531	1220	763	166	71.9	410	84	11.8	544	166	127.2	249	72	13.6
Lyons Reservoir	46	531	1439	726	159	59.7	483	95	17.0	449	141	81.4	311	86	29.1
Summit Stn	47	531	1662	705	162	62.6	542	112	26.6	427	136	70.0	249	75	14.8
Pinecrest	48	531	1905	601	138	42.5	467	94	15.9	385	119	47.3	239	80	17.9
Dodge Ridge	49	531	2119	686	151	54.5	461	97	16.3	439	134	68.9	271	82	19.8
Chowchilla Mtn	50	532	1851	769	168	72.8	544	113	25.0	503	150	98.3	238	72	12.5
N Fk Willow Ck	51	532	1846	683	155	57.6	546	115	26.6	500	152	99.3	249	78	15.4
Shaver Lake	52	533	1787	769	167	70.1	536	112	24.6	473	142	81.5	324	92	27.7
Landslide	53	534	1697	713	150	55.5	465	94	15.5	381	112	44.6	292	82	22.0
Happy Gap	54	534	1741	772	165	69.8	492	93	16.9	451	140	78.8	299	84	18.3
Lockwood Grove	55	534	2002	690	152	54.8	485	90	16.0	393	118	49.0	237	66	11.3
Hume Lake Rd	56	534	2030	746	165	70.5	499	98	18.7	399	125	57.7	251	77	15.8
Burton Mdws	57	534	2322	*np*	*np*	*np*	*np*	*np*	*np*	392	120	54.7	234	66	11.4
Hossack Mdw	58	534	2050	693	159	64.8	477	94	15.7	*np*	*np*	*np*	*np*	*np*	*np*
Black Mtn Grove	59	540	1910	731	152	58.1	500	105	19.7	*np*	*np*	*np*	*np*	*np*	*np*
Peyrone Camp	60	540	1803	673	147	51.6	463	91	16.5	*np*	*np*	*np*	*np*	*np*	*np*
Cunningham Gr	61	540	1860	761	165	72.2	488	95	17.6	*np*	*np*	*np*	*np*	*np*	*np*
Bull Run Basin	62	540	1910	674	141	47.6	578	118	28.8	*np*	*np*	*np*	*np*	*np*	*np*
Greenhorn Sum	63	540	1921	737	157	59.3	559	109	24.3	*np*	*np*	*np*	*np*	*np*	*np*
Junipero Serra	64	120	1768	626	142	44.9	378	70	8.0	379	109	42.5	261	73	15.3
San Gabriels	65	993	2119	604	128	33.4	423	81	8.9	*np*	*np*	*np*	*np*	*np*	*np*
San Bernardino	66	994	2049	517	108	24.2	353	63	5.0	*np*	*np*	*np*	*np*	*np*	*np*
San Jacintos	67	997	1814	662	143	47.4	343	59	4.8	*np*	*np*	*np*	*np*	*np*	*np*
San Jacintos	68	997	2287	578	121	29.9	340	58	3.9	367	110	38.8	230	61	9.3
Parque Nacional	69		2500	571	114	26.5	404	72	8.1	*np*	*np*	*np*	*np*	*np*	*np*

[a]MC=mapcode. Sdz=Seed Zone. h17,h13=total height at 17-,13- yrs; v17,v13=total volume at 17-,13-yrs; db17=diameter breast height at 17-yrs; dg=diameter at ground at 13-yrs.

Standard errors of means: Sundown and Burnt Timber: h13=28.3 cm, dg13=8.0 mm, v13=9.5 dm^3; Cannon and Fitch-Rantz: h17=37.9 cm, db17=8.3 mm, v17=34.9 dm^3.

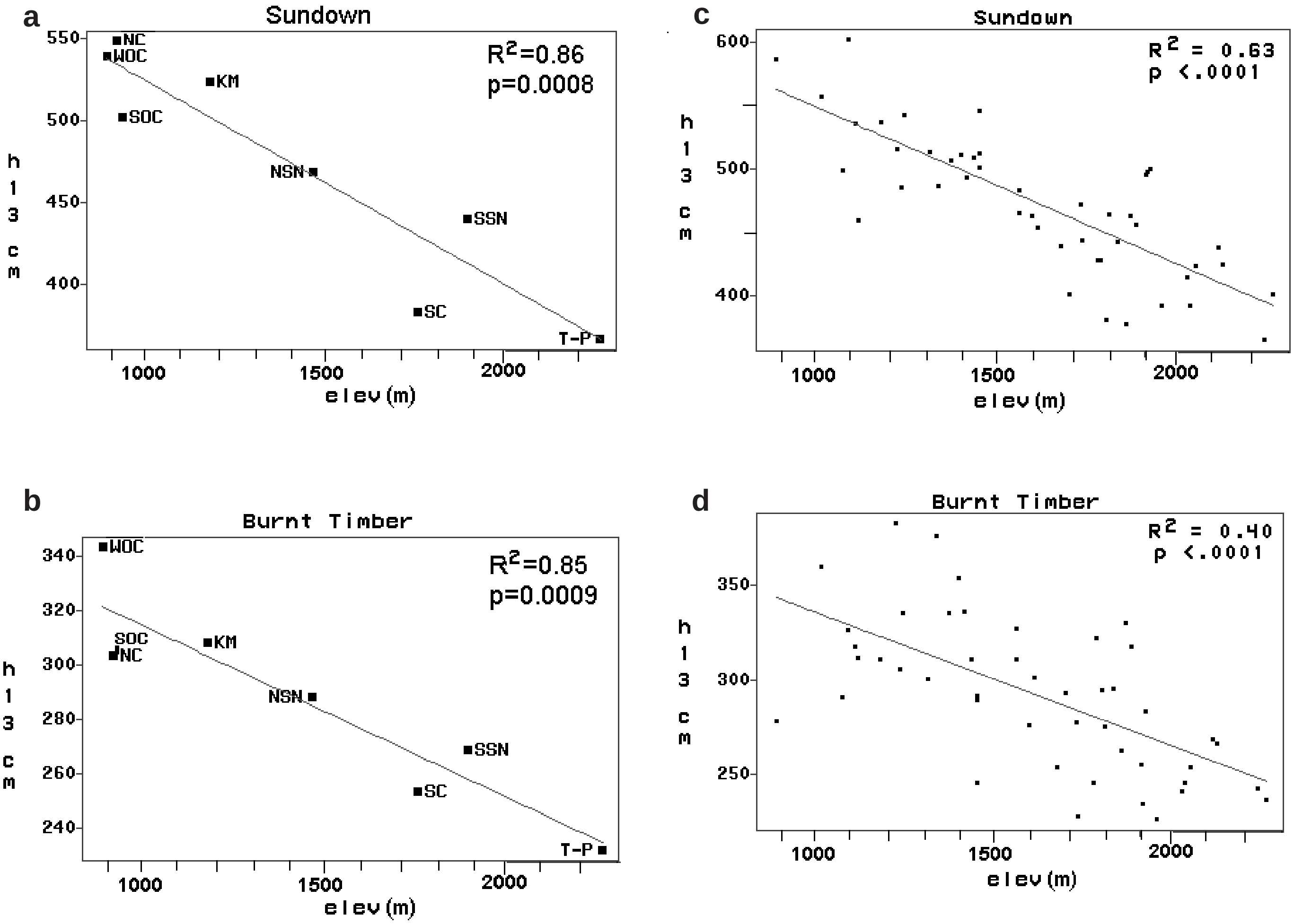


Figure 8—Association between 13-year height and origin elevation for: a. regions at Sundown, b. regions at Burnt Timber, c. provenances at Sundown, and d. provenances at Burnt Timber.

survival and growth among four contrasting test environments. These were true rank-change interactions that require restrictions in seed transfer within the species range. Only 24 percent of the sources were stable in performance across four tests, and 43 percent were highly unstable. Regions of seed origin were defined to represent broad climatic zones that might prove useful for guiding seed transfer. Region means were also interactive with test environments. Although Regions ranked similarly at the three low elevation tests, ranking changed at the FIT environment. Therefore, both regions and sources within regions with high performance on one or more test sites often had low performance on another.

Common garden test environments greatly affected performance of sugar pine. These four test environments represent a temperature and moisture gradient from the very mild, maritime climate (SUN), to moderate-stress summer, mild winter climate (BUR and CAN), to cool, short, dry summers and long, cold winters with heavy snow-packs (FIT).

Source-Test Response Patterns

SUN represents mild, humid, and productive northern maritime environments for sugar pine having low diurnal temperature fluctuation. Although high rust infection will greatly reduce survival at SUN, previous and current results show a strong regional geographic genetic variation pattern that is increasing with time. Best growth occurred for sources originating below 1220 m and most commonly from the local region. Growth at SUN was highest with mesic, mild-site western Oregon Cascade (WOC) and north coastal sources (NC). Height based on region means at SUN (as well as at BUR and CAN) followed an inverse linear elevation cline.

BUR represents warm, dry, very low elevation, south-facing environments in the interior Klamath Mountains with moderately high moisture stress for sugar pine, even though survival was high. Trees at BUR expressed weaker geographic patterns than those at SUN. Like SUN, and CAN, best growth occurred for sources originating below

1220 m. But in contrast to SUN, the more inland BUR site favored sources from moderate moisture stress sites farther inland in the western Oregon Cascades (WOC), Klamath Mountains (KM), and some Northern Sierra (NSN) sources.

CAN represents the lowest elevations for sugar pine in the Sierra Nevada, where growing seasons are long, winters are wet and mild, and summers are hot and dry. Survival at CAN was higher for southern California sources (below 38° N and above 1830 m elevation), but growth was better for sources originating above 38° N between 850 m and 1160 m elevation in the northern Sierra Nevada (up to 330 m higher than CAN). Survival based on region means at CAN closely follow a positive elevation cline. High survival of southern California sources suggests they may have higher resistance to charcoal root rot and/or higher drought resistance. Northern low elevation sources may have higher shoot growth potential due to natural selection in mesic, stable temperature climates with long growing seasons, rich, deep soils, and high competition for rapid height growth. Sites with long growing seasons and mild winters favor sources from low to middle elevation that are genetically flexible to extend their growth periods.

FIT is unique in this study in representing typical upper mixed-conifer forest sites with short, cool growing seasons and long, cold winters with persistent snow-packs. Adaptive genetic variation in survival and growth was most pronounced at FIT, and displayed opposite geographic patterns from those at the other test locations. Short-growing seasons and harsh cold winters with persistent snow packs favor trees that cease growth and enter dormancy early enough to escape freeze damage and those that heal stem injuries from snow bend and tearing of primary branches. Winter damage occurred annually, but reduced growth of low elevation sources was detected only after 7 years at FIT (Jenkinson 1996). This environment presented strong selection pressure against sources from distant origins at low or high elevations relative to the test site. Best survival, health, and growth developed for certain sources originating from 1550 m to 2020 m in the Sierra Nevada, with a higher likelihood of success for sources originating within 150 m elevation of the site. At FIT, southern Sierra Nevada sources (and 3 northern, eastside sources: Black Hills, Stover Mt, and Sierraville) tended to exhibit greater diameter and volume than others.

Seed Transfer Considerations: Matching Seed Source and Planting Site

Clearly, different seed sources should be used in mild, coastal Oregon sites like SUN than in upper mixed-conifer sites like FIT in the Sierra Nevada. Also, different seed sources should be used at low elevation sites like CAN than at middle to upper altitudes in the Sierra Nevada. Moisture-stressed sites (like CAN and BUR) at very low elevations in the Klamath and Sierra Nevada Mountains may require more site-specific matching of seed source to local site conditions, because regional effects were not as strongly expressed as they were for the two mesic sites. Such site-specific matching would be safest within local region, but some transfers across regions with known superior provenances may be successful (for example, Diamond and Breedlove to BUR, and Dutch Ck to CAN).

Extrapolation to predict safe seed transfers to other planting sites not represented in this study appears risky due to the high G-x-E interactions. Each test site displayed some selective advantage for the local climatic region and/or certain local sources. Local sources appear “safest though not necessarily best”. Even so, certain long distant transfers within a broad altitudinal band were among the best performers. The growth-elevation response pattern showed that the best source elevation was higher south of the site and was lower north of the site across a wide geographic distance. This suggests that elevation may be a “surrogate” for mean annual temperature (MAT).

MAT changes across gradients in elevation, latitude, and distance from the ocean. From long-term temperature and precipitation data recorded at 41 climate stations, MAT lapse rate was determined to be ca 1.62° C per 300 m rise in elevation and ca 0.55° C per 1° rise in latitude (ca 110 km) along the Sierra Nevada western slope (Ledig, data filed 1995). A general guide is to use provenances from ca 1 m higher in elevation than the plantation site per 1 km transfer northward. Applying this guide to the nearly 600 km span along the west-side Sierra Nevada predicts that a northernmost site should have a similar MAT as a southernmost site at 600 m higher elevation.

In general, seed transfer should be most restricted at higher altitudes. When geographic distances for transfer are shorter, the elevation range can be somewhat broader. As the distance of transfer increases, the range in acceptable elevation decreases. For high altitudes like FIT, in central Sierra Nevada, seed from northern or southern Sierra Nevada origins could be used provided source mean annual temperature (MAT), and perhaps even diurnal temperature variation (DT) during elongation, are matched closely with the temperature regime at the planting site. Transfer risk might be substantially reduced if provenance and restoration site temperatures are harmonized, and if stable, proven-superior provenances are used.

Sugar pine at mid to upper elevations has a comparatively short period of shoot elongation (51 days), only about 60 percent as long as and beginning later than ponderosa pine (81 days) and incense-cedar (*Calocedrus decurrens* Torr.) (91 days), but is similar to white fir (46 days) (Fowells 1941). Thus, sugar pine shoot growth is very rapid during a time (late May to mid July) when the temperature range is rather narrow and soil moisture is high. This suggests that sugar pine may be closely adapted to a specific and fairly narrow range of temperature for optimum growth at upper elevations. This delayed and brief growth period is still early enough to avoid drought and to allow “hardening” prior to onset of harsh weather. The temperature regime during elongation may be important to adaptation of sugar pine. Growth of ponderosa pine was inversely related to the diurnal range of temperature (DT) during elongation at the planting site (Church and others, data filed 2000). Sites with wide diurnal temperature fluctuation may selectively favor sources that grow slower (have lower energy efficiency) in a given narrow temperature range, but grow faster (have higher energy efficiency) over broader temperature ranges.

Transfer Northward to Enhance Rust Resistance

Seed transfer from south to north is the most desirable direction of movement to boost rust resistance in northern populations, where natural resistance is extremely low. Furthermore, many southern populations had higher survival potential in the low elevation plantation and high growth potential at the upper elevation site, in addition to having highest rust resistance. These results suggest significant potential advantages of seed transfer northward.

For northern (or central) Sierra Nevada sites, seed transfer northward about 220 km from central (or southern) Sierra Nevada zones may be done with success by adjusting elevation to match temperature. And, to avoid significant growth losses relative to local sources, proven (tested) high-growth provenances should be transferred. Before transfer, rust resistant families should be tested for stable high performance in contrasting environments. Similarly, transfer of northern Sierra Nevada provenances to California Klamath Mountain sites might be done successfully. Other transfers such as: Sierra Nevada or Klamath Mountains to the Cascade Range, southern California to Sierra Nevada, or elsewhere are not recommended.

References

Buck, J. M., Adams, R. S., Cone, J., Conkle, M. T. Libby, W. J., Eden, C. J., and Knight, M. J. 1970. California Tree Seed Zones. Forest Service, California Region and Forestry, Misc. Pub. 5 pp.

Campbell, R. K., and A. I. Sugano. 1987. Seed zones and breeding zones for sugar pine in southwestern Oregon. Res. Pap. PNW-RP-383. Portland, OR. USDA Forest Service, Pacific Northwest Res. Sta. 18 p.

Church, J. N., Kitzmiller, J. H., Boom, B. A., Lunak, G. A., Greenwood, K. L., and R. S. Criddle. 2002. 15-year height of *Pinus ponderosa* is correlated with mean diurnal temperature variation during bud elongation. Data filed at UC Davis Dept Environ Hort., Davis, CA.

Conkle, M.T. 1973. Growth data for 29 years from the California elevational transect study of ponderosa pine. Forest Science 19:31-39.

Critchfield, W. B. and E. L. Little, Jr. 1966. Geographic distribution of the pines of the world. USDA Forest Service Misc. Publ. 991.

Fites, J. A. 1996. Ecology of sugar pine in late successional mixed-conifer forests in the northern Sierra Nevada and southern Cascades (Abstract). *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. p. 38.

Fowells, H. A. 1941. The period of seasonal growth of ponderosa pine and associated species. Journal of Forestry 39(7):601-608.

Harry, D. E., J. L. Jenkinson, and B. B. Kinloch. 1983. Early growth of sugar pine from an elevational transect. Forest Science 29:660-669.

Jenkinson, J. L. 1996. Genotype-environment interaction in common garden tests of sugar pine. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 54-74.

Kinloch, B. B., and D. Davis. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 125-132.

Kinloch, B. B., Davis, D., and G. E. Dupper. 1996. Variation in virulence in *Cronartium ribicola*: what is the threat? *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 133-136.

Kitzmiller, J. H., and P. Stover. 1996. Genetic variation in the growth of sugar pine progenies in California plantations. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 83-98

Kitzmiller, J. H. 1976. Tree improvement master plan for the California region. USDA Forest Service, Pacific Southwest Region. San Francisco. California. 96p.

Kitzmiller, J. H. 1990. Managing genetic diversity in a tree improvement program. For. Ecol. Manage. 35:131-249.

Larson, L.T., and T.D. Woodbury. 1916. Sugar Pine. USDA Forest Service Bull. 426. Washington, DC.

Ledig, T. 1995. Data filed at Institute of Forest Genetics, Placerville, CA.

Oliver, W. W. 1996. Silvics of sugar pine: clues to distribution and management. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 28-33.

Samman, S. and J. H. Kitzmiller. 1996. The sugar pine program for development of resistance to blister rust in the Pacific Southwest Region. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 162-170.

Sniezko, R. A. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon. *In*: Kinloch, Bohun B., Jr., Mellisa Marosy, and May E. Huddleson (eds.). 1996. Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362. pp. 171-178.

Surles, S. E., T. L. White, and G. R. Hodge. 1995. Genetic parameter estimates for seedling dry weight traits and their relationships with parental breeding values in slash pine. Forest Science 41(3):546-563.

Westfall, R. D. 1991. Developing seed transfer zones. In: Fins, L. and Friedman, S. T. (Eds) Handbook of Quantitative Forest Genetics. pp. 313-395.

Willits, S, and T. D. Fahey. 1991. Sugar pine utilization: a 30-year transition. Res. Pap. PNW-RP-438. Portland, OR. USDA Forest Service, Pacific Northwest Res. Sta. 21 p.

Ecological Roles of Five-Needle Pines in Colorado: Potential Consequences of Their Loss

A.W. Schoettle

Abstract—Limber pine (*Pinus flexilis* James) and Rocky Mountain bristlecone pine (*Pinus aristata* Engelm.) are two white pines that grow in Colorado. Limber pine has a broad distribution throughout western North America while bristlecone pine's distribution is almost entirely within the state of Colorado. White pine blister rust (*Cronartium ribicola* J. C. Fisch.) was discovered in Colorado in 1998 and threatens populations of both species. Available information suggests that these species have several important ecological roles, such as (1) occupying and stabilizing dry habitats not likely to be occupied by other, less drought tolerant tree species, (2) defining ecosystem boundaries (treelines), (3) being among the first to colonize a site after fire, especially fires that cover large areas, (4) facilitating the establishment of high elevation late successional species such as Engelmann spruce and subalpine fir and (5) providing diet and habitat for animals. While the rust is not likely to eliminate five-needle pines from Colorado ecosystems, it is likely to impact species' distributions, population dynamics and the functioning of the ecosystems. These changes may well affect (1) the distribution of forested land on the landscape, (2) the reforestation dynamics after fire, (3) the rate and possibly fate of forest succession, and (4) habitat for wildlife. Our incomplete understanding of the ecology, genetic structure and adaptive variation of limber pine and Rocky Mountain bristlecone pine constrain our ability to rapidly develop and implement conservation programs.

Key words: Limber pine, *Pinus flexilis* James, Rocky Mountain bristlecone pine, *Pinus aristata* Engelm., *Cronartium ribicola* J. C. Fisch., regeneration, fire

Limber pine (*Pinus flexilis* James) and Rocky Mountain bristlecone (*Pinus aristata* Engelm.) are two white pine species that grow in Colorado. Limber pine's distribution includes habitats throughout the Rocky Mountains while the distribution of Rocky Mountain bristlecone pine is almost entirely within the state of Colorado. In southern Colorado, it is speculated that a limber pine - southwestern white pine (*Pinus strobiformis*) complex exists. The distribution of southwestern white pine extends south into New Mexico. This paper will focus on limber pine and Rocky Mountain bristlecone pine. These species are white pines (subgenus *Strobus*) yet limber pine is in section *Strobus*, subsection *Strobi* and Rocky Mountain bristlecone pine is in subgenus *Parrya*, subsection *Balfournianae* (Lanner 1990). Their often bushy growth form (fig. 1) and slow growth rate combined with the inaccessibility of the rocky sites that they dominate make them poor timber species and ones that have long been overlooked by the forestry community. The most basic ecological information, such as the forest cover, has not been quantified for these species in Colorado or throughout their ranges.

The impact of white pine blister rust (*Cronartium ribicola* J. C. Fisch.) on commercial North American white pines has been a focus of attention since its introduction from Europe in the early 1900s. In the mid-1980s, the focus expanded to

Figure 1—Limber pine on a dry site with a bushy growth form with upward reaching branches.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The author is with the USDA Forest Service, Rocky Mountain Research Station, 240 West Prospect Road, Fort Collins, CO 80526 USA. Phone: 970 498-1333. FAX: 970 498-1010. E-mail: aschoettle@fs.fed.us.

impacts of the disease to the non-commercial whitebark pine (*Pinus albicaulis* Engelm.) as forest practices shifted toward management of ecosystems. White pine blister rust's threat to whitebark pine and the resultant impacts to the habitat of the endangered grizzly bear (*Ursus arctos horribilis*) have brought whitebark pine ecosystems into view by the management and research community (for example, Schmidt and McDonald 1990, Tomback and others 2001). With the recent discovery of white pine blister rust in Colorado on limber pine in 1998 (Johnson and Jacobi 2000) and Rocky Mountain bristlecone pine in 2003 (Blodgett and Sullivan 2004), both limber pine and Rocky Mountain bristlecone pine populations are threatened. To predict the impacts of white pine blister rust on Colorado ecosystems, we must first understand the role of these five-needle pines in the absence of the rust. It is not clear how similar the ecological roles of limber pine and bristlecone pine are to the more studied whitebark pine. Therefore, in the interest of brevity, this paper will focus on research conducted on limber pine and Rocky Mountain bristlecone pine, recognizing that some information from other species may be applicable but will not be summarized here. This paper will discuss what is currently known about the ecology of limber pine and Rocky Mountain bristlecone pine in the central Rocky Mountains and the possible repercussions of white pine blister rust on these ecosystems.

Limber Pine

Limber pine is a species whose distribution has changed from continuous to patchy and presently displays metapopulation dynamics (Webster and Johnson 2000, Antolin and Schoettle 2001). Approximately 14,000 years ago, at the last glacial maximum, limber pine was widespread along the eastern slope of the Colorado Front Range in the central Rocky Mountains (Wells and Stewart 1987). Currently limber pine is characterized by a patchy distribution, spanning a broad latitudinal and elevational range (Burns and Honkala 1990) (fig. 2). In the central Rocky Mountains limber pine grows from below the lower tree line up to the upper tree line, from ~ 1600 m in the short grass steppe to > 3300 m at Rollins Pass near the continental divide (Schoettle and Rochelle 2000). Limber pine's elevational range is wider than any of its co-occurring tree species in this region (table 1). In the northern Rocky Mountains and west, limber pine is generally found at lower elevations with whitebark pine occupying the higher elevations. In the southern mountains limber pine grows at high-elevation sites with the lower elevations occupied by southwestern white pine (*Pinus strobiformis* Engelm.).

Limber pine is similar to the stone pines (subsection *Cembrae*) in so much as it has large wingless (or near wingless) seeds that depend on corvid species (for example, Clark's nutcracker, *Nucifraga columbiana* Wilson) for dispersal (Lanner and Vander Wall 1980). In contrast to the stone pines, which have indehiscent cones necessitating animals to extract the seed, limber pine cones open when dry. As for whitebark pine, seeds of limber pine can be an important food source for corvids (Tomback and Kramer 1980), black and grizzly bears (*Ursus* spp.; Kendell 1983, McCutchen 1996), red squirrels (*Tamaisciurus hudsonicus*; Hutchins and Lanner 1982) and other small rodents. The role of limber pine forests as habitat for wildlife species is unknown. The phloem, cones and seeds all provide habitat and diet for arthropod fauna (Hedlin and others 1981, Cerezke 1995, Schoettle and Négron 2001).

Limber Pine Stand Dynamics

Limber pine is often the first species to colonize an area after fire (Donnegan and Rebertus 1999). Clark's nutcrackers can cache seed many kilometers from the parent tree (Vander Wall and Balda 1977), enhancing seed dispersal across the landscape as well as into the central areas of large burns where wind-dispersed seeds of other conifer species are scarce (Tomback and others 1993). The germination of multiple seeds from one cache results in a cluster of seedlings that are often related (Carsey and Tomback 1994). The clustered distribution of seedlings facilitates successful establishment of limber pine (Donnegan and Rebertus 1999). However, as the trees mature, the clustered distribution may reduce the reproductive output (Feldman and others 1999) and lifespan of the individuals (Donnegan and Rebertus 1999) compared to trees growing singly.

The dynamic of stands containing limber pine depends on the site; limber pine form sustainable stands on dry rocky sites and tend to be limited to early succession on more mesic sites. Dry sites can be occupied by limber pine at any elevation within the species range and are often windswept and accumulate little snow. Limber pine dominates xeric sites not because they provide the optimal physical environment for limber pine growth (Lepper 1974, Schoettle and Rochelle 2000) but because the conditions are not suitable for the growth of other species and therefore competition is minimal. Competition is likely to be the largest limitation defining the realized niche of limber pine and the location of sustainable limber pine stands. On dry sites, maximum tree ages have been reported of more than 1500 years for limber pine in Colorado (Schuster and others 1995) and over 2000 years for individuals in Nevada and California (Lanner 1984). The stands tend to be low density, open, and support continual recruitment of limber pine (Knowles and Grant 1983, Stohlgren and Bachand 1997). Upon sexual maturity, which may take over 50 years (personal observation), limber pine on dry sites can produce large cone crops. Loss of apical dominance due to leader damage provides many cone bearing branches per tree. The frequency of mast years, the environmental factors that affect their periodicity, and the repercussions of them on the population dynamics of animal species deserve research attention. In addition to the extreme longevity of individuals, the lack of competing tree species and sustained regeneration, the persistence of these limber pine stands is also possible because catastrophic disturbance (i.e. wildfire) is rare on dry, rocky sites.

While rocky ridges and dry slopes are the most obvious habitat occupied by limber pine, scattered occurrence of limber pine throughout the forested region of the Colorado Front Range is typical (Marr 1961, Schoettle and Rochelle 2000). On these more mesic sites, limber pine's early post-disturbance dominance succeeds over time to other conifer species (Rebertus and others 1991). Limber pine acts as a nurse tree, mitigating the harsh open environment after disturbances and facilitating the establishment of Engelmann spruce and subalpine fir (Rebertus and others 1991,

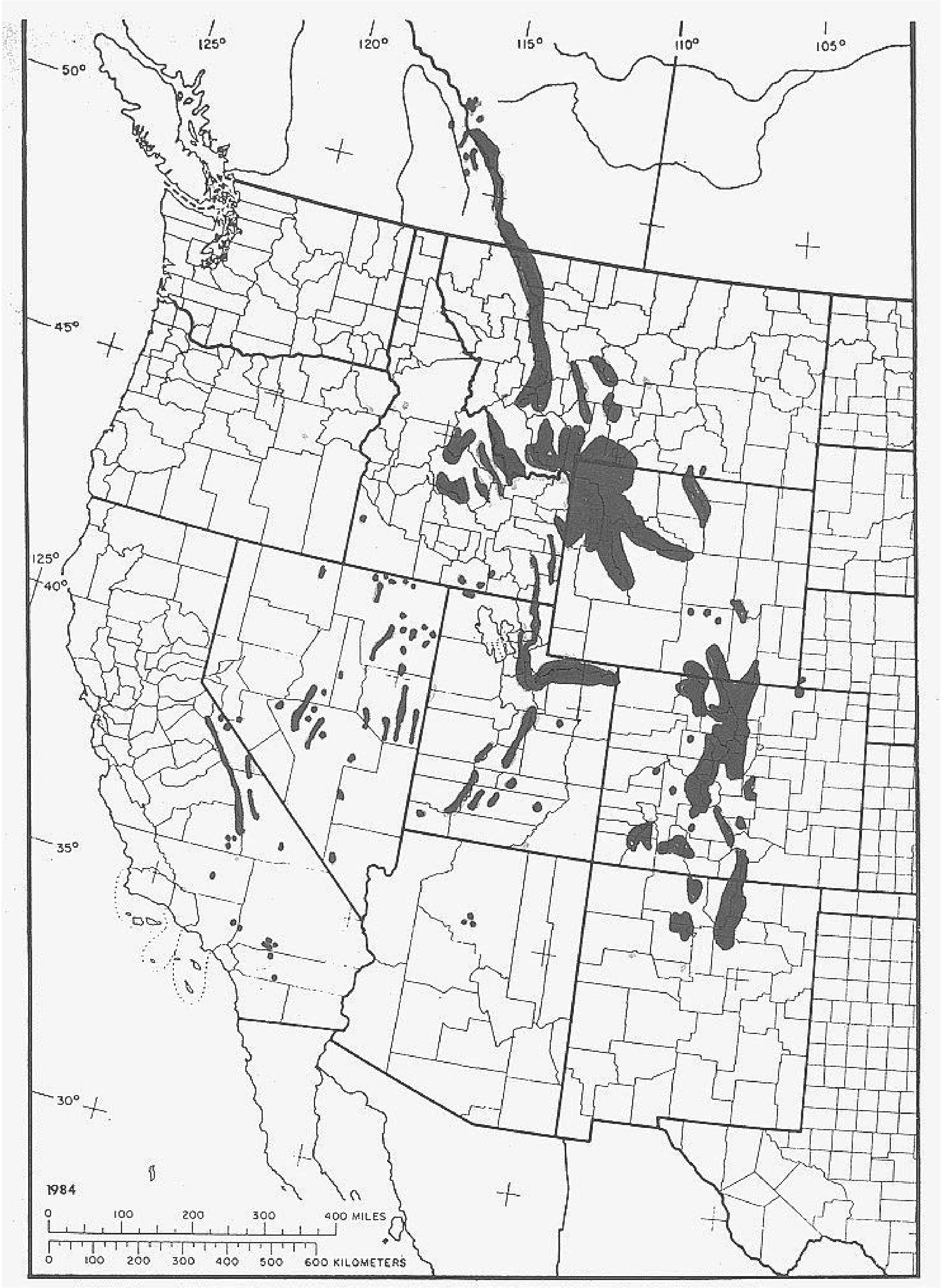


Figure 2—Distribution of limber pine (*Pinus flexilis* James). (From Burns and Honkala 1990)

Table 1—Elevation ranges of tree species in Colorado. Data from Peet (1981) and Baker (1992).

Scientific name	Common name	Elevation range (m)
Pinus flexilis James	Limber pine	1600-3400
Juniperus scopulorum Sarg.	Rocky Mountain juniper	1600-2800
Pinus ponderosa Dougl. ex Laws.	Ponderosa pine	1700-2800
Pseudotsuga menziesii (Mirb.) Franco	Douglas-fir	1700-3000
Populus tremuloides Michx.	Quaking aspen	2000-3400
Pinus contorta Dougl. ssp. *latifolia* Bailey	Lodgepole pine	2300-3300
Picea engelmannii Perry ex Engelm.	Engelmann spruce	2400-3500
Pinus aristata Engelm.	Rocky Mountain bristlecone pine	2750-3670
Abies lasiocarpa (Hook.) Nutt.	Subalpine fir	2500-3500

Donnegan and Rebertus 1999). Such facilitation accelerates limber pine's mortality due to the close proximity of, and competition by, the succeeding species. Seedlings of limber pine occur frequently throughout all stand types along the elevational gradient, yet successful establishment in late successional stands on mesic sites is rare (Stohlgren and others 1998).

Seral limber pine is likely to maintain apical dominance and retain an erect forest tree form and is suspected to produce fewer cones per tree than those trees on drier sites (Lepper 1974). Seed yields for limber pine can also be reduced by some of the same cone and seed insects that affect co-occurring conifer species (Hedlin and others 1981, Schoettle and Négron 2001). Due to the lower seed yields of successional stands, it is unclear what proportion of seed from these sites is consumed on site by animals versus dispersed and cached. Therefore, the relative contribution of progeny from seral compared to persistent limber pine stands to the recolonization of nearby disturbances has not yet been established.

Limber Pine Population Genetics

Despite limber pine's wide range and patchy distribution, it shows little genetic differentiation related to elevational changes (Latta and Mitton 1997, Schuster and others 1989, Schuster and Mitton 1991, 2000). Other species with long distance dispersal of seed by birds show similar apparent lack of genetic structure (Bruederle and others 1998). This is in contrast to species that depend on the wind for dispersal of seed; these species show not only local genetic differentiation, but also differentiation within local populations (see Rehfeldt 1997). Genetic studies of limber pine indicate that within local populations, pollen is dispersed evenly among trees (Schuster and Mitton 2000) but that seed dispersal patterns result in local clusters of related individuals (Schuster and Mitton 1991). Differences in pollen phenology along elevation gradients could limit gene flow via pollen between local populations (Schuster and others 1989), but low between-population differentiation suggests gene flow by stepping-stone pollination across intermediate populations. Long-distance seed dispersal by birds (Lanner and Vander Wall 1980) also contributes to gene flow across the elevation gradient. Currently, the only large genetic differences in limber pine that have been identified are on a regional geographic scale that may reflect isolation in Pleistocene refugia on the Great Plains east of the Rocky Mountains and in the Great Basin west of the Rocky Mountains (Latta and Mitton 1997; Mitton and others 2000).

Limber pine appears to be a genetic generalist based on presumably selectively neutral genetic markers, yet extensive common garden and genetic by environment interaction experiments have not been conducted to evaluate local adaptation. One common garden study of several seed sources for limber pine suggests some geographic variation in seedling growth characteristics (Heit 1973). Seed transfer rules for limber pine have not been established.

Limber Pine Adaptive Variation

Despite living in metapopulations along a broad elevational gradient, limber pine shows remarkably low morphological variation (Schoettle and Rochelle 2000). The genetic basis for the morphological variation or lack thereof has not yet been assessed. Schoettle and Rochelle (2000) hypothesized that if limber pine lacked elevational races, the environmental effect of elevation on growth and resultant phenotype would be greater for limber pine than for species that have undergone adaptations to local environments. Contrary to this hypothesis, the environmental stress of increasing elevation that is apparent in the growth patterns of other tree species was less obvious for limber pine (fig. 3). Leaf longevity, ranging from 4 to 10 years, was one of the few characteristics to vary along an elevational gradient (Schoettle and Rochelle 2000). Limber pine appears less stressed than other species by the environmental gradients associated with elevation (Schoettle and Rochelle 2000).

How can limber pine uncouple its growth from the environmental differences from the upper to the lower tree line? The rates of most physiological and biochemical processes are a function of temperature. Limber pine seedlings from four of five populations from Wyoming, Nevada and California revealed a typical photosynthetic temperature optimum (15 °C) but an unusually broad response curve with a variation in photosynthetic rate of only 12 percent from the maximum over the temperature range of 10-35 °C (Lepper 1980). This is in contrast to the sharper temperature response of photosynthesis of balsam fir (*Abies balsamea* (L.) Mill., Fryer and Ledig 1972) and Great Basin bristlecone pine (then called *Pinus aristata* Engelm. but now recognized

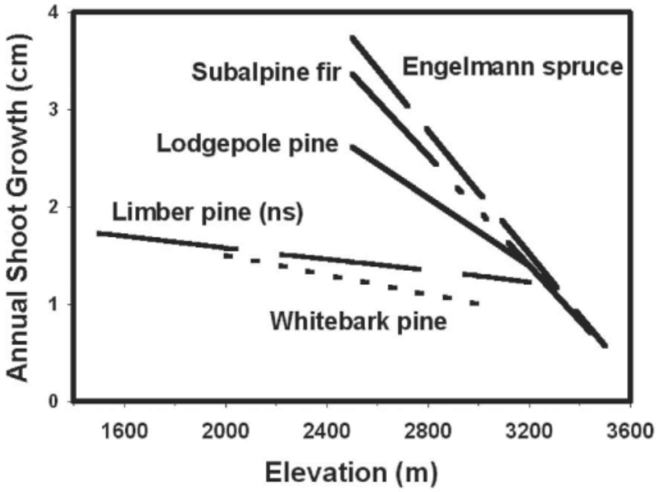


Figure 3—Effect of elevation on the annual twig growth of mature conifers. Data for Engelmann spruce and subalpine fir are from Hansen-Bristow (1986), limber pine are from Schoettle and Rochelle (2000), and lodgepole and whitebark pine are from Schoettle (unpublished data). (Adapted from table 7 of Schoettle and Rochelle, 2000)

as *Pinus longaeva* Bailey) according to Bailey (1970) Mooney and others (1964) where photosynthesis fell 63 percent and 87 percent, respectively, below the maximum rates within the range of 5°C below and 20°C above the optimum temperature for photosynthesis (fig. 4). Strong variation in photosynthetic capacity between mature trees at the elevational extremes (Schoettle, unpublished data) also suggests considerable adaptive physiological variation for limber pine. Limber pine also has a high degree of variation in other physiological traits, both among individuals as well as within individuals (Barrick and Schoettle 1996, Schoettle and Rochelle 1996). Therefore physiological plasticity or broad physiological tolerances appear to contribute to limber pine's wide fundamental niche with respect to temperature.

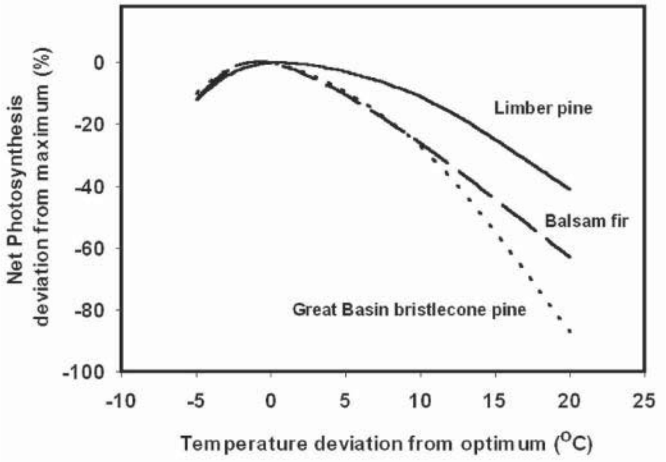


Figure 4—Relative temperature response of net photosynthesis of seedlings of three conifer species. The optimum temperature for photosynthesis for each species is that temperature that the maximum rate of photosynthesis was recorded. To enable comparison among species, photosynthesis is expressed as a percentage reduction from the maximum rate.

Limber pine seedlings, similar to the stone pines, have large root to shoot ratios. How or if this allocation pattern varies among habitats hasn't been studied. This pattern of carbon allocation is often associated with shade intolerance as well as drought tolerance and avoidance. Both limber pine seedlings and mature trees demonstrate drought tolerant behavior, compared to co-occurring species, by maintaining leaf gas exchange even under severe soil drying (Lepper 1980; Pataki and others 2000). The hypothesis that, on xeric sites, the long roots of limber pine are able to access ground water sources not within reach of other conifer species has not been tested. Mature limber pine also demonstrates drought avoidance behavior by closing its stomata more readily than associated species during periods of atmospheric dryness (high vapor pressure deficit) (McNaughton 1984, Pataki and others 2000). Stomatal closure may prevent xylem cavitation but also sacrifices photosynthetic carbon gain; this pattern of water conservation at the expense of carbon assimilation may contribute to limber pine's poor competitive abilities.

Limber pine may be a case where turnover of local populations, combined with high dispersal and gene flow, results in evolution of a generalist lifestyle capable of tolerating a wide variety of environmental circumstances (Schoettle and Rochelle 2000; Antolin and Schoettle 2001). It is unclear at this time if being a poor competitor is the "cost" associated with the generalist lifestyle for limber pine.

Rocky Mountain Bristlecone Pine

In 1970, Bailey (1970) split the North American bristlecone pine (*Pinus aristata* Engelm.) into two species, the Rocky Mountain bristlecone pine (retaining the name *Pinus aristata* Engelm.) and Great Basin bristlecone pine (newly named *Pinus longaeva* Bailey). Most of the research on bristlecone pines before 1970 was conducted on Great Basin bristlecone pine; very little research has been conducted on Rocky Mountain bristlecone pine. Both species are recognized as charismatic and are appreciated by the public for their majestic and artistic tree form and their extreme longevity (fig. 5). Great Basin bristlecone pine can reach ages in excess of 4,000 years (Schulman 1958, Curry 1965), while the oldest Rocky Mountain bristlecone pine is just over 2,400 years of age (Brunstein and Yamaguchi 1992). Both species of bristlecone pine have been utilized in dendrochronology studies (such as Kreb 1973, LaMarche and Stockton 1974).

The distribution of Rocky Mountain bristlecone pine is primarily in Colorado and extends south into New Mexico along the Sangre de Cristo Mountains and includes a disjunct population on the San Francisco Peaks in central Arizona (fig. 6). It is thought that during the Pleistocene glacial periods there was nearly continuous habitat for bristlecone pine between the New Mexico and Arizona stands, suggesting that the Arizona stand is a relic of a formerly larger distribution (Bailey 1970). The current southern distribution of bristlecone pine appears limited by suitable habitat, however it is not known what limits bristlecone pine

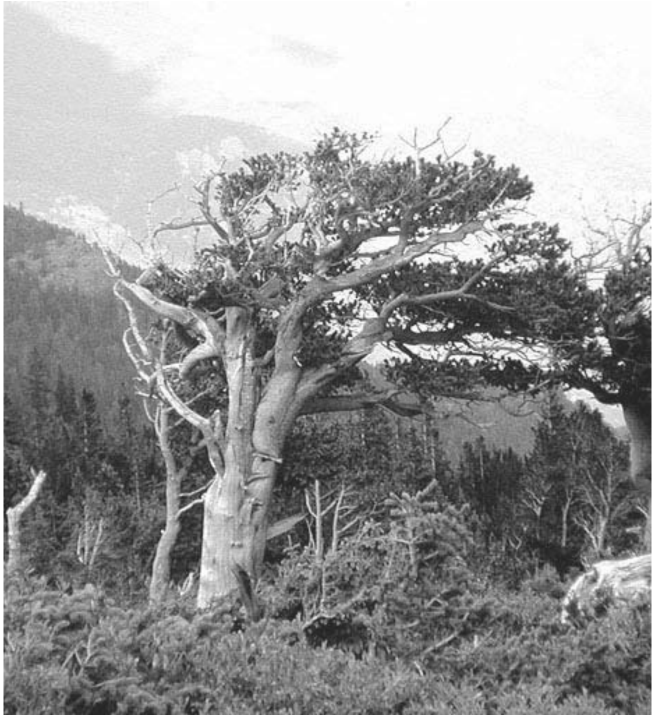


Figure 5—Rocky Mountain bristlecone pine near treeline in central Colorado. Note partial cambial dieback (see fig. 7).

from occupying apparently suitable habitat to its north. The distribution of this species may reflect a dependence on summer monsoons, restricting it from occupying higher elevation sites in northern Colorado. Rocky Mountain bristlecone pine (referred to as bristlecone pine hereafter) has a narrow elevation range and is primarily a high elevation species occupying dry sites from 2750 to 3670 m elevation (Baker 1992). Bristlecone pine forests may contain limber pine, Engelmann spruce, subalpine fir, quaking aspen, and Douglas fir.

Bristlecone Pine Stand Dynamics

The origin of bristlecone pine stands throughout Colorado is related to episodes of drought and presumably peak fire occurrence (Baker 1992). Bristlecone pine is a long-lived species that regenerates well after fires. Bristlecone pine has been identified as a component of two climax vegetation types (DeVelice and others 1986). The first is dominated by bristlecone pine with or without Engelmann spruce with an understory of *Festuca*. These sites are open and park-like. This habitat type transitions into one where bristlecone pine succeeds to the more shade tolerant spruce's competitive edge on moister sites (Moir and Ludwig 1979). On lower elevation sites, bristlecone pine dominates or co-dominates stands with Douglas fir. Using a different approach based on environmental variables and species distributions, Baker (1992) characterized sixty-five bristlecone pine stands into 6 forest structures that are distinguished by (1) the time since the last disturbance (age of the oldest tree in the stand), (2) presence of young quaking aspen, and (3) relative amounts and sizes of Engelmann spruce and subalpine fir (Baker 1992). Baker (1992) reports that bristlecone pine regenerates well only on recently burned sites and therefore attributes the persistence of old stands of bristlecone not to climax stand dynamics but to the long lifespan of the individual pioneer trees in the absence of competition and fire. However, Baker's data reveal some bristlecone pine regeneration in most of the sampled bristlecone pine stands. This raises the question of how much regeneration is necessary to sustain bristlecone pine on sites with little to no competition.

Regardless of whether one subscribes to climax vegetation theory or not for very long-lived species, it is clear that the rate of succession from bristlecone pine to other species varies with site and the transition may proceed very slowly (>1000 yrs) on dry high elevation sites and may be preempted by disturbance. Ranne and others (1997) followed up on Baker's work and characterized the vegetation characteristics of the six bristlecone pine forest groups. Vegetation in bristlecone forests is influenced primarily by elevation and soil pH and secondarily by substrate, soil texture, topographic position, and geographic location (Ranne and others 1997).

The relative role of wind versus animal-dispersal of seeds for bristlecone pine regeneration within existing stands and colonization of burned areas is not known. Bird-dispersal of seeds appears common at higher elevations while wind-dispersal may predominate at lower elevations for Great Basin bristlecone pine (Lanner 1988). Clustered individuals, indicative of animal-mediated seed dispersal, are apparent in mature high elevation Rocky Mountain bristlecone pine stands in central Colorado (Torick and others 1996), as well as in the young seedlings establishing in those stands (personal observation, 2001). The frequency of clustered individuals on sites that have been recently burned, those at lower elevation stands or those in southern Colorado has not been assessed. Therefore it is not clear if long-distance animal-mediated seed dispersal of bristlecone pine plays a major role in recolonization of disturbed areas.

Although bristlecone pine is a pioneer species after fire, its role in mediating the environment to facilitate the establishment of late successional species has not been fully explored. At the forest - alpine ecotone, bristlecone pine growing in the krummholz form facilitate the establishment of Engelmann spruce and subalpine fir (personal observation). In the subalpine zone, bristlecone pine forests tend to have relatively clear boundaries with bristlecone pine densities abruptly falling as elevation decreases and moisture regimes change.

Although bristlecone pine has delayed sexual maturity, its extreme longevity enables each tree to be a seed source for many years. During a good cone year, cone production per tree appears to increase with increasing elevation within a stand, including good production by krummholz trees at tree line (Schoettle, unpublished data, 2001). The gradient in cone production may be a function of differences in the number of cones initiated or rates of cone damage or abortion. Cone insects were common on low elevation trees and absent from trees growing at the higher elevations (personal observation, 2001), similar to the findings for limber pine (Schoettle and Négron 2001). As with limber pine, squirrels are very efficient at harvesting bristlecone pine cones and create large cone caches within the forests. Again, similar to

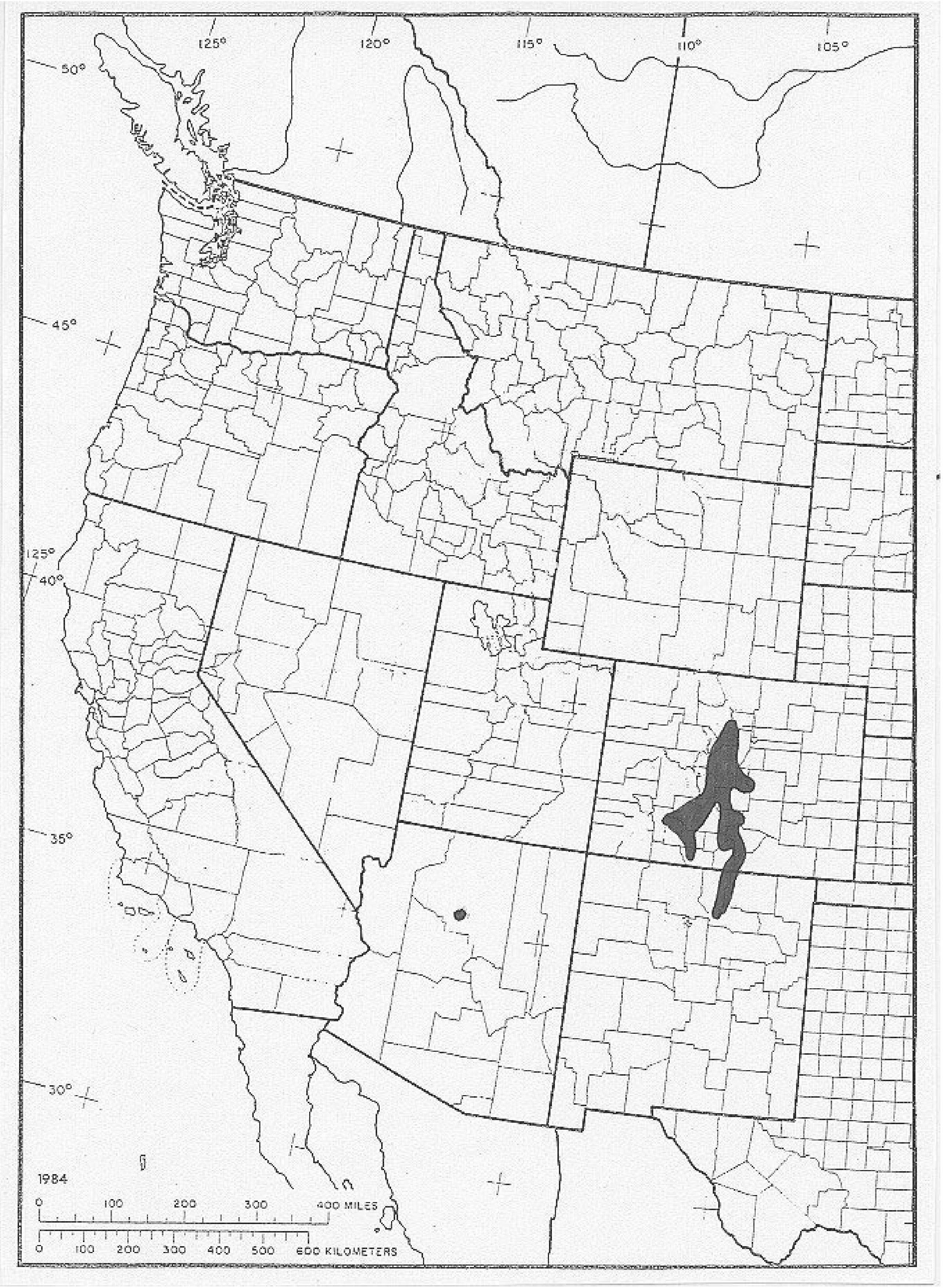


Figure 6—Perimeter of the distribution of Rocky Mountain bristlecone pine (*Pinus aristata* Engelm.) based on data from Bailey (1970), Brunstein and Yamaguchi (1992) and Ranne and others (1997).

limber pine, the frequency of mast years, the environmental factors that affect their periodicity, and the repercussions of them on the population dynamics of animal species deserve research attention.

Bristlecone Pine Population Genetics

Very little is known about the population genetics of bristlecone pine. Recent research has shown that stands as close as 11 km from one another near the northern extreme of the species distribution differed from one another in allele frequencies and the distribution and presence of certain alleles, suggesting a strong founders effect (Oline 2001). This pattern may suggest that long-distance transport of seed by birds for this otherwise wind-dispersed species may play a significant role in the establishment of bristlecone pine stands. As mentioned above, the caching behavior of birds also results in fine scale genetic structure for bristlecone pine, similar to that of the other bird-dispersed pines (Torick and others 1996). As with limber pine, common garden and genetic by environment interaction experiments have not been conducted for bristlecone pine.

Bristlecone Pine Adaptive Variation

Phenotypic variation associated with elevation has been observed for bristlecone pine (Ewers and Schmid 1981) yet the genetic basis for the differences has not been studied. Bristlecone pine has several traits that may contribute to its longevity. This species has considerable plasticity with respect to leaf longevity, ranging from 7 to over 15 years, and has the unusual ability to maintain high physiological function of leaves as they age. Both of these traits may contribute to the absence of growth declines in aging bristlecone pine trees that are commonly observed in other species (Schoettle 1994). Bristlecone pine and limber pine both express partial cambial dieback, resulting in a strip of dead bark extending from dead roots to dead branches (fig. 7) (Schauer and others 2001). It is speculated that partial cambial dieback contributes to the exceptional longevity of individuals by effectively isolating damaged roots, stem or branches from remaining healthy tissues and thereby maintaining a favorable photosynthetic to non-photosynthetic tissue ratio (Schulman 1954, LaMarche 1969).

Similar to limber pine, bristlecone pine seedlings allocate a large amount of resources below ground. How this allocation pattern affects the performance of seedlings regarding stress tolerance or competitive abilities has not been studied, yet this pattern is usually reflective of poor shade tolerance (Tilman 1988).

Threat of White Pine Blister Rust

The most immediate threat to limber pine and bristlecone pine is the exotic disease white pine blister rust caused by the fungus *Cronartium ribicola* J.C. Fisch. This pathogen was introduced into North America in the early 1900s and has caused significant impacts to white pines throughout North America. For a summary of the biology of the rust and the impacts of this disease to white pines, see McDonald and Hoff (2001). The rust has been affecting limber pine since 1945 in the Northern Rocky Mountains and down into southern Wyoming since the 1970s (Brown 1978) and was identified in Colorado in 1998 (Johnson and Jacobi 2000). White pine blister rust was first reported on Rocky Mountain bristlecone pine in 2003 in the Sangre De Cristo Mountains of Colorado (Blodgett and Sullivan 2004).

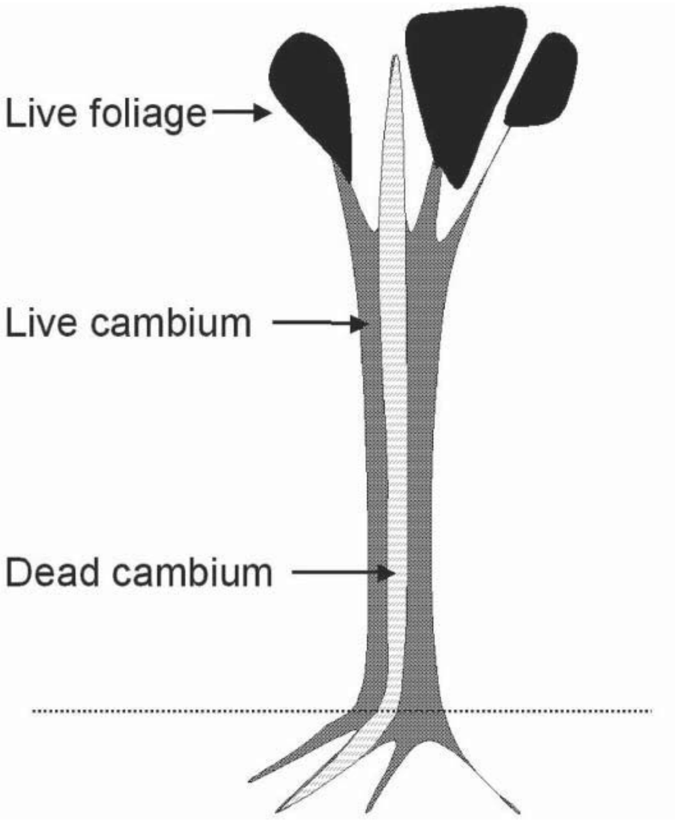


Figure 7—Schematic of partial cambial dieback. Note that the dead cambial strip connects a dead root with a dead branch.

The white pine blister rust spores enter trees through the stomatal openings of young leaves (McDonald and Hoff 2001). The effectiveness of older leaves as infection sites needs to be assessed for Colorado white pines since more than 90 percent of their foliage is greater than 1 year old (Schoettle 1994). The rust causes cankers that girdle the infected branch or stem killing the distal tissue. Cankers on the main stem of a tree will usually kill the individual. Branch cankers often will not kill the tree until the reduction in leaf area is so great that the tree cannot survive or the canker grows to affect the main stem. The contribution of rust-caused branch mortality to an increase in sensitivity of the tree to other stresses such as drought, competition, and bark beetle attacks deserves research attention to fully assess the impacts of the disease. Very old trees that have significant partial cambial dieback, such that all of the tree's surviving foliage is supported on a few branches, may be rapidly killed by white pine blister rust. Alternatively, it is possible that those trees that support foliage on many upwards-reaching branches may prolong the time between canker formation and tree mortality.

Effects of white pine blister rust on recolonization of disturbed areas may well precede the mortality of existing, mature white pine trees (fig. 8). While a tree may survive with white pine blister rust cankers it is likely to experience substantial branch mortality and reduced cone and seed production. If seed yields are low, it is unclear if Clark's nutcrackers will visit and cache seeds from these stands. In addition, even if seed is available for colonization and regeneration, white pine blister rust exerts strong selective pressure at the seedling – sapling stage and can cause high rates of seedling mortality within several years of infection.

White pine blister rust has its own set of environmental constraints as influenced by the tolerances of its biology as well as the distribution of its two hosts, the five-needle pines and *Ribes* spp. The degree of overlap between the rust's potential habitat with that of limber pine and bristlecone pine's distributions has not been fully defined. While the selective pressure exerted by the rust on these five-needle pines will not be uniform across their distribution, existing information on *Ribes* distributions suggests that it may be extensive; three-fourths of the limber pine sites sampled along the elevation gradient of Colorado's Front Range contained *Ribes* spp. (8 of 12 stands; Schoettle and Rochelle 2000) and more than half of the bristlecone pine sites evaluated by Ranne and others (1997) contained *Ribes* spp. (27 of 50 stands). Many of these stands support *Ribes cereum* Douglas, a species that has been thought to be a poor alternate host for white pine blister rust in other parts of North America (Van Arsdel and others 1998), yet it may serve as a host for the disease in Colorado, southern Wyoming and South Dakota (Lundquist and others 1992, Johnson and Jacobi 2000). *Ribes* spp. may also be present and be potential sources of blister rust spores near white pine stands that do not support it directly. Long-distance dispersal of white pine blister rust spores needs research attention before it will be possible to assess the risk to white pine patches based on the spatial relationships among hosts and the rust.

The white pine populations in other parts of North America that have been severely affected by white pine blister rust have all shown some level of genetic resistance to the disease (e.g. Hoff and others 1980, Kinloch and Dupper 2002, Sniezko and others this proceedings). A bulk seed lot from one Colorado Front Range limber pine population showed evidence of the presence of a hypersensitive reaction to the rust at moderate frequencies, although the bulk seed lot precluded an estimation of the incidence or inheritance of the resistance mechanism within the population (Kinloch and Dupper 2002). No data is currently available on the presence of other resistance mechanisms in limber pine. The loss or near loss of limber pine on xeric sites will likely transition the sites to treeless vegetation communities with currently unknown implications on slope stability, hydrology and wildlife. The impact of the loss of nurse trees on the establishment success of late successional species on mesic sites has yet to be understood. Exclusion of limber pine from some

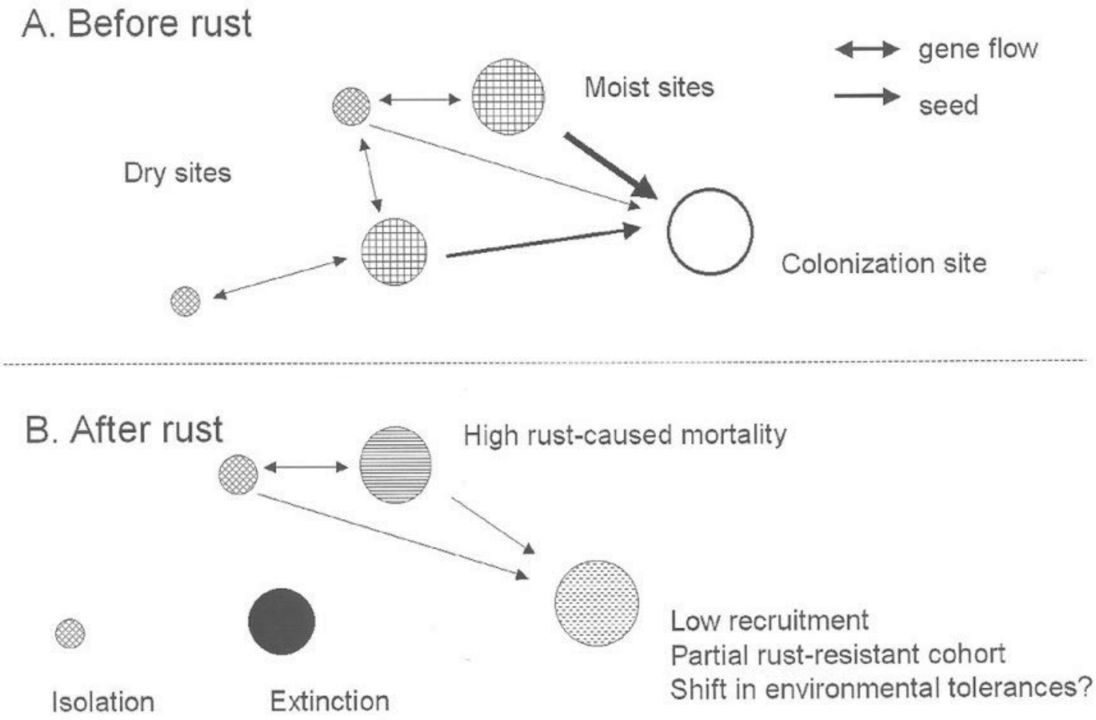


Figure 8—Schematic of potential effects of white pine blister rust on limber pine and bristlecone pine populations. The rust may cause extinction of some stands and isolation of others while also affecting reforestation of disturbed sites. See text for further discussion.

habitats by the selective pressure of the blister rust may isolate surviving patches with implications on gene flow among patches and recolonization success of forest disturbances (fig. 8). As a result, the extinction rate of limber pine patches, although already different for persistent and seral patches, is likely to be disrupted by this exotic disease with implications on the genetic structure of the limber pine metapopulation. The impacts of white pine blister rust on high elevation stands of bristlecone pine will likely lower treeline in those locations and transition the cover to a subalpine understory/alpine species mixture. The presence, nature, and geographic distribution of resistance mechanisms in bristlecone pine have not been studied.

In addition to the obvious population effects of rust-caused tree mortality, the rust may also affect the environmental tolerances of the future rust-resistant population. It is well known in plant ecology that the allocation of resources to defense, be it from herbivory or pests and pathogens, diverts resources from other plant functions. It is not known if the physiological cost on the part of the white pines associated with expressing resistance to white pine blister rust may alter a tree's sensitivity to environmental stresses, potentially causing the rust-resistant trees to have a different fundamental niche from that of the original population. After being challenged by white pine blister rust, the resultant populations of limber pine and bristlecone pine may have a different suite of environmental tolerance and competitive abilities than we see today.

Interaction of Five-Needle Pines, White Pine Blister Rust, and a Changing Fire Regime

The effects of white pine blister rust on five-needle pines will interact with the changing fire regimes in the Rocky Mountains. As fire regimes get more frequent and unpredictable due to past fire suppression and forest practices, large wildfires may jeopardize the usually less-flammable five-needle pine ecosystems on dry sites. In addition, branch and tree mortality caused by white pine blister rust may contribute to fuel loading in white pine stands, increasing the susceptibility of these stands to sustain and be consumed by fire. In the event of larger fires, especially those covering a larger area than can be seeded effectively by wind dispersal mechanisms, the loss of bird-dispersed pines as colonizers may be especially pronounced. Alternatively, if fires do not burn five-needle pine dominated stands and white pine blister rust does not affect Clark's nutcracker dispersal and caching behavior, burned areas offer recolonization opportunities for the establishment and natural selection of rust resistant pine genotypes (fig. 8).

Fire regimes may also change as a result of climatic changes in temperature and precipitation patterns. Again depending on the availability of seed and the scale and location of the fires, this may isolate stands or provide colonization opportunities. However, because persistence of limber pine stands is so sensitive to the competitive ability of co-occurring species, the indirect effects of climatic change on the performance of other species may alter the distribution of persistent versus seral limber pine.

Conservation Strategies

In the case of Colorado white pines, there are at least two possible conservation goals: (1) conservation of the genetic diversity within each species and (2) attempt to maintain the species' existing distribution by accelerating the establishment of white pine blister rust resistant genotypes across the landscape. It is unclear if selection for rust resistance will result in the loss of some physiological traits from these species; as a result conservation of genetic diversity of each species may be critical for future breeding stock to attempt to restore the traits that confer stress tolerance in these species in the future. Both bristlecone pine and limber pine have the extraordinary capability of surviving in very harsh environments and it is not known if the selective pressure of the blister rust may cause the loss of any of these traits from the surviving populations. Because white pine blister rust has only just entered Colorado and has contributed little to mortality at this time, the opportunity to conserve the full genetic diversity of Colorado limber pine and bristlecone pine populations exists. However, the feasibility of this task is another matter. Until the genetic structures of the natural populations and seed transfer rules have been defined, the only option is to immediately collect and archive seed and pollen from throughout each species geographic range. Concurrent with this approach, seed storage protocols for the species will need to be developed.

Management to accelerate the establishment of white pine blister rust resistant genotypes across the landscape may require silvicultural treatment and identifying resistant individuals and collecting and planting the seed or seedlings from those individuals in disturbed areas (Schoettle, in press). Identifying resistant individuals can be done, as has been done for other white pines, by field assessment in areas already challenged by white pine blister rust or by screening seedlings in nursery trials with artificial inoculations (Sniezko and Kegley, this proceedings).

Summary

In summary, both limber pine and bristlecone pine are long-lived species that regenerate well after fires. They can persist on xeric sites and may facilitate establishment of late successional species on more mesic sites. Disturbances throughout the elevational gradient of forested lands open habitat for limber pine and are recolonized by the effective bird dispersal of limber pine seed. Disturbances in the higher elevations open possible habitat for bristlecone pine. The genetic structure has not been defined for either species, yet limber pine may be more of a genetic generalist than bristlecone pine, and displays metapopulation dynamics. Both species are poor competitors and dominate sites that are not suitable for other species. It is unclear at this time if being a poor competitor is the "cost" associated with the stress tolerant behavior of both species and the generalist lifestyle for limber pine.

The currently available information on limber pine and bristlecone pine suggests that these species have several important ecological roles in Colorado ecosystems. (1) These white pines are exceptionally stress tolerant and occupy and stabilize habitats not likely to be occupied by other, less tolerant tree species. (2) Often these species define ecosystem boundaries (treelines). (3) These species are among the first to colonize a site after fire, especially fires that cover large areas. (4) Limber pine, and possibly bristlecone pine, facilitate the establishment of high elevation late successional species such as Engelmann spruce. (5) The seeds of both five-needle pines provide a dietary component for several animal species, and the stands likely also provide habitat for other species.

The recent discovery of white pine blister rust in Colorado threatens limber pine and bristlecone pine populations. While the rust is not likely to eliminate the five-needle pines from Colorado ecosystems, it is likely to impact species' distributions, population dynamics and the functioning of the ecosystems. The rust may cause local population extinctions as well as greatly reduce genetic diversity and alter environmental tolerances of the species. The reduction in effective population numbers may hinder the evolutionary development or render local populations even more subject to risk. Changing fire regimes resulting from management or climatic changes will contribute to determining the future importance of the ecological role of white pines. In addition, change in the competitive interactions among Rocky Mountain conifers as a result of climatic changes may affect the future of these landscapes. The interaction of these factors with the stress of this exotic pathogen may well affect (1) the distribution of forested land on the landscape, (2) the reforestation dynamics after fire, (3) the rate and possibly fate of forest succession, and (4) habitat for wildlife. Our incomplete understanding of the ecology, genetic structure and adaptive variation of limber pine and bristlecone pine constrain our ability to rapidly develop and implement conservation programs.

References

Antolin, M.F. and Schoettle, A.W. 2001. Fragments, extinctions, and recolonization: the genetics of metapopulations. Pp. 37-46 *in* D. Joyce and J.D. Simpson, eds. Proceedings of the 27th Biennial Conference of the Canadian Tree Improvement Association. Natural Resources Canada, Fredricton, NB Canada.

Bailey, D.K. 1970. Phytogeography and taxonomy of *Pinus* subsection *Balfourianae*. Ann. Mo. Bot. Gard. 57:210-249.

Baker, W.L. 1992. Structure, disturbance, and change in the bristlecone pine forests of Colorado, USA. Arctic and Alpine Research 24:17-26.

Barrick, K. A. and Schoettle, A.W. 1996. A comparison of the foliar nutrient status of elfinwood and symmetrically formed tall trees, Colorado Front Range, USA. Canadian Journal of Botany 74: 1461-1475.

Blodgett, J.T. and Sullivan, K.F. 2004. First report of white pine blister rust on Rocky Mountain bristlecone pine. Plant Disease 88:311.

Brown, D.H. 1978. Extension of the known distribution of *Cronartium ribicola* and *Arceuthobium cyanocarpum* on limber pine in Wyoming. Plant Disease Reporter 62:905.

Bruederle, L.P., Tomback, D.F., Kelly, K.K. and Hardwick, R.C. 1998. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (Pinaceae). Can. J. Bot. 76: 83-90.

Brunstein, F.C. and Yamaguchi, D.K. 1992. The oldest known Rocky Mountain bristlecone pines (*Pinus aristata* Engelm.). Arctic and Alpine Research 24:253-256.

Burns, R.M. and Honkala, B.H. 1990. Silvics of North America, vol. 1, Conifers. USDA Forest Service Agricultural Handbook 654.

Carsey, K. S. and Tomback, D.F. 1994. Growth form distribution and genetic relationships in tree clusters of Pinus flexilis, a bird dispersed pine. Oecologia 98: 402-411.

Cerezke, H.F. 1995. Egg gallery, brood production, and adult characteristics of mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in three pine hosts. The Canadian Entomologist 127:955-965.

Curry, D.R. 1965. An ancient bristlecone pine stand in eastern Nevada. Ecology 46:564-566.

DeVelice, R.L., Ludwig, J.A., Moir, W.H. and Ronco, F. 1986. A classification of forest habitat types of northern New Mexico and southern Colorado. USDA Forest Service General Technical Report RM-131, 59 p. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo.

Donnegen, J.A. and Rebertus, A.J. 1999. Rates and mechanisms of subalpine forest succession along an environemtal gradient. Ecology 80:1370-1384.

Ewers, F.W. and Schmid, R. 1981. Longevity of needle fascicles of Pinus longaeva (bristlecone pine) and other North American pines. Oecologia 51:107-115.

Feldman, R., Tomback, D.F. and Koehler, J. 1999. Cost of mutualism: competition, tree morphology, and pollen production in limber pine clusters. Ecology 80: 324-329.

Fryer, J.H. and Ledig, F.T. 1972. Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient.. Can. J. Bot. 50:1231-1235.

Hansen-Bristow, K. 1986. Influence of increasing elevation on growth characteristics at timberline. Canadian Journal of Botany 64: 2517-2523.

Hedlin, A.F., Yates III, H.O., Tovar, D.C., Ebel, B.H., Koerber, T.W. and Merkel, E.P. 1981. Cone and seed insects of North American conifers. Canadian Forestry Service, United States Forest Service, and Secretaria de Agricultura y Recursos Hidraulicos, Mexico. 122 pp.

Heit, C.E. 1973. Testing and growing limber and Mexican border pines. American Nurseryman 137 (11): 8-9 and 64-74.

Hoff, R.J., Bingham, R.T., McDonald, G.I. 1980. Relative blister rust resistance of white pines. European Journal of Forest Pathology 10:307-316.

Hutchins, H.E. and Lanner, R.M. 1982. The central role of Clark's nutcracker in the dispersal and establishment of whitebark pine. Oecologia 55:192-201.

Johnson, D.W., Jacobi, W.R . 2000. First report of white pine blister rust in Colorado. Plant Disease 84:595

Kendell, K.C. 1983. Use of pine nuts by black and grizzly bears in the Yellowstone area. International Conference on Bear Research and Management 5: 166-173.

Kinloch, B.B. Jr. and Dupper, G.E. 2002. Genetic specificity in the white pine/blister rust pathosystem. Phytopathology 92[3]: 278-280.

Knowles, P. and Grant, M.C. 1983. Age and size structure analyses of Engelmann spruce, pondersoa pine, lodgepole pine, and limber pine in Colorado. Ecology 64:1-9.

Kreb, P.V. 1973. Dendrochronology of bristlecone pine (*Pinus aristata* Engelm.) in Colorado. Arctic and Alpine Research 5:149-150.

LaMarche, V.C. 1969. Environment in relation to age of bristlecone pines. Ecology 50:53-59.

LaMarche, V.C. and Stockton, C.W. 1974. Chronogies from temperature-sensitive bristlecone pines at upper treeline in western United States. Tree-Ring Bulletin 34:21-45.

Lanner, R.M. 1980. Avian seed dispersal as a factor in the ecology and evolution of limber and whitebark pine. IN B. P. Dancik and K. O. Higginbotham [eds.], Proceedings of sixth North American Forest Biology Workshop, 15-48. University of Alberta, Edmonton, Alberta, Canada.

Lanner, R.M. 1984. Trees of the Great Basin. University of Nevada Press, Reno, NV.

Lanner, R.M. 1988. Dependence of Great Basin bristlecone pine on Clark's nutcracker for regeneration at high elevations. Arctic and Alpine Research 20:358-362.

Lanner, R.M. 1990. Biology, taxonomy, evolution, and geography of stone pines of the world. Proceedings – Symposium on whitebark pine ecosystems: Ecology and management of a high-mountain resource. General Technical Report INT-270, Intermountain Research Station, Ogden UT. pp. 14-24.

Lanner, R.M. and Vander Wall, S.B. 1980. Dispersal of limber pine seed by Clark's nutcracker. Journal of Forestry 78: 637-639.

Latta, R.G. and Mitton, J.B. 1997. A comparison of population differentiation across four classes of gene marker in limber pine (*Pinus flexilis* James). Genetics 146: 1153-1163.

Lepper, M.G. 1974. *Pinus flexilis* James and its environmental relationships. Ph.D. dissertation, University of California Davis, Davis, California, USA.

Lepper, M.G. 1980. Carbon dioxide exchange in *Pinus flexilis* and *Pinus strobiformis* (Pinaceae). Madroño 27: 17-24.

Lundquist, J.E., Geils, B.W. and Johnson, D.W. 1992. White pine blister rust on limber pine in South Dakota. Plant Disease 76:538.

Marr, J.W. 1961. Ecosystems of the eastern slope of the Front Range in Colorado. Series in Biology No. 8. University of Colorado Press, Boulder, Colo.

McCutchen, H.E. 1996. Limber pine and bears. Great Basin Naturalist 56(1): 90B92.

McDonald, G.I. and Hoff, R.J. 2001. Blister rust: an introduced plaque. *In* Whitebrak Pine Communities, *edited* by D.F. Tomback, S.F. Arno and R.E. Keane. Island Press, Washington, D.C. pp193-220.

McNaughton, G.M. 1984. Comparative water relations of *Pinus flexilis* at high and low elevations in the Central Rocky Mountains. Master's thesis, University of Wyoming, Laramie, Wyoming, USA.

Mitton, J.B., Kreiser, B.R. and Latta, R.G. 2000. Glacial refugia of limber pine (*Pinus flexilis* James) inferred from the population structure of mitochondrial DNA. Mol. Ecol. 9: 91-97.

Moir, W.H. and Ludwig, J.A. 1979. A classification of spruce-fir and mixed conifer habitat types of Arizona and New Mexico. USDA Forest Service Research Paper RM-207, 47 p. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo.

Mooney, H.A., Wright, R.D. and Strain, B.R. 1964. The gas exchange capacity of plants in relation to vegetation zonation in the White Mountains of California. American Midland Naturalist 72: 281-297.

Oline, D.K. 2001. Allozyme variation in the Rocky Mountain bristlecone pine (*Pinus aristata*) at the regional and local scales. Published abstract. International IUFRO Conference on Breeding and Genetic Resources of five-needle pines: Growth, adaptability and pest resistance. Medford, OR. July 23-27, 2001. 1p.

Pataki, D.E., Oren, R. and Smith, W.K. 2000. Sap flux of co-occurring species in a western subalpine forest during season soil drought. Ecology 81:2557-2566.

Peet, R.K. 1981. Forest vegetation of the Colorado Front Range: composition and dynamics. Vegetatio 43: 3-75.

Ranne, B.M., Baker, W.L., Andrews, T. and Ryan, M.G. 1997. Natural variability of vegetation, soils and physiography in the bristlecone pine forests of the Rocky Mountains. Great Basin Naturalist 57:21-37.

Rebertus, A.J., Burns, B.R. and Veblen, T.T. 1991. Stand dynamics of *Pinus flexilis*-dominated subalpine forests in the Colorado Front Range. Journal of Vegetation Science 2: 445-458.

Rehfeldt, G.E. 1997. Quantitative analysis of the genetic structure of closely related conifers with disparate distributions and demographics: the *Cuppressus arizonica* (Cuppressaceae) complex. Am. J. Bot. 84: 190-200.

Schauer, A.J., Schoettle, A.W. and Boyce, R.L. 2001. Partial cambial mortality in high-elevation *Pinus aristata* (Pinaceae). Am. J. Bot. 88:646-652.

Schmidt, W.C. and McDonald, K.J., compilers. 1990. Proceedings – Symposium on whitebark pine ecosystems: Ecology and management of a high-mountain resource. General Technical Report INT-270, Intermountain Research Station, Ogden, UT.

Schoettle, A.W. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. Tree Physiol. 14:1055-1068.

Schoettle, A.W. in press. Developing proactive management options to sustain bristlecone and limber pine ecosystems in the presence of a non-native pathogen. In: Shepperd, Wayne, Compiler. Silviculture in special places: Proceedings of the 2003 National Silviculture Workshop. 2003 September 7-11. Granby, CO. Proceedings RMRS-P-XX. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Schoettle, A.W. and Negron, J.F. 2001. First report of two cone and seed insects on *Pinus flexilis*. Western North American Naturalist 61:252-254.

Schoettle, A.W. and Rochelle, S.G. 1996. Effect of light on shoot structure and function of mature subalpine conifer trees. IN C. Edelin [ed.], L'ARBRE Biologie et Developement. Naturalia Monspeliensia, Montpellier, France.

Schoettle, A.W. and Rochelle, S.G. 2000. Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevation. Am. J. Bot. 87: 1797-1806.

Schulman, E. 1954. Longevity under adversity in conifers. Science 119:396-399.

Schulman, E. 1958. Bristlecone pine, oldest known living thing. Natl. Geogr. Mag. 113:355-372.

Schuster, W.S.F., Mitton, J.B., Yamaguchi, D.K. and Woodhouse, C.A. 1995. A comparison of limber pine (*Pinus flexilis*) ages at lower and upper treelines east of the Continental Divide in Colorado. Am. Midl. Nat. 13:101-111.

Schuster, W.S.F., Alles, D.L. and Mitton, J.B. 1989. Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational gradient. Am. J. Bot. 76: 1395-1403.

Schuster, W.S.F. and Mitton, J.B. 1991. Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. Heredity 67: 41-48.

Schuster, W.S.F. and Mitton, J.B. 2000. Paternity and gene dispersal in limber pine (*Pinus flexilis* James). Heredity 84: 348-361.

Stohlgren, T.J. and Bachand, R.R. 1997. Lodgepole pine (Pinus contorta) ecotones in Rocky Mountain National Park, Colorado, USA. Ecology 78:632-641.

Stohlgren, T.J., Bachand, R.R., Onami, Y. and Binkley, D. 1998. Species environment relationships and vegetation patterns: effects of spatial scale and tree life stage. Plant Ecology 135:215-228.

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, N.J. 360 p.

Tomback, D.F. and Kramer, K.A. 1980. Limber pine seed harvest by Clark's nutcracker in the Sierra Nevada: timing and foraging behavior. Condor 82:467-468.

Tomback, D.F., Sund, S.K. and Hoffmann, L.A. 1993. Post-fire regeneration of *Pinus albicaulis*: height-age relationships, age structure, and microsite characteristics. Can. J. For. Res. 23:113-119.

Tomback, D.F., Arno, S.F. and Keane, R.E., editors. 2001. Whitebark Pine Communities. Island Press, Washington, D.C. 440 p.

Torick, L.L., Tomback, D.F. and Espinoza, R. 1996. Occurrence of multi-genet tree clusters in "wind-dispersed" pines. Am. Midl. Nat. 136:262-266.

Van Arsdel, E.P. Conklin, D.A., Popp, J.B. and Geils, B.W. 1998. The distribution of white pine blister rust in the Sacramento Mountains of New Mexico. Finnish Forest Research Institute Research Papers 712: 275-283.

Vander Wall, S.B. and Balda, R.P. 1977. Coadaptation of the Clark's nutcracker and the pinon pine for efficient seed harvest and dispersal. Ecol. Monogr. 47:89-111.

Webster, K.L. and Johnson, E.A. 2000. The importance of regional dynamics in local populations of limber pine (*Pinus flexilis*). Ecosci. 7: 175-182.

Wells, P.V. and Stewart, J.D. 1987. Cordilleran-boreal taiga and fauna on the central Great Plains of North America, 14,000 - 18,000 years ago. Am. Midl. Nat. 118: 94-106.

Genetic Variation of *Pinus cembra* Along an Elevational Transect in Austria

Raphael Thomas Klumpp
Marcus Stefsky

Abstract—The genetic variation of *Pinus cembra* was analyzed by means of isozyme gene markers along an elevational transect at Koetschach Valley (Salzburg State/Austria). Mature stands and their natural recruitment were studied at three elevation levels: subalpine zone, high montane zone, and middle montane zone. Sample included juvenile and mature individuals. Thirteen enzyme systems encoding for 22 gene loci were scored. The results showed increasing allelic multiplicity with increasing altitude in mature stands and decreasing polymorphism with increasing altitude in juvenile populations. Surprisingly high values of allelic multiplicity and hypothetic gametic multilocus diversity were found in middle elevation populations, which have potential for generating genetically diverse gametes in future generations. Seed dispersal by a nutcracker bird species, as well as gene flow by local wind systems, may be the reason for this phenomenon, which is obviously strengthened by strong selection forces.

Key words: *Pinus cembra*, isozyme, elevation, population, genetic diversity, seed dispersal, *Nucifraga caryocatactes*.

Introduction

Pinus cembra L., a locally important species in Europe, has had multiple uses over centuries. The species has been used for timber, when indoor use for country style furniture and wall ornaments were in fashion, and the large seeds (nuts) were used to improve the diets of farmers living in alpine pastures. Presently, the blue cones are harvested for preparing liquor, which leads to crown damage and problems for species that depend on the seeds for food like nutcracker birds (*Nucifraga caryocatactes* L.).

It is well known that *P. cembra* was eliminated in lower elevations by competition. It is less commonly known that virgin forests at the timberline in alpine mountains were harvested in order to extend alpine pastures, even up to the 20th century (Fromme 1957). Furthermore, large amounts of timber from these forests were needed and utilized. Hence, distortion of genetic architecture is anticipated in all natural populations. In spite of these facts, we hypothesize that there is still a relatively high level of genetic variation in high elevations, as those forests have not been regularly managed. Furthermore, the competitiveness of *P. cembra*, as well as the mutualism with nutcracker birds at these elevations, may lead to more effective preservation of the gene pool. In contrast, populations at lower elevation may have suffered a reduction of genetic diversity due to regular management activities and limited activity of nutcrackers in such dense forests. Isoenzyme studies in *P. cembra* and related species have been conducted by different authors. Heterozygosity is low in comparison to other pine species (Szmidt 1982), and species can be easily differentiated (Goncharenko and others 1992, Politov and Krutovskii 1994). Different numbers of gene loci have been found to be encoding the same enzyme systems of different pine species (Bergmann and Hattemer 1995), but *P. cembra* and its relatives from subgenus *Strobus* Lemm. section *Strobus* exhibit the same number of gene loci controlling the enzyme system of 6PGDH (Bergmann and Gillet 1997).

Genetic architecture in conifer populations can be affected by the age of the population. Investigations on the natural recruitment in *P. sylvestris* L. revealed that there is an excess of homozygotes in the embryo stage, which decrease as age advances according to the species' life cycle (Muona and others 1987, Yazdani and others 1985). Similar results have been found in other coniferous species, such as *P. radiata* D. Don. (Plessas and Strauss 1986), *Pseudotsuga menziesii* [Mirb.] Franco (Shaw and Allard 1982), and *Abies alba* Mill. (Hussendoerfer 1998).

Variation along elevational transects has been observed by means of isozyme gene markers in several species. Mitton and others (1980) found clinal variation at two gene loci in ponderosa pine (*P. ponderosa* Laws.). Ruetz and Bergmann (1989) found variation of allelic structures along one elevational transect in Norway spruce (*Picea abies* (L.) Karst.). However, other studies, for example Neale and Adams (1985) in balsam fir (*Abies balsamea* (L.) Mill.), could not confirm this finding. Similarly, some publications on Norway spruce (Konnert 1991) and European beech (*Fagus sylvatica* L.) (Loechelt and Franke 1995) did not show clinal variation along elevational gradients. These studies were conducted on populations in southwestern Germany, where silvicultural activities by humans over centuries may have lead to distortion of natural variation.

The reproductive potential of this species is currently in danger, as the local forest authority is unable to control the harvesting of cones in Austria. As this tree species is less competitive than other species, we initiated an investigation on genetic diversity along 11 elevational transects in the

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Raphael Thomas Klumpp and Marcus Stefsky are with the Institute of Silviculture, University of Natural Resources and Applied Life Sciences, Peter Jordan Str. 70 A-1190, Vienna, Austria. Contact Klumpp: Phone 0043 1 47654 4063. Fax 0043 147654 4092. Email: raphael.klumpp@boku.ac.at

Austrian Alps (Alpine Mountains). This study will provide information to guide gene conservation/restoration efforts and can be used as well to indicate changes in genetic diversity due to global warming over time. In this paper, initial results will be reported using isozyme gene markers to study diversity along a single Austrian transect.

Materials and Methods

The Koetschach Valley in the State of Salzburg, Austria, is found in the eastern part of the central Alps, which exhibits a subcontinental, cold winter climate. Sampling areas were designated on a single slope with northern exposition at three different elevation levels (table 1). The transect number is AT1, and the sampling area was designated as "U" for the subalpine zone, "M" for the high montane zone, and "L" for the middle-montane zone. Samples were taken from adult and juvenile trees (51 to 149 individuals per population) and recorded by age class.

Isoenzyme analyses were conducted following the methods described by Cheliak and Pitel (1984) and Hertl (1997). The analyses used 13 enzyme systems, which encode for 22 gene loci (table 2). Data were scored using the GSED pro-

Table 1—Characteristics of the sampling area.

Transect AT 1			
Altitudinal zone	**Subalpine (U)**	**High montane (M)**	**Middle montane (L)**
Elevation	1900 – 2100 m	1600 – 1700 m	1200 – 1300 m
Forest community	*P. cembra* – larch forest with *Pinus mugo* Turra	Larch – Norway spruce forest with *P. cembra*	Spruce forest with some *P. cembra* and larch
Soil	Semipodsol		
Precipitation	900 – 1100 mm		
Total number of individuals:	**149**	**148**	**51**
Adult population	99	100	34
Juvenile population	49	48	17

Table 2—Analysed enzyme systems and gene loci.

Nomenclature	Enzyme system	Analysed loci
Alaninaminopeptidase (E.C. 3.4.11.2)	AAP	AAP-A AAP-B
Aspartataminatransferase (E. C. 2.6.1.1)	AAT	AAT-A AAT-B AAT-C
Aconitase (E. C. 4.2.1.3)	ACO	ACO-A
Diaphorase (E.C. 1.6.4.3)	DIA	DIA-A DIA-B
Glutamatdehydrogenase (E. C. 1.4.1.2)	GDH	GDH-A
Isocitrat-Dehydrogenase (E. C. 1.1.1.42)	IDH	IDH-B
Leucin-Aminopeptidase (E. C. 3.4.11.1)	LAP	LAP-A LAP-B
Malat-Dehydrogenase (E. C. 1.1.1.37)	MDH	MDH-A MDH-B MDH-C
Menadion-Reduktase (E. C. 1.6.99.2)	MNR	MNR-A
6-Phosphogluconic-Dehydrogenase (E. C. 1.1.1.44)	6-PGDH	6-PGDH-A 6-PGDH-C
Phosphoglucose-Isomerase (E. C. 5.3.1.9)	PGI	PGI-A PGI-B
Phosphoglucomutase (E. C. 2.7.5.1)	PGM	PGM-A
Shikimat-Dehydrogenase (E. C. 1.1.1.25)	SKDH	SKDH-A

gram (Gillet 1994). We used allelic multiplicity (number of alleles M), relative allelic multiplicity (M/Mmax) and the average number of alleles per locus (A/L) for describing allelic variation (Hattemer and others 1993). Genetic diversity was described using average (observed) heterozygosity (Ho), diversity of the gene pool (V) and hypothetic gametic multilocus diversity (Vgam) (Gregorius 1978, 1987). The genetic variation within each population was quantified by calculating the percentage of polymorphic loci (P_{95}), where the frequency of the most common allele does not exceed 95 percent.

Results and Discussion

The *P. cembra* subpopulation from the middle montane zone on the valley floor is scattered in a spruce-dominated forest that also includes larch. Correspondingly, only 34 adult and 17 young individuals were found, which represented approximately 80 percent of the whole population in this area. This bias in sampling was considered when drawing some conclusions from this study.

The number of observed alleles was found to obviously increase with elevation in the adult populations. However, the highest value (M=36) was found at medium elevation in juvenile populations (table 3). In the high montane zone, 75 percent of the known variants were represented in the juvenile population, as a total of 48 alleles over 22 gene loci, were found in this valley. Closer inspection revealed that variants with allele frequencies of more than 5 percent were found to comprise between 58.3 and 64.6 percent of the total known variants. Adult populations obviously possessed more rare variants with an allele frequency of 1 percent, such as AT1-U: M/Mmax=83.3 and M(f>1)/Mmax=77.1, than in young populations, such as AT1-U: 70.8: 70.8 (table 3).

Surprisingly, the populations of the middle elevation exhibited not only the highest values of observed alleles, but also high values of relative allelic multiplicity (M/Max), which was 75 percent of the known variants (table 3). Stone pine appears to be different from other species, where clinal variation was not found in allozyme gene markers (see for example Moran and Adams 1989), or was only found only in certain loci (for example Mitton and others 1980). This may be due to strong selective forces such as the harsh climate at the timberline, or competition with spruce in the valley floor may influence the gene pool composition of this species. Moreover, trees resulting from movement of seed by birds (compare Marzluff and Balda 1992) may have enhanced the existing gene flow (primarily by pollen transport up and down the slopes), which leads to high level of genetic multiplicity.

A comparison of selected parameters of genetic variation in mature stands from different elevations shows a slight increase of multiplicity (M) with elevation but nearly no trends in the other parameters (fig. 1). In contrast, no trend was found in the heterozygosity in the juvenile stands, and polymorphism decreased with elevation. Multiplicity as well as hypothetic gametic multilocus diversity (the potential for generating genetically diverse gametes in future generations) is highest in the middle elevation (fig. 2). This indicates that the genetic architecture in the juvenile populations at the middle elevation has been preserved better than at other locations/age classes (fig. 3; table 3).

Conclusions

The higher allelic multiplicity in juvenile populations at the mid-montane zone is due to a combination of birds and wind. Nutcracker birds transport seed to the middle slopes from higher and lower elevations, as the middle slopes provide less snow cover and easy access. Snow cover at the timberline and the dense forest in the valley are not as attractive for habitats for the bird, and allelic multiplicity is correspondingly lower. Pollen transport by local wind systems cause gene flow among populations up and down slopes, thereby the mid-montane zone has an influx of genes from populations at both higher and lower elevations. Selection forces are obviously active, which to a certain extent keeps the gene pool of the timberline population different

Table 3—Genetic variation at transect AT1[a] in the Koetschach valley, Salzburg State / Austria

	AT1 - U		**AT1 – M**		**AT1 - L**		
	A[b]	**J[c]**	**A**	**J**	**A**	**J**	**Average**
Number of individuals	100	49	100	48	34	17	—
Number of loci	22	22	22	22	22	22	22
Number of observed alleles (M)	40	34	38	36	34	32	35.7
M/M_{max} (%)	83.3	70.8	79.2	75.0	70.8	66.7	74.3
A/L	1.82	1.54	1.73	1.64	1.54	1.45	1.62
A/L ≥ 5 %	1.27 (58.3)	1.27 (58.3)	1.32 (60.4)	1.32 (60.4)	1.36 (62.5)	1.41 (64.6)	1.33 (60.6)
A/L ≥ 1 %	1.68 (77.1)	1.55 (70.8)	1.64 (75.0)	1.64 (75.0)	1.55 (70.8)	1.41 (64.6)	1.58 (72.2)
P_{95} in %	23	18	18	27	32	32	25
Ho (observed)	0.095	0.081	0.083	0.083	0.083	0.086	0.085
Gene pool diversity (V)	1.10	1.09	1.12	1.13	1.13	1.12	1.12
Hyp. gam. diversity (V_{gam})	28.3	24.6	27.3	37.1	26.4	24.8	28.1

[a] Where U refers to the subalpine zone, M refers to the high montane zone, and L refers to the middle montane zone of transect AT1.
[b] A - Adult population
[c] J - Juvenile population

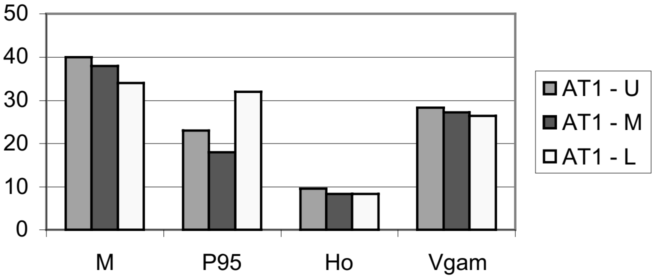


Figure 1—Average genetic variation for selected parameters in mature stands from different elevation.

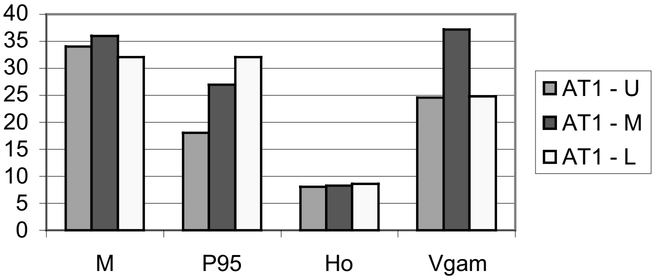


Figure 2—Average genetic variation for selected parameters in the juvenile population from different elevation.

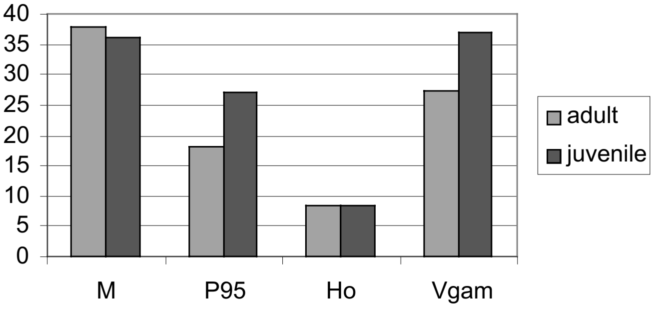


Figure 3—Average genetic variation for selected parameters in adult and juvenile populations at sample area AT1- M (high montane zone).

from that of the valley population. Strong competition with the dominating spruce at the ground of the valley reduces *P. cembra* populations to the extent that the opportunity for rare variants in the gene pool is limited.

Acknowledgments

This study was funded by the European Union as part of the project on “biodiversity of alpine forest ecosystems” (EU CT96-1949). We thank Eva, Tajana, Herwig, and Wolfgang for technical assistance in collecting the samples as well as for laboratory assistance. The advice and many helpful comments by Prof. Gerhard Mueller-Starck (Munich/Germany) is greatly appreciated. Furthermore, we thank Scott Schlarbaum and Richard Sniezko for helpful comments on an earlier draft.

References

Bergman, F. Hattemer, H., H., 1995: Isozyme gene loci and their allelic variation in *Pinus sylvestris* L. and *Pinus cembra* L. Silvae Genet. 44 (5-6): 286 - 289.

Bergman, F., Gillet, E., 1997: Phylogenetic relationships among *Pinus* species (Pinaceae) inferred from different numbers of 6 - PGDH loci. Plant Syst. Evol. 208: 25 - 34.

Cheliak, W.M., Pitel, J.A., 1984: Techniques for starch gel electrophoresis of enzymes from forest tree species. Information report PI-X-42, Petawawa National Forestry Institute. 49 pp.

Fromme, G., 1957: Der Waldrueckgang im Oberinntal (Tirol) [On the deforestation in the upper Inn-Valley (Tyrol)]. Mitt. Der forstl. Bundesversuchansanstalt Mariabrunn Vol. 54. 221pp. (German, title translated by the author of this paper).

Gillet, E., 1994: GSED, Genetic structures from electrophoresis data. Vers.1.1. Institute of Forest Genetics and Treebreeding. University of Goettingen, Germany. 49 pp.

Goncharenko, G.G., Padutov, V.E., Silin, A.E., 1992: Population structure, gene diversity and differentation in natural populations of Cedar pines (*Pinus* subsect. *Cembrae, Pinaceae*) in the USSR. Plant Syst. Evol. 182: 122 – 134.

Gregorius, H.-R. 1978. The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math. Bioscience 41: 253-271.

Gregorius, H.-R. 1987. The relationship between the concepts of genetic diversity and differentiation. Theoret. Appl.Genetics 74: 397-401.

Hattmer, H.H., Bergmann, F., Ziehe, M., 1993: Einführung in die Genetik für Studierende der Forstwirtschaft, J.D. Sauerläder’s Verlag, Frankfurt am Main, 2 Auflage. 492 pp. (in German).

Hertl, H., 1997: Biochemisch-genetische Untersuchung bei Kiefer (*Pinus sylvestris* L.). Anleitung zur Trennmethodik und Auswertung der Zymogramme, Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft Hamburg, Nr. 186. 59 pp. (German with English summary).

HussendOErfer, E., 1998. Genetische Inventuren im Bannwald Schwarzahalden, Mitt. Ver. Forstl. Standortskunde u. Forstpflanzenzüchung 39: 103-108. (German with English summary).

Konnert, M., 1991: Die Fichte im Schwarzwald: Genetische Variation und Korrelationen. Forstw. Cbl. 110: 84 - 94. (German with English summary).

Loechelt, S., Franke, A., 1995 Bestimmung der genetischen Konstitution von Buchen-Bestaenden (*Fagus sylvatica* L.) entlang eines Hoehentransektes von Freiburg auf den Schauinsland. Silvae Genet. 44 (5-6): 312-318. (German with English summary).

Marzluff, J. M., Balda, R. P., 1992: The pinyon jay: behavioral ecology of a colonial and cooperative corvid. London, Poyser. 317 p.

Mitton, J.B., Sturgeon, K.B., Davis, M.L., 1980: Genetic differentiation in ponderosa pine along a steep elevational transect. Silvae Genet. 29 (3-4): 100-103.

Moran, G.F., Adams, W.T., 1989: Microgeographical patterns of allozyme differentiation in Douglas-fir from Southwest Oregon. Forest Sci. 35: 3-15.

Muona, O., Yazdani, R., Rudin, D., 1987: Genetic change between life stages in *Pinus sylvestris*: allozyme variation in seeds and planted seedlings. Silvae Genet. 36 (1): 39-42.

Neale, D.B., Adams, W.T., 1985: Allozyme and mating system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. Can. J. Bot. 63: 2448-2453.

Plessas, M. E., Strauss, S. H., 1986: Allozyme differentiation among populations, stands, and cohorts in Monterey pine. Can. J. For. Res. 16: 1155-1164.

Politov, D. V., Krutovskii, K. V., 1994: Allozyme polymorphism, heterozygosity, and mating system of stone pines. pp 36 - 42 *in*: Schmidt, W.C., Holtmeier, F.-K. (compilers), 1994: Proceedings - International workshop on subalpine Stone pines and their environment: the status of our knowledge. USDA For. Serv. Gen. Tech. Rep. INT-GRT-309. 321 pp.

Ruetz, W., Bergmann, F., 1989: Moeglichkeiten zum Nachweis von autochthonen Hochlagenbestaenden der Fichte (*Picea abies*) in den Berchtesgadener Alpen. Forstw. Cbl. 108: 164-174. (German with English summary).

Shaw, D.V., Allard, R.W., 1982: Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. Silva Fennica 16: 115-121.

Szmidt, A.E., 1982: Genetic variation in isolated populations of stone pine (*Pinus cembra*). Silva Fennica 16 (2): 196-200.

Yazdani, R., Muona, O., Rudin, D., Szmidt, A.E., 1985: Genetic structure of a *Pinus sylvestris* L. seed-tree stand and naturally regenerated understory. Forest Sci. 31 (2): 430-436.

Five-Needle Pines in New Zealand: Plantings and Experience

J.T. Miller
F.B. Knowles
R.D. Burdon

Abstract—Five-needle pines that have been tried as plantation crops in New Zealand are: *Pinus strobus* L., *P lambertiana* Dougl., *P. monticola* Dougl. *ex.* D. Don., and *P. wallichiana* A.B. Jacks. Total plantation areas have reached approximately 1,300 ha, 75 ha, 20 ha and 10 ha, respectively but have generally dropped in recent years. A partial picture of provenance variation in performance has been obtained. These species have grown well on a range of sites but have tended to be affected by drought and exposure. While they are sometimes affected by out-of-season frosts, the tolerance of partly continental conditions is generally good. Disease has been little problem, except for root disease in *P. strobus* on poorly drained soils and *Dothistroma* needle blight on *P. lambertiana* at some sites. However, animal damage (mainly deer and Australian possums) has often been troublesome and has led to much malformation. Productivity can be high, with mean annual increments of approximately 25 m^3/ha/year stemwood in *P. strobus* and *P. lambertiana*, and equal to or greater than 30 m^3/ha/year in *P. monticola*, given favourable sites and appropriate provenances. One other species, *P. ayacahuite*, has shown promise; while fairly frost-tender and prone to animal damage, it can have the fastest height growth and produce 24 m^3/ha/year or so in stem volume. Eight other species are known to have grown successfully in New Zealand without arousing commercial interest, and a few others have failed to grow. Despite some good performances, five-needle pines are eclipsed in New Zealand by Monterey pine (*P. radiata* Don.), Douglas-fir and some eucalypts. However, there has been a recent reintroduction of a provenance collection of *P. lambertiana*.

Key words: *Pinus strobus, Pinus monticola, Pinus lambertiana, Pinus wallichiana, Pinus ayacahuite*, provenance, genecology, growth, yield, site adaptation, browse damage, fungal diseases.

History in New Zealand

Five-needle pines were first introduced into New Zealand well over 100 years ago, including: *P. strobus* in 1866, *P. wallichiana* in 1868, *P. lambertiana* in 1869; *P. monticola* in 1905 and 1907, and *P. ayacahuite* in 1915.

The first plantings were often few trees and mostly on private estates. Later plantings were more extensive, and predominantly within state forests, either as part of routine plantings or in designated research trials. Plantings have been mostly in the central North Island (Lats 37-39°S, Long. 176°E), but plantations have been established as far as 46°S. Elevations of planting have varied from near sea level to around 600 m, mainly in areas of 1,000 to 1,600 mm annual precipitation.

In the case of *P. strobus*, state plantings between 1931 and 1935 comprised more than 50 percent of the eventual total area. By 1957 there were 1,282 ha in pure stands (1210 ha in the North Island and 73 ha in the South Island), with 39 ha in mixture with other species. By 1960 there were estimated to be 1,307 ha under all ownerships, over 1,130 ha being in the central North Island. *Pinus strobus* has proved to be vigorously invasive in some localities. It has regenerated freely around Rotorua, spreading up to 3 km from the parent source into scrubland. It has also become naturalised in the South Island in Nelson, North Canterbury, and at Mt Linton (Lat. 46°S).

Plantings of *P. monticola* were small by comparison. State trials were planted in the North Island about 1910 and in the South Island in 1932 and 1933. Further experimental plantings occurred in the central and lower North Island between 1947 and 1958, and by 1961 the total area had increased to 21 ha of which 98 percent was in the North Island.

The main plantings of *P. lambertiana* took place in the central North Island between 1928 and 1931, with small areas being added in the South Island about the same time. By 1957 the total area of *P. lambertiana* in State Forests was approximately 74 ha, of which only 5 ha were in the South Island. By 1961 this was down to 68 ha, over 80 percent of which was in the North Island.

Small areas of *P. ayacahuite* were planted by the state forestry agency in 1915, and in species trials between 1957 and 1959. Further plantings were confined to provenance trials established between 1959 and 1963 at nine locations throughout the country.

Pinus wallichiana has been fairly widely planted in New Zealand but usually singly, as small groups as an ornamental, or with in species trials. By 1956 there were approximately 9 ha in State Forests, nearly all in Golden Downs Forest in the north of the South Island.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the New Zealand Forest Research Institute, Private Bag 3020, Rotorua, New Zealand. J.T. Miller's current address is Regency Park Estate, Te Ngae Road, Rotorua, New Zealand. F.B. Knowles' current address is Ridge Road, Lake Okareka, Rotorua, New Zealand. R.D. Burdon, the corresponding author, may be reached at phone +64 7 343 5742; fax +64 7 348 0952; e-mail Rowland.burdon@forestreach.co.nz.

Other five-needle pines occasionally found growing satisfactorily in New Zealand arboreta include *P. flexilis* James, *P. aristata* Engelm., *P. peuce* Griseb., *P. koraiensis* Sieb. & Zucc., *P. cembra* L., *P. maximartinezii* Rzedowski, and *P. morrisonicola* Hayata.

A species that failed was *P. chiapensis* (Martinez) Andresen. Planted in two North Island trials, it suffered heavy initial losses from frost, and then succumbed to browsing by the Australian brush-tailed possum (*Trichosurus vulpecula*).

Little record exists of the seed origins of New Zealand white pine imports. Seed for some of the *P. strobus* plantings of the 1920s came from Ontario in Canada. Seed of *P. monticola* was imported from interior British Columbia in 1927 and from a Washington, USA, supplier in 1941. A small amount of *P. lambertiana* seed came in 1869 from a San Francisco supplier, but the sources of other early importations are unknown. Origins of seed imported by the Forest Service between 1927 and 1930 included Butte, Eldorado, and Placer Counties in California, and, in 1947 and 1948, from the northern Sierra Nevada of California and the southern Cascades into Oregon.

In recent years the areas of plantings of five-needle pines have declined sharply, largely through widespread replacement with the Monterey pine (*P. radiata*).

Site Tolerances

Most of the five-needled pines grown in New Zealand can tolerate a wide range of local soil types. However, they prefer fertile, moderately deep and well-drained soils, with poor drainage favouring root disease. While fastest growth, particularly in early years, tends to occur on warm, mild sites, and out-of-season frosts can be damaging, the temperate species tolerate well the partly continental conditions of the central South Island. Drought, particularly on poor soils, can be associated with mortality, and tolerance of exposure tends to be limited.

Pinus monticola, P. lambertiana, P. flexilis, P. aristata, P. cembra and *P. peuce* have been planted in the Craigieburn Ranges (Lat. 43°S) in Canterbury. Although growth over early years was often slow, on the better sites below 1,000 m elevation (above sea level) they have generally been healthy, of good form and have performed consistently. At higher altitudes and on depleted soils there has been poor survival. Here it should be noted that timberline in New Zealand is low in relation to latitude.

Pinus strobus

In New Zealand *P. strobus* tolerates a fairly wide range of sites and climatic conditions including poorer dry sites but grows best on moderately fertile, deep, fresh soils. It has grown particularly well in damp, sandy, deep soils adjacent to streams in Canterbury. Reasonably good shelter is preferred and *P. strobus* is generally unsuitable for too exposed sites. It has grown successfully at elevations of 60 to 900 m in the North Island and 60 to 600 m in the South Island. Early growth is generally quite slow, requiring control of weed growth on fertile sites. It grows best in full light when young but some shade is tolerated.

Pinus monticola

Pinus monticola has been tried mostly in cooler parts of the North Island and northern South Island with annual rainfall of 760 to 2,030 mm, and appears to be the least demanding species. It is hardy and has withstood –14°C of frost. It has grown well on pumice soils in the central North Island and will also tolerate fairly poor drainage and rather sour clay soils. It has succeeded at elevations of 60 to 670 m in the North Island and 275 to 410 m in the South Island.

Pinus lambertiana

This species has been planted in the central North Island, Wellington, Canterbury, and Otago in localities with annual rainfall of 760 to 1,650 mm. Establishment has often been slow and variable. Young trees need shelter for successful establishment and on exposed sites they can be checked or killed by frosts. It failed at Hanmer (Lat. 43°S, elevation approximately 400 m) due to frost; however, older trees have withstood frosts of –14°C. *Pinus lambertiana* has succeeded on well-drained pumice soils in the central North Island where optimum growth and form occurred at 300 m elevation. In Canterbury it has grown strongly in well-drained, sandy or silty sites but cannot tolerate waterlogging, while older trees growing on dry shingly soil at Greendale in Canterbury have died, probably due to drought. It has been reported to favor alkaline soils.

Pinus wallichiana

Pinus wallichiana has been tried in Wellington, Nelson, and Canterbury within a rainfall range of 760 to 1,400 mm and has grown well on fertile, alluvial soils and light loams with adequate moisture. It is relatively wind-tolerant, but growth is poor on exposed sites, and trees in a trial at 490 m in Kaingaroa Forest were badly malformed. The species is hardy and has withstood frosts of –14°C.

Pinus ayacahuite

In most New Zealand trials of *P. ayacahuite* growth and survival have been poor. Frost damage occurred when the trees were young, and they proved susceptible to possum damage. On some sites, however, growth is excellent.

Pests and Diseases

Five-needle pines grown in New Zealand have generally been free of serious pests and diseases. The white pine blister rust fungus (*Cronartium ribicola* J.C. Fisch.) is absent. Even if it did arrive, the absence in forests of naturally occurring alternate hosts (*Ribes* spp.) should make it unimportant, although the genus *Ribes* does occur as some orchards and a few naturalised populations. Poorly drained soils tend to be associated with attack by root pathogens. Among other pathogens, the root-rot fungi, *Armillaria* spp., can affect most species, *Diplodia* sp. can kill occasional shoots, and *Dothistroma pini* can cause severe needle cast, depending on species and site. Various insect pests have been recorded on five-needle pines; they are usually unimportant, but the wood wasp *Sirex noctilio* can sometimes cause significant mortality. Browsing damage by mammals (especially deer, goats, and possums) has tended to be troublesome on almost all species, but the phenomenon of preferential attack of minority species may well have been an important contributing factor.

Pinus strobus

The root-disease fungus *Leptographium procerum* (previously *Verticicladiella procera*) has been involved in considerable mortality of *P. strobus* in New Zealand. However, it is mostly associated with sticky, poorly drained soils and sometimes with root damage associated with road and access track construction. The foliage of infected trees becomes light green before wilting and turning rusty brown, and a black stain appears in the roots and the wood at the base of the trunk. The root-rot fungi, *Armillaria* spp., have been recorded from the roots and butts of *P. strobus.* The needle-cast fungi, *Dothistroma pini* and *Cyclaneusma minus*, have both been recorded from the foliage but not as significant pathogens. Other minor disease-causing fungi recorded for *P. strobus* include *Diplodia* spp., *Hypoderma* sp., and *Lophodermium* sp.

In New Zealand various insect pests have been recorded as present but not usually troublesome on *P. strobus*. *Sirex noctilio*, with its fungal symbiont, has caused damage in thinned stands.

Pinus monticola

In New Zealand *P. monticola* has generally been healthy and there are few recorded pest and disease problems. Disease-causing fungi recorded include *Armillaria* spp. (apparently troublesome in provenance trials but not in plantations), and *Dothistroma pini, Diplodia* sp., *Leptographium* sp., *Lophodermium* sp. and *Rhizosphaera* sp., generally as minor pathogens.

Pinus lambertiana

Dothistroma needle blight can cause severe defoliation on trees of *P. lambertiana* on New Zealand sites with summer humidity. *Cylindrocladium scoparium* has caused troublesome root rot in 2-year-old nursery stock. Otherwise, fungal pathogens and insect pests are generally unimportant.

Growth and Yield

Available growth data from a selection of measurement plots are summarized for different species in tables 1 and 2. They cover a range of latitudes, although latitude is not critical in itself. Elevations varied but none were extreme. Stem volume production could be high, despite modest height growth. Data from other sources are also considered below.

Pinus strobus

In New Zealand early growth generally rates as slow, but *P. strobus* is ultimately capable of high stem volume production with mean annual increments (m.a.i.) at 35 to 40 years ranging up to over 24 m^3/ha/year, depending much on site quality. Near Rotorua (Lat. 38°S), 50-year-old unthinned stands on good sites planted at 2,224 stems/ha had mean top height (m.t.h.) of 30 m, a mean diameter at breast height (d.b.h.) of 25 cm, a volume of 1,220 m^3/ha and a m.a.i. of 24.4 m^3/ha/year (Weston 1957). Mean top height is defined as the mean height of the 100 largest diameter trees per hectare. Small piece sizes can limit recovery rates: the 39-year-old stand with a total volume of 927 m^3/ha (table 1) had a recovered volume of 795 m^3/ha. Higher productivity could be expected with optimal provenances (see later).

Pinus monticola

In New Zealand *P. monticola* has, on the whole, grown slightly faster than *P. strobus*, with observed m.a.i. ranging up to equal or greater than 30 m^3/ha/year (table 1). In the early years annual height growth is generally 0.3 to 0.6 m. At Patunamu (Lat. 39°S), a fertile, low-elevation site, mean top height of 16-year-old trees was 15.8 m and the largest tree was 32 cm in diameter at breast height. In species trials in inland Canterbury (Lat. 44°S), a few 17-year-old trees averaged 7.9 m in height. In Kaingaroa Forest (Lat $38^1/_2$°S, elevation 488 m), a 22-year-old unthinned stand carried a total volume of 108 m^3/ha (m.a.i.= 5 m^3/ha/year) in 587 stems with m.t.h. of 15 m and a mean d.b.h. of 19.6 cm.

Pinus lambertiana

In New Zealand, on favorable sites, height growth of *P. lambertiana* in early years is 0.3 to 0.6 m annually. In well-stocked stands, trees in the upper crown classes are of good form; however, the proportion of double-leadered trees tends to be high, greater than 50 percent in some areas. At low stockings, especially on fertile sites, stem taper can be severe. On a site of moderate quality in Kaingaroa Forest (Lat $38^1/_2$°S), a 26-year-old stand (containing about 40 percent of malformed stems) has produced an estimated total volume of 452 m^3/ha (m.a.i.= 17.4 m^3/ha/year) in 1268 stems/ha averaging 13 m tall and 29.5 cm d.b.h. In a small plot on a good site nearby, the best dominant trees grew to about 27.4 m in height and 63 cm d.b.h. in 48 years (Weston 1957); at age 59 the same stand had a m.t.h. of 39.6 m, and a mean d.b.h. of 38 cm with 30 percent malformed, the largest tree being 89 cm d.b.h. A stand in lowland Canterbury (Lat. 44°S) on relatively dry fluvial gravels (precipitation approximately 700 mm/annum), planted in 1931 at 400 stems/ha, with stocking 260 stems/ha remaining, had a basal area of 12.5 m^2, a mean d.b.h. of 39 cm and a mean height of 11.9 m. Height growth was uniform but the trees were rough at this low stocking. Individual trees over 60 years old have reached 35 m height and around 100 cm d.b.h.

Table 1—Growth and yield of—*Pinus strobus*, *P. monticola*, *P. ayacahuite* and *P. lambertiana* in New Zealand[a].

Species	Age (yrs)	No. of plots	Stems/ha	D.B.H. (cm)	Ht (m)	BA (m^2/ha)	Volume (m^3/ha)	MAI/yr (m^3/ha)	Latitude (°S)
Pinus strobus									
Age 20-29 yrs	22	6	350	30.2	14.6	19.8	124	5.6	40
	27	4	920	33.1	19.4	41.7	331	12.3	38
	28	1	459	48.8	20.3	46.4	316	11.2	38
	28	1	1156	36.4	20.0	79.4	557	19.9	$39^{1}/_{2}$
		mean	721	37.1	18.6		332	12.2	
Age 30-39 yrs	34	1	1416	29.2	17.8	51.9	251	7.4	46
	36	1	287	48.7	30.3	42.7	513	14.2	39
	38	1	1211	34.0	17.3	65.6	515	13.5	46
	39	1	1538	-	-	88.3	927	23.8	38
		mean	1113	37.3	21.8	62.1	551	14.7	
Age 40-49 yrs	41	1	1800	32.0	18.5	90.4	772	18.8	46
	42	1	2743	29.5	21.3	102.4	861	20.5	46
	43	1	1495	40.4	23.5	99.6	923	21.5	46
	44	1	1424	42.8	28.5	88.2	999	22.7	38
	45	3	626	44.7	28.5	56.3	638	14.2	$38^{1}/_{2}$
	46	4	644	46.0	27.2	66.2	666	13.9	$38^{1}/_{2}$
	48	1	1139	40.0	25.3	88.1	810	16.9	38
	48	1	941	45.1	25.4	78.3	833	17.3	38
		mean	1351	32.2	21.2	83.7	813	18.2	
Age 50-59 yrs)	52	2	607	46.5	27.1	63.9	673	12.9	38
	53	3	909	41.9	26.0	60.0	622	11.7	38
	53	3	946	38.1	22.1	67.4	947	17.9	43
	56	1	356	48.5	31.0	47.4	580	10.4	38
		mean	704	43.7	26.5	59.7	705	13.2	
Age 60 yrs +	62	3	784	51.8	35.1	93.3	960	15.5	$38^{1}/_{2}$
	61-65	1	593	49.5	28.3	80.1	720	11.4	46
	69	4	694	50.5	34.8	75.7	1035	15.0	$38^{1}/_{2}$
		mean	690	50.6	32.7	60.4	905	14.0	
P. monticola									
Age 30-39 yrs	34	1	949	39.5	17.7	53.6	371	10.9	38
	34	1	1480	36.3	18.5	86.6	624	18.4	$39^{1}/_{2}$
		mean	1215	37.9	18.1	70.1	497	14.6	
Age 40-49 yrs	49	1	476	49.7	33.8	63.1	1560	31.8	38
P. ayacahuite									
Age 30-39 yrs	34	1	1400	45.3	23.4	116.4	863	25.4	$39^{1}/_{2}$
	36	1	536	55.6	33.8	73.0	792	21.9	38
		mean	968	50.4	28.6	94.7	827	23.6	
P. lambertiana									
Age 30-39 yrs	36	1	536	53.4	20.5	62.5	406	19.2	38
	39	1	1183	63.2	21.2	156.4	1068	27.4	$39^{1}/_{2}$
		mean	58.3	20.8	109.4	737	23.3		

[a] Source: New Zealand Forest Research Institute Ltd permanent sample plot system.

Table 2—Summary figures (mainly from table 1) for mean annual volume increment and mean height growth, by species.

Species	Total plots	Mean annual increment—stemwood (m^3/ha/yr) (>30 years old)		Height growth (m/yr) (Range)
		Range	Mean	
P. strobus	30	7-24	15.5	0.42-0.84
P. lambertiana	2	19-27	23.3	0.54-0.57
P. monticola	4	11-32	20.4	0.52-0.99
P. wallichiana	2	-	-	0.42-0.48
P. ayacahuite	2	22-25	23.6	0.69-0.94

Pinus wallichiana

At Golden Downs Forest (Lat. $41^1/_2$°S), a 21-year-old unthinned stand with about 1,000 stems/ha developed evenly, with tree heights 9 to 12 m and average diameter about 15.2 cm. In lowland Canterbury (Lat. 44°S) trees measured at 17 years averaged 8.2 m tall, while farther inland a few trees in species trials averaged 7.6 m in height at 18 years. An exceptional specimen, in the North Island, was 38.6 m tall with a d.b.h. of 105 cm when felled.

Wood Properties and Uses

New Zealand-grown timber of *P. strobus* tends to be slightly less dense than that from its native habitat, but otherwise the properties are similar. The relatively low density (370 kg/m^3 at 12 percent moisture content), low strength, and poor nail-holding ability make *P. strobus* unsuitable for structural purposes. Indications are that end joints machine poorly. Uniform texture and dimensional stability allow certain specialist uses, however, including ice cream spatulas and similar products, and for toy making, shoe heels, picture frames, and rulers. Its good woodworking properties make it useful for panelling. Timber assessed in pilot studies was seriously degraded by the frequent small encased knots, with insufficient lengths free of defects; this largely reflects the relatively short rotations that are deemed to be economic in New Zealand.

Pinus strobus has a large percentage of heartwood that has a high moisture content, leading to drying and seasoning difficulties, requiring relatively long drying times in either the kiln or open air. Drying is further complicated by the development of an irregular brownish stain resulting from enzymes acting on the sugars in the wood. Compared with *P. radiata,* the high proportion of heartwood in *P. strobus* renders it unattractive for pulping and also makes the product difficult to season and preserve for use as posts.

Timber tests on *P. monticola* (Harris and Kripas 1959) have indicated a general similarity to imported American material, including early formation of heartwood (34 percent at 24 years) and a high moisture content in the heartwood.

Establishment and Silviculture

Five-needle pines, while often being vulnerable to exposure, often need vigorous weed control for satisfactory establishment and good early growth in New Zealand. Usually the preferred way to achieve this is by the use of chemical sprays, best practice depending very much on local conditions. Good control of browsing animals is also important.

In general, five-needle pine stands in New Zealand have tolerated high stockings, with little difference between heights of dominant and other trees, yet only moderate mortality from mutual suppression. However, in dense stands the crowns are small, leaving long lengths of stem with persistent dead branches. Response to delayed thinning appears to be rather slow.

Although the five-needle pines in New Zealand are monocyclic or 'uninodal', the knot clusters are typically too closely spaced and far too persistent to yield the relatively long' 'internodal' clear-cuttings grades that have come readily from *P. radiata*. Because of this and their low wood density, standard silvicultural regimes do not exist. Appropriate regimes, if the species are to be grown further, would be governed by some largely conflicting considerations, including the desirability of early pruning to produce clear timber; the desirability of maintaining high stockings to realize the inherent productivity; and the time required to obtain clear timber from pruning, particularly if stockings are kept high.

Stem forking has been notorious in *P. strobus* in Southland. This is assumed to be due to wind, snow, and unseasonable frosts, but it could also be attributable in part to both animal damage and genetic origin.

High initial stocking for *P. lambertiana* also allows for selection of good-form trees at mean top height 15 m or later. The unusual ability of this species to sustain a high rate of growth to advanced ages argues for long rotations that, however, would make effective growing costs high by local standards.

Provenance Variation and Genetic Improvement

Provenance testing is the only level at which within-species genetic variation has been studied. Some limited cross-referencing of species has been done within provenance trials. Tree-to-tree genetic variation is not obvious in the way that it is in *P. radiata*.

Pinus strobus

Provenance trials were planted in 1970 with 77 seedlots of *P. strobus* on three New Zealand sites: Rotoehu (Lat. 38°S) and Gwavas (Lat. $39^3/_4$°S) in the North Island, and Golden Downs (Lat. $41^1/_2$°S) in the South Island. Seed was provided by various suppliers in the United States and represented a fairly complete sample of the geographic range of the species (Lats $34^1/_2$-48°N), albeit weighted toward the southern part. (Note: One lot was recorded as having been collected from 54°N in Manitoba, outside the limit of 52°N shown by Critchfield and Little (1966), but without appearing to be an outlier for growth rate). Ten-tree row-plots were used. Heights were measured at ages 3 and 5 years from planting (Shelbourne and Thulin 1976), while diameters and incidence of malformation were assessed at Rotoehu and Gwavas at 18 years, in 1988 (Chen 1989). Six seedlots of *P. monticola* and one of *P. wallichiana* were also included.

The early heights were closely correlated (negatively) with latitude of origin, the southernmost provenances being almost twice as tall as the northerly ones (Lat. ≥45°N) (Shelbourne and Thulin 1976). This was essentially irrespective of planting site, provenance x site interaction being only minor despite marked differences among sites at that stage. In fact, the estimated genetic correlation (compare Burdon 1977) between year-18 diameter across the two sites and latitude of origin was equal to or greater than 0.9. Thus most of the best provenances were from the south of the species' range; from the southern Appalachian region, northern Georgia, western North Carolina, West Virginia, and eastern Tennessee. These trees at 18 years averaged up to 30 percent greater in diameter than the overall mean, and had higher survival and less forking. This pattern matched closely the results of Sluder and Dorman (1971) in a trial in the southern Appalachians. However, heights of provenances from the New England States were about the same as those from Virginia, Maryland, and Pennsylvania – that is, 7 to 10 percent above the overall mean for provenances from the same latitudinal range.

Consistency of provenance rankings within the three assessments suggested that early selection of *P. strobus* is possible at ages 3 to 5 years from planting, at least at the provenance level. *Pinus monticola,* and *P. wallichiana* had the poorest growth over all sites.

Some of the results remain puzzling. While the southern Appalachian provenances promised significantly greater productivity in New Zealand than Ontario material, the local seedlots from Kaingaroa Forest performed markedly better than their reported origins in Ontario would suggest, even allowing for any likely effects of both release from the neighbourhood inbreeding of natural stands and natural selection in the New Zealand environment. Thus, the reported origins from the Ontario region come into question. Generally, growth was best at two milder North Island trial sites, suggesting by itself that a higher altitude and warmer climate are more suited to this species.

The relatively poor showing of the *P. monticola* provenances included in these trials was also puzzling, in the light of stand growth data (tables 1 and 2). However, the lack of 18-year height data prevented rigorous testing of the hypothesis that the slower early growth of the *P. monticola* was a transient phenomenon.

Pinus monticola

Provenance trials were established using seven seedlots of *P. monticola*, comprising five native provenances collected by a New Zealand operator in 1956, plus one from the Institute of Forest Genetics at Placerville and another from a stand in Kaingaroa Forest. The native provenances came from the Sierra Nevada (as far south as $38^1/_2$°N), and the Southern Cascades, extending as far north as Lat. $45^1/_2$°, elevations ranging from 425 m to 2,300 m. They were planted out at three South Island sites in 1963 and four North Island sites in 1964. In addition four demonstration rows were planted out at Rotorua. The provenance from Kaingaroa Forest, derived from selected parent trees within an approved seed stand (N.Z. Forest Service), which reportedly originated in interior British Columbia, grew best among the tested origins, and was obviously well adapted. No formal assessments have been carried out, but inspection notes and observation of the demonstration planting revealed a strong pattern of inverse relationship between height and elevation of origin, within that part of the natural range. This pattern contrasts strongly with the weak elevational differentiation reported for the species in Idaho (Anon. 2000). Also surprising, in the light of provenance tests for various other species, were the indications that material originating from British Columbia was among the most vigorous samples. A suspicion arises that material from the part of the native range sampled for the provenance trials may be relatively prone to *Armillaria* root rot. *Pinus monticola* in general maintained better stem form than *P. strobus*.

Pinus lambertiana

Seven provenances were tested in trials established in 1960 to 1961 at nine New Zealand sites (two North Island, seven South Island; Lats 38-46°S). The seedlots used were collected from localities in California ranging in mean elevation from 850 m (1.6 km southeast of Koberg) to 2,000 m at Jordan Peak in Sequoia National Park.. Five of the trials remained current by 1999, showing, as expected, considerable within-population variation in tree size. Inspection notes from well-grown trials in each island showed that the relatively low elevation (760 to 1,030 m) seedlot from Lassen National Forest in northern California yielded trees with the best height and diameter growth.

In 1997 a wide provenance range of *P. lambertiana* seed was obtained from the United States. A total of 279 single-tree progenies representing eight provenance groups were sown in the research nursery at Rotorua in September 1977. Coastal Californian, and certain central Sierra lots showed vigorous early growth and good survival in the first 18 months in the nursery bed. However, other lots from the central Sierra area were among the poorest in growth and survival among this material, pointing to the necessity of further testing over time.

Pinus wallichiana

Little information is available on genetic variation in this species, and it has not been planted in provenance trials in New Zealand. One seedlot of *P. wallichiana* from a single tree was included in the provenance trials of *P. strobus* planted in 1970. At age 18 years, survival of this lot was comparable to *P. strobus* (about 70 percent), but diameter was 18 percent less than the mean of the 77 *P. strobus* lots in the trials. The only other known planting in New Zealand of *P. wallichiana* is as a forest stand of 9 ha planted in 1932 through 1938 at Golden Downs Forest (Lat $41^1/_2$°S) and in arboretum-scale plantings in inland Canterbury in the South Island (Lat. $43^1/_2$°S).

Pinus ayacahuite

Ten provenances of *P. ayacahuite* from locations in Mexico and Guatemala were planted at each of nine New Zealand locations between 1962 and 1968. Most plantings survived poorly and grew slowly, with high malformation rates mainly due to browsing by possums, and most were abandoned. Exceptions were two trials at Kaingaroa Forest and two at Gwavas Forest (Lats 38-$39^1/_2$°S). Survival at the best of these were over 90 percent and the average diameter in 1983, at age 15 years, was 20 cm. Although the incidence of multileadering was high, much of this could have been corrected by thinning. There was little provenance variation in growth.

Two plots of *P. koraiensis* were incorporated in a trial in Karioi Forest, at over 700 m elevation, in 1968, but all trees were killed by frost.

Future Roles in New Zealand

Despite often excellent growth by standards of natural ranges, ability to thrive on a range of sites, and resistance to certain diseases, five-needle pines are not foreseen as having any major role in New Zealand, because they suffer so much by comparison with the preferred species *P. radiata*, Douglas-fir, two cypresses, and various eucalypts. The required long rotations, plus some other factors that increase effective growing costs, in conjunction with limitations of timber quality and limited site tolerance all mitigate against any important role, even with use of better provenances.

Pinus monticola, despite lesser heights in some trials, emerges preferred over *P. strobus* in New Zealand because of its generally good growth and form (less stem forking) and its tolerance of difficult sites. *Pinus lambertiana,* despite its attractions as a virgin timber in its native sites and its high long-term productivity, is seen as seriously disadvantaged by a need for long rotations. *Pinus ayacahuite*, despite its growth rate, appears to be to site-demanding, apart from its timber being unproven.

A major collection of *P. strobus* remains, but the 'land races' represented in the commercial plantings have largely disappeared. However, a significant genetic collection of *P. lambertiana* has recently been established.

References

Anon. 2000. Forestry Compendium Global Module. CD Publication. CAB International, Wallingford, U.K.

Burdon, RD 1977. Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. Silvae Genetica. 26: 168-175.

Chen, Jianxin 1989. Provenance selection of *Pinus strobus* in New Zealand. Ministry of Forestry Forest Research Institute, Project Record No. 2339, 22 pp. (unpubl.).

Critchfield, WB; Little, EL 1966. Geographic distribution of the pines of the world. USDA Forest Service Miscellaneous Publication No. 991, v + 97 pp.

Harris JM, Kripas S 1959. Notes on the physical properties of ponderosa pine, monticola pine western red cedar, and Lawson cypress grown in New Zealand. NZ Forest Service, FRI Research Note No. 16, 24 pp.

Shelbourne, CJA; Thulin, IJ 1976. Provenance variation in *Pinus strobus*; heights five years after planting in New Zealand. New Zealand Forest Service, FRI, Genetics & Tree Improvement Internal Report No. 100, 23 pp. (unpubl.).

Sluder, ER 1963. A white pine provenance study in the Southern Appalachians. USDA Forest Service Southeastern Forest Experiment Station Research Paper SE-2, 16 pp.

Weston, GC. 1957. Exotic Forest Trees of New Zealand. New Zealand Forest Service, FRI Bulletin No. 13, 103 pp.

Genetic and Environmentally Related Variation in Needle Morphology of Blister Rust Resistant and Nonresistant *Pinus monticola*

Kwan-Soo Woo
Lauren Fins
Geral I. McDonald

Abstract—This paper compares the results of two studies (differences related to genotype versus differences related to growing environment) that have been reported in previous publications (Woo and others 2001; Woo and others 2002) and highlight information that may be useful to tree breeders in refining rust resistance evaluation procedures. The objectives of these studies were to assess genetic and environmentally related variation in needle surface traits in rust–resistant western white pine (*Pinus monticola* Dougl.). Statistically significant differences were found in 14 needle traits (needle length and width, number of stomatal rows, number of stomata per row, total stomata per needle, adaxial surface area, stomatal density, major axes of stomata, stomatal shape, stomatal area, stomatal occlusion, epistomatal wax degradation, weight of wax per dry weight of needle, and the contact angles of water droplets) of western white pine seedlings grown from the same seed orchard source in three nurseries in northern Idaho. Waxes on needle surfaces were tubular in structure and the amount of surface wax appeared to be associated with surface wettability. In a separate study, stomata on needles from susceptible families were found to be significantly wider and larger than those from genetically resistant families and from genetically improved bulked lots from the seed orchard. Neither the percent of stomatal occlusion nor the amounts of degraded epistomatal wax were statistically different among the seed sources. Contact angles of water droplets on needles of the resistant families were significantly larger than those of the susceptible families and the seed orchard lots. Results of both the genetic study and the nursery study should be more broadly tested to determine their generality and applicability to refining rust screening procedures. Information from this study may be useful in refining protocols for selecting genotypes for tree improvement programs and/or for quantifying levels of rust resistance in selectively bred western white pine stocks. Rust screening protocols may be made more efficient if any of the traits prove to be reliable indicators of resistance.

Key words: Epistomatal wax, *Pinus monticola* Dougl., wettability, stomatal occlusion, blister rust, needle morphology, rust resistance

Introduction

Routine tests of western white pine (*Pinus monticola* Dougl.) for blister rust resistance rely on successful inoculation of seedlings with spores of the fungus *Cronartium ribicola* J. C. Fisch. in Rabenh., and subsequent ocular evaluations for rust symptoms and expression of the resistance mechanisms. Presumably, phenotypic variation that has a genetic basis will play a critical role in infection success; for example, a specific resistance type may prevent entry of germinating rust spores into needle tissue. However, phenotypic variation caused by a particular nursery regime that temporarily prevents or diminishes successful inoculation of seedlings may hamper attempts to identify genetically resistant stocks for tree improvement programs or attempts to quantify realized levels of resistance in selectively bred stock. The studies reported here were designed to assess and quantify phenotypic variation in a variety of needle surface traits that may be associated with genetic differences in resistant versus susceptible genotypes and which may also be affected by differences associated with nursery growing regimes and or their environments. Results of the individual studies (differences related to genotype versus differences related to growing environment) have been reported in previous publications (Woo and others 2001; Woo and others 2002). The purpose of this paper is to compare the results of the studies and highlight information that may be useful to tree breeders in refining rust resistance evaluation procedures.

Background

White pine blister rust, a devastating disease caused by the fungus *Cronartium ribicola* J. C. Fisch. in Rabenh., was introduced into western North America in 1910 on eastern

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Kwan-Soo Woo is with the Department of Forest Genetic Resources, KFRI, Korea Forest Service, Suwon, Kyonggido 441-350, South Korea. Telephone: (031) 290-1106, fax: (031) 292-4458, e-mail: woo9431@yahoo.co.kr. Lauren Fins is with the Department of Forest Resources, University of Idaho, Moscow, ID 83844-1133, U.S.A. Telephone: (208) 885-7920, fax: (208) 885-6226, e-mail: lfins@uidaho.edu. Geral I. McDonald is with the USDA Forest Service, Rocky Mountain Research Station, 1221 South Main Street, Moscow, ID 83843, U.S.A. Telephone: (208) 883-2343, fax: (208) 883-2318, e-mail: gimcdonald@fs.fed.us.

white pine seedlings imported from Ussy, France, to Point Grey, near Vancouver, British Columbia. The rust appears to have reached Idaho about 1923 (Mielke 1943). Blister rust is largely responsible for the drastic reduction in white pine cover type (greater than 90 percent) in the inland northwestern United States (eastern Washington, northeastern Oregon, northern Idaho, and northwestern Montana) since 1923 (Fins and others 2001). A breeding program designed to increase white pine's resistance to blister rust was initiated in the late 1940s as part of an effort to restore white pine to inland Northwestern ecosystems (Bingham 1983). The program produces genetically improved stocks that outperform unimproved (susceptible) stocks in operational plantings and field tests throughout the region (Fins and others 2002).

Rust Screening

As a key component of the ongoing breeding program, white pine seedlings are routinely evaluated for rust resistance after inoculation with *C. ribicola* basidiospores. Although most seedlings subjected to the screening process are grown at the USDA Forest Service Coeur d'Alene (Idaho) nursery, occasionally seedlings grown elsewhere have been included in the rust inoculations. At these times, differences in infection levels, mortality, variation in needle thickness, and the amount of water that collects on needle surfaces were observed between seedlings grown in different nurseries, suggesting that inoculation success may vary not only with the genetic backgrounds of the seedlings, but also with the nursery in which the seedlings are grown.

The relative number of needle spots that appear on white pine seedlings after exposure to blister rust has been shown to be under genetic control (Hoff and McDonald 1980; Meagher and Hunt 1996) and it appears to be a type of "rate reducing resistance" (Rossi and others 1999) that reduces infection efficiency by 10 times (Hoff and McDonald 1980).

At least two blister rust resistance types, "no spot" and "reduced needle lesion frequency," are recognized by the lack of or low numbers of needle spots that appear on needles after exposure to blister rust spores. "No spot," which occurs in high frequencies in Eurasian white pine species and occasionally in North American white pine species, is rare in western white pine, but the "reduced needle frequency" type of resistance can be found in higher frequencies (Hoff and others 1980). Although the exact mechanisms by which seedlings avoid or reduce infection are not known, it is known that *C. ribicola* germ tubes enter needles through their stomata (Patton and Johnson 1970), indicating that stomatal features are likely to be important to the infection process. Whether or how specific variations in stomatal features of western white pine needles are critical to infectability by blister rust is also not known.

Surface Wettability

"Wettability" refers to the tendency of a surface to retain water. Water beads up and remains on nonwettable surfaces longer than on wettable surfaces. Because water droplets take different shapes on wettable versus nonwettable surfaces, the angles they make with the surfaces on which they rest can be used as a measure of surface "wettability" (see Woo and others 2002 for images). A higher contact angle (produced by a more globular, rounder bead) indicates lower wettability of the surface (Cape 1983; Leyton and Juniper 1963). This phenomenon may be important in blister rust infection because water collection on needle surfaces is essential for fungal infections (Huttunen 1984), and the amount and distribution of free moisture have been reported to be the most important factors for germination of *C. ribicola* basidiospores and subsequent infection of eastern white pine needles (Spaulding and Rathbun-Gravatt 1926; Hansen and Patton 1977).

Study Objectives

The objectives of these studies were to describe and compare differences in needle surface traits, including surface wettability, stomatal size, frequency and distribution, and other needle traits that may be influenced by differences in nursery environment and/or genetic constitution. Our hypothesis was that both nursery environment and genetic differences affect needle surface traits that are related to infectability by *C. ribicola*.

Materials and Methods

Nursery Study

Seeds of western white pine were sown in 1997 and grown for 2 years (1997 and 1998) at three nurseries in northern Idaho, USA: the USDA Forest Service nursery in Coeur d'Alene, Potlatch Corporation's nursery in Lewiston, and the University of Idaho Forest Research nursery in Moscow. All seedlings used for the comparisons between nurseries originated from F_2 open pollinated seeds collected from the R.T. Bingham White Pine Seed Orchard in Moscow, ID. This stock has been reported as approximately 66 percent resistant to blister rust at 2.5 years after inoculation (Hoff and others 1973).

Morphometric traits were measured on three needles per seedling collected from each of 30 seedlings per nursery, one needle from each of three directions on the current stem of each seedling. Stomatal size (major and minor axes) and area were assessed on additional collections of one needle from each of two fascicles per seedling from 25 seedlings per nursery. Details on field, laboratory and statistical methods can be found in Woo (2000) and Woo and others (2002).

Genetics Study

For the genetics study, needle samples were collected from western white pine seedlings grown at the USDA Forest Service nursery in Coeur d'Alene as part of a routine rust screening operation of 271 entries that included open-pollinated families from phenotypic selections in natural stands, woods-run check lots, and open-pollinated bulk F_2 seed orchard lots from the R.T. Bingham White Pine Seed Orchard in Moscow. The seeds were sown in 164 cm^3 single super cells in spring 1993 and remained in a greenhouse for two growing seasons. They were inoculated in August 1994 by suspending blister rust-infected *Ribes* leaves over them in an inoculation chamber at 100 percent relative humidity.

In May 1995, the seedlings were transplanted to five outdoor nursery beds where they remained for the next 3 years (Mahalovich and Eramian 1995). Four families with low spot frequency (3062, 3653, 4437, and 4922), four families with high spot frequency (3162, 3233, 4110, and 4778), and two open-pollinated F_2 resistant bulked lots from the R.T. Bingham White Pine Seed Orchard (lots 4815 and 4816) were selected for this study (mean spot frequencies: 0.065, 3.32, and 0.20) (Woo and others 2001). One family in the "resistant" group (4437) also had 73 percent zero spot individuals 1 year after inoculation. Of the two seed orchard lots, lot 4815 was a general seed orchard collection, and lot 4816 was collected from parent trees that had been selected for the "short shoot" type of resistance (needle lesions appear after inoculation but rust infection is stopped between the needle and the branch), but seedlings from both lots exhibit a variety of rust resistance mechanisms (Rust 1998). Needle samples consisted of one needle from the current stem from each of three fascicles from each of 21 seedlings per source (seven seedlings per source per replication). Details on field, laboratory and statistical methods used can be found in Woo (2000) and Woo and others (2001).

Results

Nursery Study

Significant differences were found among the three nurseries (l=5.67; P<0.0001) and among seedlings within nurseries (l=4.17; P<0.0001) for the eight measured needle characteristics and three of the four stomatal measurements; that is, major axes, stomatal shape, and mean stomatal area (P<0.001) (table 1). Minor axes of stomata (stomatal width) were nearly identical among the three nurseries (P=0.94).

Relatively larger deposits of waxes were commonly distributed along the stomatal rows and over epistomatal chambers. Stomata with severely degraded epistomatal waxes were found side by side with stomata whose waxes were completely intact. Percent of occluded stomata was similar on needles from the Coeur d'Alene (86 percent) and Moscow nurseries (90 percent), and both had significantly more occluded stomata than needles from the Lewiston nursery (56 percent, P=0.0001). Needles from the Lewiston nursery produced more wax per dry weight of needle than those from either the Coeur d'Alene or the Moscow nursery, but those from the Moscow nursery had statistically higher levels of degraded wax than either of the other two (table 1).

Mean contact angles of water droplets on the needle surfaces differed significantly among the three nurseries and were highest on needles from the Lewiston nursery, both with and without the presence of surface waxes (table 1). Needles from the Moscow nursery had the smallest contact angles.

Genetics Study

In the genetics study, we found significant differences among the 10 seed sources (P≤0.10) for nearly all of the traits (Woo and others 2002). When grouped by resistance type (resistant, susceptible, and Moscow Seed Orchard), most of the comparisons were no longer statistically significant. Several exceptions stood out, however, including needles from the four resistant families were significantly shorter than those from the seed orchard lots (P=0.015), and the stomata of the susceptible families were significantly wider and greater in area than stomata on the seed orchard lots and the resistant families. The stomata of the susceptible families were also "rounder" in shape (smallest ratio of

Table 1—Means and standard errors of western white pine needle traits (from Woo and others 2002).

		Nursery			
Variables	No. of needles	Coeur d'Alene (mean ± SE)	Lewiston (mean ± SE)	Moscow (mean ± SE)	P-value
Needle length (mm)	270	54.3 ± 0.78	65.7 ± 1.08	81.2 ± 1.12	0.0001
Needle width 1 (mm)	270	0.8 ± 0.006	0.7 ± 0.008	0.9 ± 0.01	0.0001
Needle width 2 (mm)	270	0.8 ± 0.006	0.7 ± 0.008	0.9 ± 0.01	0.0001
Stomatal rows/plot	270	2.4 ± 0.05	3.3 ± 0.07	3.6 ± 0.07	0.0001
Stomata/row	270	13.1 ± 0.11	13.5 ± 0.13	13.8 ± 0.15	0.0228
Stomata/needle	270	3449 ± 76.9	5811 ± 158.7	8048 ± 246.7	0.0001
Adaxial surface area (mm^2)	270	82.8 ± 1.46	93.2 ± 1.92	146.2 ± 3.58	0.0001
Stomatal density	270	41.8 ± 0.63	62.2 ± 0.97	55.0 ± 0.92	0.0001
Major stomatal axes (mm)	150	59.5 ± 0.61	54.8 ± 0.55	57.9 ± 0.66	0.0001
Minor stomatal axes (mm)	150	33.2 ± 0.27	33.1 ± 0.31	33.0 ± 0.28	0.9369
Stomatal shape:					
Major axes/Minor axes	150	1.80 ± 0.03	1.66 ± 0.02	1.76 ± 0.02	0.0001
Mean stomatal area (mm^2)	150	1549 ± 19.41	1426 ± 24.41	1502 ± 23.73	0.001
Mean wax degradation	150	2.46 ± 0.06	2.58 ± 0.06	3.27 ± 0.05	0.0003
Wax per dry weight (mg/mg)	75	6.26	8.48	4.38	0.0002
Wax per surface area (mg/mm^2)	75	9.48	7.76	6.07	0.0719
Contact angles with wax	270	90.8 ± 0.79	105.4 ± 0.74	62.9 ± 1.68	0.0001
Contact angles, no wax	30	94.7 ± 1.31	101.0 ± 0.93	55.3 ± 2.3	0.0051

major to minor axes) than stomata on the seed orchard lots, and contact angle of water droplets on needles surfaces was significantly larger on resistant families than on susceptible families and Moscow Seed Orchard lots.

Discussion

Nursery Study

Growth Environment and Nursery Regimes—The differences we found in needle traits of the same seed source grown in three northern Idaho nurseries likely reflect differences in nursery growth regimes, temperature levels, container size, and/or sowing dates. Some of these nongenetic differences may be associated with variation in blister rust infection levels. For example, differences among nursery samples in stomatal traits are potential candidates as predictors of differences in initial infection levels because the blister rust fungus enters white pine needles through their stomata (Patton and Johnson 1970). We did not test this hypothesis in our current study. However, incidental evidence suggests a link between nursery growth regimes and rust infection following artificial inoculation, as on several occasions, attempts to inoculate seedlings grown in the Lewiston nursery had resulted in low infection levels compared to seedlings grown in Coeur d'Alene and inoculated at the same time.

Different growth regimes in each nursery (amounts and timing of water, temperature, light, growth medium and fertilization) likely contribute to differences in needle morphology and possible differences in infectability by rust infection. Fertilization, for example, may generally increase seedling growth and vigor but also increases susceptibility of southern pines to fusiform rust (Schmidt and others 1972; Blair and Cowling 1974; Rowan and Steinbeck 1977). Another potential association links differences in infection to a "functional resistance" associated with stomatal behavior (Hart 1929; Hirt 1938). If, for example, wider and larger stomata close slowly or incompletely compared to small narrow stomata, fungal germ tubes may more easily invade the larger ones.

Surface Waxes—Other, less obvious, surface traits may be implicated in differences in infection. For example, the high proportion of degraded waxes on seedlings from the Moscow nursery, which may be related to the use of surfactants and other treatments, such as the use of an acid rinse following fertilization, may be associated with rust infection. This relationship is suggested by a study showing that fungal hyphae penetrated Norway spruce needles more easily when surface or epistomatal waxes were degraded compared to when the wax structures were well-preserved (Huttunen 1984; Elstner and others 1985).

Stomatal Occlusion—Previous researchers have suggested a possible link between reduced blister rust infection and occlusion of the stomatal antechamber on needles of *Pinus strobus* L. (Patton and Johnson 1970; Patton and Spear 1980). However, seedlings from the Coeur d'Alene nursery (86 percent occluded) have historically been more easily infected than those from the Lewiston nursery (56 percent occluded). If previous infection patterns hold true, it would suggest that factors other than stomatal occlusion may be relevant to infectability, but this relationship was not tested in the current study.

Needle Wettability—The distribution and amount of water on a needle is important for basidiospore germination (Spaulding and Rathbun-Gravatt 1926; Hansen and Patton 1977). Thus, the wettability of a needle will likely affect infectability because "nonwettable" surfaces hold more water and for a longer time than "wettable" surfaces (Leyton and Juniper 1963; Cape 1983; Haines and others 1985). In routine rust screenings in 1989 and 1992, water tended to bead up more and remain longer on seedlings from the Coeur d'Alene nursery compared to seedlings from the Lewiston nursery; they also contracted higher infections (Eramian, personal communication). In our study, however, the contact angles on needles from the Lewiston nursery were significantly higher than those from the Coeur d'Alene nursery suggesting either that nursery regimes have changed since the mid 1990s or that is the contact angle of water droplets is not informative with regard to predicting levels of infection with blister rust fungus. We did not, however, test these hypotheses in this study.

We hypothesized that the amount of wax on the needle surface would be associated with needle wettability. Surface waxes on needles of Scots pine are more hydrophobic than the cuticle itself (Cape 1983), but the amount of wax is not critical to the hydrophobic properties of a leaf surface (Silva Fernandes 1965; Holloway 1969a and 1969b). In our study, the amount of extracted surface wax, expressed over needle dry weight, varied among nurseries and did appear to be associated with differences in needle wettability.

Genetics Study

Perhaps the most noticeable (and potentially the most important) finding came from the genetics study, in which the susceptible families had larger and rounder stomata and smaller contact angles of surface water droplets than either or both the resistant families and the seed orchard bulk lots.

Gansel (1956) investigated seven white pine needle traits based on the hypothesis of direct penetration of *C. ribicola* germ tubes through the epidermis but found no differences in the traits between four uninfected (but untested) and four susceptible individual trees growing in natural stands. Our results are generally consistent with Gansel's findings for needle traits other than stomatal size.

Stomatal Traits—Stomatal width and area may be important and potentially definitive traits that distinguish trees with "reduced needle lesion frequency" from those that are genetically susceptible. It has been suggested that the reduced needle lesion frequency may be related to occlusion of stomata by wax, which was hypothesized to reduce the chance of infection of *Pinus strobus* L. by *C. ribicola* (Patton and Johnson 1970; Patton and Spear 1980). However, we found no evidence that resistant families had different proportions of stomatal occlusion compared to susceptible families. Most of the observed stomatal chambers were occluded in all sampled families and lots.

Surface Waxes—At the time we sampled the seedlings, which was 3 years after inoculation, we found no differences among families and bulked lots in the proportions of degraded epistomatal wax on needles. It is possible that there were differences among families in the amounts of degraded wax and/or the proportions of occluded stomata at the time the seedlings were inoculated, possibly due to differences in wax chemistry or different melting temperatures. Alternatively, environmental factors may play a greater role than genetic differences in epistomatal wax degradation and stomatal occlusion (Cape 1983).

Needle Wettability—Previous research has indicated that the most important factor in basidiospore germination and needle infection of *Pinus strobus* L. with *C. ribicola* is the distribution and amount of free moisture on the needle surface during incubation (Spaulding and Rathbun-Gravatt 1926; Hansen and Patton 1977). However, our finding of larger contact angles of the water droplets on needles of the resistant families compared to both the susceptible families and the R.T. Bingham Seed Orchard bulked lots, whose contact angles were similar to each other, suggests relatively more moisture was retained on needles of the resistant families. Unless wettability changed differentially among the families from the time of inoculation to the time they were sampled, infectability does not appear to be a simple function of needle wettability. We note too that trees in the R.T. Bingham Seed Orchard were selected for resistance mechanisms other than low needle lesion frequency (although many of the trees exhibit this resistance trait as well) (Bingham 1983). Thus, if there were a relationship between needle wettability and specific resistance mechanisms, it would not be surprising to find differences in wettability between the Moscow bulks and the resistant families.

Summary and Conclusions

Most of the needle traits examined in these studies exhibited significant phenotypic plasticity, varying across samples of the same genetic stock grown in three nurseries. But only a few of the traits varied genetically (that is, across stocks of different rust resistance types grown in a single nursery). Although not tested in this study, our results suggest that variation in stomatal traits and/or the characteristics of surface water on pine needles may be critical features in the dynamics of blister rust infection. If some nursery regimes produce seedlings with needle surface characteristics similar to those of resistant genotypes, such seedlings may exhibit lower initial infection rates when planted under field conditions, even in “high rust hazard” areas. Nonetheless, such an effect would likely be short-lived as the needles produced in the nursery senesced and new ones were produced under field conditions. Furthermore, seedlings produced under these nursery regimes would be unsuitable for use in rust screening procedures, as initial infection is required for detection of most of the rust resistance traits.

Whether the variations in needle surface properties we observed are associated with differences in infectability by white pine blister rust has yet to be determined. Differences in infectability are not likely to be associated with stomatal occlusion, which did not vary among nurseries or between resistant and susceptible families. Larger wax deposits were associated with higher contact angles and lower wettability on western white pine needle surfaces. This apparent relationship may reflect a broader or more even distribution of wax as the quantities increased. Factors such as chemical composition of the waxes or surface roughness may play an important role in surface wettability and may be related to fungal spore attachment and germination. The physiological condition of seedlings may also be related to infectability because *C. ribicola* absorbs nutrients from its hosts.

Further exploration of nursery environments and growth regimes for effects on the morphology of seedling needles is warranted. Additional studies are needed to explore the relationships between the chemical and physical nature of needle surfaces (particularly at the time of inoculation) and their infectability by the blister rust fungus. Finally, the initial finding of differences in stomatal size and shape between resistant and susceptible families should be more fully explored and verified using a larger number of families.

References

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: The story of the first 25 years of the research and development program. General Technical Report INT-146, USDA For. Serv. Intermountain. For. and Range Exp. Sta., Ogden, Utah. 45 p.

Blair, R.L., and Cowling, E.B. 1974. Effect of fertilization, site, and vertical position on the susceptibility of loblolly pine seedlings to fusiform rust. Phytopathology 64:761-762.

Cape, J.N. 1983. Contact angles of water droplets on needles of Scots pine (*Pinus sylvestris*) growing in polluted atmospheres. New Phytol. 93:293-299.

Elstner, E.F., Osswald, W., and Youngman, R.J. 1985. Basic mechanisms of pigment bleaching and loss of structural resistance in spruce (*Picea abies*) needles: Advances in phytomedical diagnostics. Experientia 41:591-596.

Eramian, A. Personal communication. 1997. USDA Forest Service, Coeur d’Alene nursery, Coeur d’Alene, Idaho. U.S.A.

Fins, L., Byler, J., Ferguson, D., Harvey, A., Mahalovich, M.F., McDonald, G., Miller, D., Schwandt, J. and Zack, A. 2001. Return of the Giants. Idaho Forest, Wildlife, and Range Experiment Station Bulletin 72, University of Idaho, College of Natural Resources, Moscow, ID. 20p.

Fins, L., Byler, J., Ferguson, D., Harvey, A., Mahalovich, M.F., McDonald, G., Miller, D., Schwandt, J. and Zack, A. 2002. Return of the Giants: Restoring Western White Pine to the Inland Northwest. J.For. 100(4):20-26.

Gansel, C.R. 1956. A comparison of some morphological features of needles from blister rust- resistant and non-resistant western white pine. M.S. Thesis, University of Idaho, 24 p.

Haines, B.L., Jernstedt, J.A., and Neufeld, H.S. 1985. Direct foliar effects of simulated acid rain. II. Leaf surface characteristics. New Phytologist 99:407-416.

Hansen, E.M., and Patton, R.F. 1977. Factors important in artificial inoculation of *Pinus strobus* with *Cronartium ribicola*. Phytopathology 67:1108-1112.

Hart, H. 1929. Relation of stomatal behavior to stem-rust resistance in wheat. J. Agr. Res. 39:929-948.

Hirt, R.R. 1938. Relation of stomata to infection of *Pinus strobus* by *Cronartium ribicola*. Phytopathology 28:180-190.

Hoff, R.J., Bingham, R.T., and McDonald, G.I. 1980. Relative blister rust resistance of white pines. Eur. J. For. Path. 10:307-316.

Hoff, R.J., and McDonald, G.I. 1980. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. Can. J. Bot. 58:574-577.

Hoff, R.J., McDonald, G.I., and Bingham, R.T. 1973. Resistance to *Cronartium ribicola* in *Pinus monticola*: structure and gain of resistance in the second generation. USDA For. Serv. Res. Note INT-178, Intermountain Forest and Range Experiment Station, Ogden, Utah. 8 p.

Holloway, P.J. 1969a. The effect of superficial wax on leaf wettability. Annals of Applied Biology 63: 145-153.

Holloway, P.J. 1969b. Chemistry of leaf waxes in relation to wetting. J. of the Science of Food and Agriculture 20:124-128.

Huttunen, S. 1984. Interactions of disease and other stress factors with atmospheric pollution. p. 321-356. *In* Treshow, M. (ed.). Air pollution and plant life. John Wiley and Sons, New York.

Kirk, R.E. 1995. Experimental design: procedures for the behavioral sciences. 3rd ed. Brooks/Cole Publishing Company, Pacific Grove, California. 921 p.

Leyton, L., and Juniper, B.E. 1963. Cuticle structure and water relations of pine needles. Nature 198:770-771.

Mahalovich, M.F., and Eramian, A. 1995. Breeding and seed orchard plan for the development of blister rust resistant white pine for the Northern Rockies. Draft tree improvement plan. USDA Forest Service Northern Region. 59 p.

Meagher, M.D. and Hunt, R.S. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. Silvae Genetica 45(2-3):75-81.

Mielke, J.L. 1943. White pine blister rust in western North America. Yale Univ., Sch. For. Bull. 52, 155 p.

Patton, R.F., and Johnson, D.W. 1970. Mode of penetration of needles of eastern white pine by *Cronartium ribicola*. Phytopathology 60:977-982.

Patton, R.F., and Spear, R.N. 1980. Stomatal influences on white pine blister rust infection. Phytopathologia Mediterranea 19:1-7.

Rossi, V., Giosue, S., and Racca, P. 1999. A model integrating components of rate-reducing resistance to Cercospora leaf spot in sugar beet. J. Phytopathology 147:339-346.

Rowan, S.J., and Steinbeck, K. 1977. Seedling age and fertilization affect susceptibility of loblolly pine to fusiform rust. Phytopathology 67: 242-246.

Rust, M. (1998). Performance of Moscow Seed Orchard lots in artificial inoculation trials. Twenty-second Progress Report of the Inland Empire Tree Improvement Cooperative. University of Idaho, Moscow, Idaho 83843. 130p.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6.4th ed. SAS Institute Inc., Cary, N.C.

Schmidt, R.A., Foxe, M.J., Hollis, C.A., and Smith, W.H. 1972. Effect of N, P, and K on the incidence of fusiform rust galls on greenhouse-grown seedlings of slash pine. Phytopathology 62: 788 (Abstr.).

Silva Fernandes, A.M.S., 1965. Studies on plant cuticle. VIII. Surface waxes in relation to *water*-repellency. Ann. Appl. Biol. 56: 297-304.

Spaulding, P.C., and Rathbun-Gravatt, A. 1926. The influence of physical factors on the viability of sporidia of *Cronartium ribicola* Fischer. J. Agric. Res. 33:397-433.

Trimbacher, C., and Eckmullner, O. 1997. A method for quantifying changes in the epicuticular wax structure of Norway spruce needles. Eur. J. For. Path. 27:83-93.

Woo, K-S. 2000. Variation in needle morphology of blister rust susceptible and resistant western white pine. Ph.D. dissertation, University of Idaho, Moscow. 161 p.

Woo, K-S., Fins, L., McDonald, G.I, Wenny, D.L. and Eramian, A. (2002). Effects of nursery environment on needles morphology of *Pinus monticola* Dougl. and implications for tree improvement programs. New Forests 24:113-129.

Woo, K-S., Fins, L., McDonald, G.I, and Wiese, M.V. 2001. Differences in needle morphology between blister rust resistant and susceptible western white pine stocks. Can. J. For. Res. 31:1880-1886.

Eight-Year Growth and Survival of a Western White Pine Evaluation Plantation in the Southwestern Oregon Cascades

Andrew D. Bower
Richard A. Sniezko

Abstract—An evaluation plantation of western white pine (*Pinus monticola*) was established in southwestern Oregon in 1991 using 3-year-old seedlings. The planting was comprised of 98 full-sib families from Dorena seed orchards and 42 wind-pollinated families from parents in natural stands from a wide range in elevation, latitude, and longitude. All parent trees had previously been selected for above average resistance to white pine blister rust (caused by *Cronartium ribicola*) in seedling testing at Dorena. Growth, survival, and level of blister rust infection were assessed in 1993 at age 5 and in 1998 at age 10. Overall survival in 1993 and 1998 was 72.1 and 66.7 percent, respectively, and 7.5 percent of the trees were infected with blister rust by 1998. Significant differences were found between families within each seed type (both seed orchard and natural stand seed collections) for mean height increment and survival percentage, but not for rust infection. Within a subset of families from the Rogue River National Forest, significant differences were found between seed types for both height increment and survival percentage, and differences in infection percentage were nearly significant. Families from this forest originating from orchard seed were found have larger height increment (93.03 vs. 70.71 cm), higher survival (73.8 vs. 61.4 percent), and lower infection (6.0 vs. 9.6 percent) than trees from wild seed. In a stepwise regression, height increment of trees from orchard seed was only associated with seed weight, but trees from wild seed sources local to the planting site (when northerly and easterly sources were excluded) were only associated with latitude of the female parent. Large differences in height among families provide good potential for future selection in growth. Despite very slow initial growth, the plantation is progressing on a trajectory that suggests that we have successfully regenerated the site. Recent site visits indicate little additional mortality, low rust infection and increasing annual growth as well as some cone production on this site which was problematic for previous Douglas-fir (*Pseudotsuga menziesii*) reforestation efforts.

Key words: western white pine, *Pinus monticola*, white pine blister rust, *Cronartium ribicola*, height growth, survival, geographic location

Introduction

Western white pine (*Pinus monticola* Dougl.) was once a much larger component of forests in the Pacific Northwest than it is today. Historically and ecologically, western white pine has been considered important in the Pacific Northwest, yet information on its status is limited (Goheen 2000). In areas of Idaho and the Inland Empire, the white pine cover type spanned an area of five million acres (Fins and others 2001). However, due to logging, white pine blister rust (caused by *Cronartium ribicola* J.C. Fisch. in Rabenh.), mountain pine beetle (*Dendroctonus ponderosae* Hopk.) attack, and the exclusion of fire, it now is a minor component throughout its range with only 5 to 10 percent of the original five million acres of white pine cover type in the inland northwest still carrying a significant component of white pine (Fins and others 2001). A comparison of surveys done in 1957 and the mid-1990s in southwest Oregon showed a drop from 60 to 40 percent in the number of plots with 5-needle pines, and a drop in pine cover of five percent in 10 years from the 1980s into the 1990s (Goheen 2000).

Many areas where western white pine dominated historically are now populated by stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.), grand fir (*Abies grandis* (Dougl.) Lindl.), and western hemlock (*Tsuga heterophylla* (Rafn.) Sarg.). Western white pine is more suitable for wetter, root disease-prone sites (Harrington and Wingfield, 1998) because it is less susceptible to laminated root rot (*Phellinus weirii*), Annosus root rot (*Heterobasion annosum*) (Hadfield and others 1986), and insect attacks than Douglas-fir and grand fir (Fins and others 2001). Western white pine is also more tolerant to drought than western hemlock (Fins and others 2001) and is among the most tolerant conifers to frost (Burns and Honkala 1990). Despite these adaptive advantages, western white pine has been largely ignored by managers in Oregon and Washington as a species to plant in burned and harvested areas because of white pine blister rust. Despite the availability of resistant stock, western white pine has not achieved its reforestation potential or been used in restoration plantings, even on lands where historically it was present.

White pine blister rust has caused widespread mortality, but even in areas of heavy infection, a low percentage of trees remained disease free (Bingham and others 1953). The presence of naturally resistant stock formed the basis for an operational breeding program to produce genetically resistant western white pine that began in the USDA Forest Service Region 6 (Oregon and Washington) in the late 1950s, and has been based at the Dorena Genetic Resource Center

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the USDA Forest Service, Dorena Tree Improvement Center, 34963 Shoreview Road, Cottage Grove, Oregon, 97424, USA. Andrew Bower's current address is Forest Sciences Department, University of British Columbia, Vancouver, B.C. V6T 1Z4 CANADA, Phone: (604) 822-1951, Fax: (604) 822-9102, Email: adbower@interchange.ubc.ca.

(Dorena) since 1966 (Sniezko 1996, Sniezko and others these proceedings).

Screening for rust resistance with artificial inoculation has revealed several different resistant mechanisms in western white pine (Struckmeyer and Riker 1951, Hoff 1984, 1986, Hoff and McDonald 1971, 1980, McDonald and Hoff 1970, 1971, McDonald 1979; Kinloch and others 1999). Field plantings have also shown differences in rust infection among families (Sniezko and others 2000, Sniezko and others, this proceedings), and breeding programs have resulted in offspring with increased resistance to white pine blister rust. The ultimate goal of these breeding programs is to produce stocks for reforestation that will be able to survive despite the presence of white pine blister rust.

The site described in this paper was planted with the purpose of assessing growth as well as rust resistance for families from a range of geographic origins in Oregon and Washington (fig. 1). Little information is available on family variation in growth and survival of western white pine in southwestern Oregon. Most of the seedlots included in this field planting represent the part of southwestern Oregon in which the planting occurs. The additional seedlots from throughout Oregon and Washington will provide basic information on adaptability of the species.

This report has two objectives: to present our results regarding the magnitude and potential sources of genetic variation in growth, survival, and blister rust infection in a field planting, and to use these results to illustrate that despite high rust hazard in many localities, there are areas in which western white pine is a suitable choice for reforestation, especially where other species may be unsuccessful.

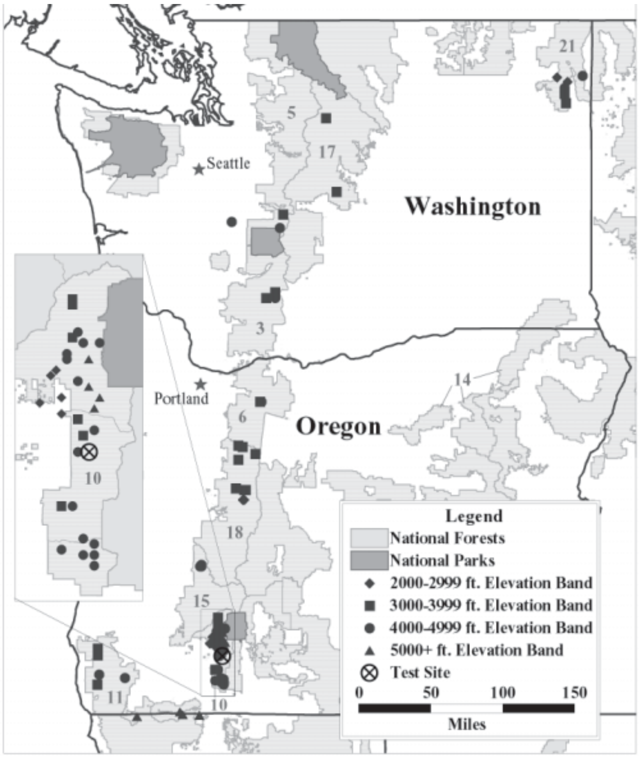


Figure 1—Map of parent test site and parent tree locations.

Materials and Methods

Study Site Description

A 10-acre site in southwestern Oregon that had been logged in the early 1980s was chosen for the evaluation planting. The site is located on the Butte Falls Ranger District on the Rogue River National Forest at an elevation of approximately 1230 m (4035 ft) (fig. 1). The site is located in a white fir (*Abies concolor* (Gord. & Glend.) Lindl.)/Shasta red fir (*Abies magnifica* A. Murr.) mixed conifer plant association with an ENE aspect and gentle slope (10 percent). The soil is a sandy clay grading from relatively deep on the south and east to very rocky on the north half of the unit. The surrounding overstory is composed of Douglas-fir, white fir, englemann spruce (*Picea englemannii* Parry), Shasta red fir, and western white pine. Understory regeneration includes golden chinquapin (*Chrysolepsis chrysophylla* (Dougl.) A. DC.), pacific yew (*Taxus brevifolia* Nutt.), white fir, Shasta red fir, Douglas-fir, englemann spruce, western white pine, and some ponderosa pine (*Pinus ponderosa* Laws), with *Ribes* (the alternate host of *C. ribicola* in N. America) present in the shrub population. Although the site had been cleared for several years, there was little grass or brush growing before the site was planted with white pine in 1991. The location of the site and the surrounding canopies of mature trees make the site susceptible to frost. This is illustrated by the nearly complete mortality of the original Douglas-fir evaluation planting in the mid-1980s due to frost (Jim Hamlin pers. comm.). The local plant associations along with the environmental conditions made this site a good candidate for regeneration with western white pine.

Seed from 140 western white pine families were sown in containers at Dorena in spring of 1988. The population consisted of 42 wind-pollinated families from phenotypically rust-resistant trees selected in natural stands (“wild”) from ten national forests (table 1), and 98 controlled cross families of clonal grafts growing in the Dorena orchards. All trees in the Dorena orchards had previously been screened for blister rust resistance at Dorena. Resistant individuals within families were then selected for inclusion in the orchards for future seed production of resistant stock. Selection was based entirely on the presence of one or more resistance mechanisms, without regard to growth. The 42 ‘wild’ parents had also been selected as above average for rust resistance using artificial inoculation of progeny. Most seedlots (126) represented the southwestern portion of Oregon in which the planting site is located, but a small number (14) of seedlots representing a wider range of Oregon and Washington were also used. In all, 106 different female parents were used in at least one (but up to four) different crosses, and 66 male parents were used in usually one (but up to five) crosses. Forty-four trees were used as both male and female parents. Seedlings were transplanted from containers to 100 cm x 115 cm boxes with two families per box and 45-50 trees per family after the first growing season. They were grown outdoors for an additional two-and-one-

Table 1—Number of parent trees by source and elevation range (in meters).

Map #[a]	Source	Seed type	No. of females	Elevation mean	Elevation range
3	Gifford Pinchot NF	Orchard	1	1325	
5	Mt. Baker-Snoqualmie NF	Orchard	2	1250	1140-1355
6	Mt. Hood NF	Orchard	1	1200	
10	Rogue River NF	Orchard	23	1150	800-1463
		Wild	26	1450	900-1900
11	Siskiyou NF	Wild	6	1150	985-1290
14	Umatilla NF[b]	Wild	2		n/a
15	Umpqua NF	Orchard	34	1390	1310-1480
17	Wenatchee NF	Wild	2	990	930-1050
18	Willamette NF	Orchard	32	1075	645-1385
21	Colville NF	Wild	6	1060	800-1415
	Roseburg BLM	Orchard	3		n/a
	Eugene BLM	Orchard	2		n/a

[a] Refers to national forest number as shown in figure 1.
[b] Latitude and longitude data not available.

half years. In February of 1991 seedlings were lifted and stored near 0° C. until planting. Seedlings were planted in April and May of 1991 at 3 m x 3 m spacing in a randomized complete block design with seven replications (reps). Families were represented by four-tree or three-tree (when seedlings were limited) row plots. Weight per 100 seed (in grams) was available for 111 of 140 families.

The trees were assessed in 1993 for survival (S5), height growth (HT5) and percentage of trees with blister rust infection (RUST5) after two years in the field when trees were five years old from seed. A more comprehensive assessment was done in 1998 at age 10, with growth measurements including survival (S10), incidence of rust infection (RUST10) and tree height (HT10), and status/health measurements including the height, number, and type of blister rust cankers, canker activity (active or inactive), damage and severity of damage. To help remove differences in early height growth that may have been influenced by differences in seed weight, height increment from 1993 to 1998 was used to test for differences in growth. Only results on height increment from age 5 to age 10 (HTINCR), survival, and infection are presented here.

Statistical Analysis

The GLM, REG and CORR procedures of the SAS system were used for all statistical analyses (SAS Institute 1989). For height increment, individual tree data were used in an analysis of variance (ANOVA) to test for differences between reps and families for each seed type (orchard origin and wild stand origin) separately with the following model:

$$Y_{ijk} = \mu + R_i + F_j + RF_{ij} + e_{ijk}$$

Where μ is the overall mean, R_i is the effect of the ith replication, F_j is the effect of the jth family, RF_{ij} is the interaction of rep by family, and e_{ijk} is the error term. The rep and family effects were both tested using the rep-by-family interaction as the error term.

Plot means (using family rep means) were used in a separate ANOVA to test for differences in survival and rust infection using a similar model.

Due to the differences in distribution between orchard and wild families from different forests (see Figures 4 and 5, and Table 1), we could not test the significance of the differences in height growth, survival, and infection between seed types using data from all families. The Rogue River National Forest was the only seed origin location that included both orchard and wild seed types (table 1), so this subset of families was used to test for differences between the two seed types. For testing height increment, an additional ANOVA was performed using the following model:

$$Y_{ijk} = \mu + R_i + S_j + RS_{ij} + F(S)_{jk} + R(F(S))_{ijk} + e_{ijk}$$

Where μ is the overall mean, R_i is the effect of the ith replication, S_j is the effect of the jth seed type (orchard vs. wild stand origin), RS_{ij} is the interaction of rep by seed type, $F(S)_{jk}$ is the effect of the kth family within the jth seed type, $R(F(S))_{ijk}$ is the interaction of rep by family within seed type, and e_{ijk} is the error term, with appropriate error terms used as needed to test each effect. Seed type was tested with a composite error term as determined by SAS that was approximately ($RS_{ij} + F(S)_{jk} - R(F(S))_{ijk} - e_{ijk}$). For survival and infection percentage, the same model was used but without the rep x family within seed type interaction and the family within seed type term used as the error for testing the source main effect.

Pearson correlations were calculated using family means for five-year height increment (from 1993 to 1998) with seed weight, as well as between height increment, survival, infection percentages and seed weight with latitude, longitude, and elevation of both the female and male parent (when available). These correlations were used to examine the relationship of source location of the mother tree with seed weight, and the relationship of height growth with both mother tree location and seed weight for all orchard and wild seedlots. Infection and survival percentages were calculated on a family mean basis by rep, then averaged for each family. All trees killed by something other than blister rust were excluded when calculating infection percentage.

Families from the Colville and Wenatchee National Forests are from the eastern side of the Cascade Range, and the Colville families are also geographically disjunct from the other families in this test which are on the western side of the Cascades (see fig. 1). Geographic and environmental differences of the source locations of these families may strongly influence the correlations of height growth with seed weight, latitude, longitude, and elevation. Therefore, these correlations were recalculated excluding these families.

To better understand the relationship between height and seed weight and parental location, regression was used twice; once for all families and once excluding the families from the Colville and Wenatchee National Forests. Variables with moderate correlations were included in stepwise simple regressions with an alpha = 0.05 level used for inclusion and retention in the model. PROC REG (SAS 1989) with height increment for both seed types as the independent variable and latitude, longitude, and elevation of the female parent, and seed weight as the independent variables was used in the regression analyses.

Results and Discussion

Overall Infection and Survival

Mean survival over all families in 1998 (S10) was 66.7 percent (a slight decrease from 72.1 percent in 1993) with a range in family means from 28.6 to 96.4 percent (fig. 2).

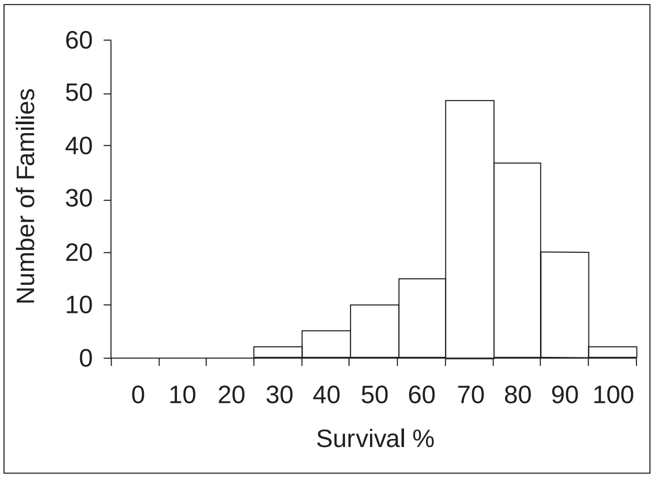


Figure 2—Distribution of survival percent in 1998 for 140 families.

Survival by seed type (orchard (S10o) and wild (S10w)) in 1998 was 69.5 and 60.2 percent, respectively, down from 1993 when it was 74.9 and 65.7 percent (table 2). Most of this early mortality appears to be due to gophers (Marc Ellis, pers. comm.) rather than blister rust. Survival at this site is within the range of western white pine survival reported elsewhere. (Parent 1998 and 1999, Bower 1987, Harrington and others 2003, Steinhoff 1981)

Significant differences ($p < 0.01$) were found between reps and families for orchard seed (S10o) and between families only for wild seed (S10w) for survival percentage. Rep mean survival percentage for orchard seed ranged from 61.7 to 73.5 percent with a general trend of the reps lower on the slope having lower survival than the higher reps. For wild seed, mean rep survival percentage ranged from 56.0 to 69.0 percent with a similar but weaker trend. The mean infection percentage for both orchard and wild seed was approximately 7.5 percent, with a range in family means from 0 to 31.25 percent. Significant differences in infection percentage were found only between reps for orchard seed. Neither reps nor families were significantly different for wild seed. Although reps were different for orchard seed, there was no clear pattern among reps that indicated that infection was higher or lower at a specific position on the slope. The lack of differentiation for infection is most likely due to the low level of infection present on this site.

Overall Height Growth

Mean height in 1993 (two years after planting and five years from seed) and in 1998 for both seed types is presented in table 2. When all families are included, overall mean height increment (HTINCR) was 87.2 cm, with a mean for the orchard families (HTINCRo) of 92.2 cm and a mean for the wild families (HTINCRw) of 75.7 cm. Height increment ranged from 72.6 cm in rep 1 to 106.9 cm in rep 7 for orchard seed and from 65.5 cm in rep 1 to 90.7 cm in rep 7 for wild seed. Height increment was significantly different ($p<0.01$) for rep, family, and the rep x family interaction for both orchard and wild seed. Clear differences in height growth were visible between reps with height increment increasing from bottom to top of the slope. This would be expected due to the pooling of cold air lower on the slope. Tree heights at the time of planting are unavailable, so height increment

Table 2—Mean survival percent, height growth, and seed weight by seed type.

Variable	Orchard Seed		Wild Seed	
	n	mean	n	mean
Survival 1993	98	74.9% (1.12) [b]	42	65.7% (2.18)[b]
Survival 1998	98	69.5% (1.17)	42	60.2% (2.28)
Height 1993 (cm)	98	36.78 (0.53)	42	30.42 (0.86
Height 1998 (cm)	98	128.93 (1.88)	42	106.65 (3.07)
Height Increment	98	92.2 (1.48)	42	75.73 (2.45)
Seed Weight[a]	70	2.376 (0.046)	41	2.13 (0.075)

[a] Seed weight in grams, weights were not available for all seed lots.
[b] Standard errors are in brackets

during the first two years in the field cannot be compared with height increment during the next five years. Although growth has been slow, site visits in summer of 2001 showed that growth had begun to accelerate, and many trees appeared to be at the age where rapid growth was commencing. Early height growth of western white pine is relatively slow compared with other white pine species until about age 10 to 15 when it begins to accelerate rapidly. This onset of rapid growth is usually later in natural stands than in plantations, but growth in both can continue at 30 – 90 cm per year for more than 100 years (Bingham and others 1972). The range among families in height increment was 50.27 to 138.25 cm which supports previously reported results showing considerable genetic variation in height growth of this species. Significant differences between family mean height have been reported in several studies for both controlled-cross and open-pollinated western white pines <15-years-old (Rehfeldt and Steinhoff 1970, Steinhoff 1979, Bower and Yeh 1988). These reports agree with earlier results that showed that although western white pine is highly variable, most of the variation is related to individual trees within a family or stand (Rehfeldt and Steinhoff 1970, Hanover and Barnes 1969). The range in height growth that was observed on this site indicates a good potential for selection opportunities in the future for growth, in addition to the rust resistance for which these families were originally selected.

Orchard vs. Wild Seed Source Type Comparison

Height Growth and Survival—Using only the subset of families from the Rogue River National Forest, significant differences were detected between orchard and wild seed for both height increment (p = 0.0002) and survival percentage (p = 0.0017). The orchard seedlots had greater height increment (93.0 vs. 71.6 cm) and higher survival (73.8 vs. 61.4 percent) than the wild seedlots. The differences between orchard and wild seedlots could be the result of a number of factors, including differences in the geographic ranges (even within the Rogue River N.F.) of the parents of these families (see Figures 4 and 5). Sites in southwestern Oregon are very diverse, covering a wide range of elevations, rainfall, and soil types, all of which most likely differ from the more stable environmental conditions experienced by the families from the orchard. In addition, orchard seed are generally harvested at the point of optimum ripeness before the cone scales begin to flare. Cones collected from wild stands may have been harvested at a point when they were accessible and available but may not have achieved the same ripeness. Potential differences in seed maturity may also contribute to the differences between orchard and wild seed (Jerry Berdeen, pers. comm.)

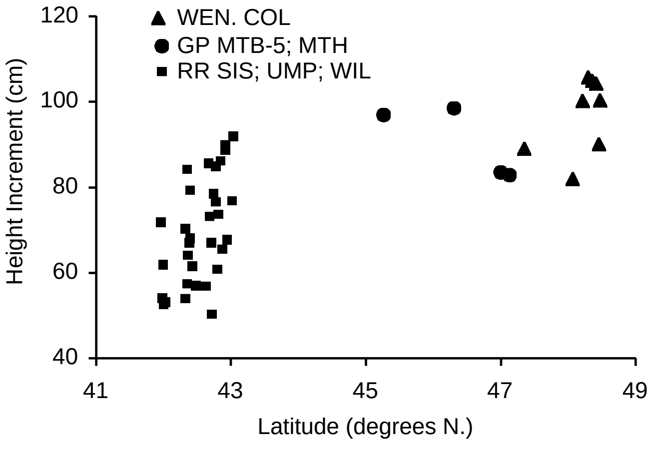


Figure 3—Scatterplot of latitude vs. family mean height increment by forest* for 44 families from wild seed.
*see table 1 for abbreviations

Infection—Using the same subset of families from the Rogue River National Forest, differences between seed types in infection percentage approached significance (p = 0.0854) with the wild seed having a higher level of infection than the orchard seed (15.9 vs. 9.3 percent). The seedlots from the orchard would be expected to show higher rust resistance since both the male and female contribution to the seed would be from resistant parents.

Seed Source Location Effects

Height growth—Previous reports of ecotypic variation in western white pine have been inconsistent. Squillace and Bingham (1958) reported that progeny from high elevation parents were shorter at four years than progeny from lower elevation parents within the same watershed when grown together at a low elevation site, but they were taller when grown on a high elevation site. Evidence of local differentiation at this small scale has not been substantiated by other research (Rehfeldt 1979); however, differentiation has been found on a larger scale. Steinhoff (1979) found that seedlings from higher elevation parents grew slower than seedlings from lower elevation parents at low- and mid-elevation sites, but he did not find them to be taller at high elevation sites. Townsend and others (1972) found no evidence of racial differentiation in monoterpenes, photosynthesis, or growth for 4-year-old seedlings, despite marked differences in elevation and geographic separation of certain sources.

In contrast, other studies have shown patterns of differentiation among western white pine populations. Results from these studies indicate that differences in height growth, isozymes, and cold hardiness have separated the western white pine range into a relatively small southern population (restricted to the Sierra Nevada mountains in California) and a broad northern population (covering the northern part of the species distribution, including the Washington Cascades), with a transition zone in the Southern Cascades and Warner Mountains in Oregon, but with no differentiation related to the elevation of the seed source (Steinhoff and others 1983, Rehfeldt and others 1984, and Meagher and Hunt 1998). The northern populations are characterized by relatively high growth potential and low cold hardiness, while the southern populations have lower growth potential and higher hardiness, with the transition zone population from southern Oregon (Cascades and Warner Mountains) arranged along a steep latitudinal gradient linking the two populations. In a western white pine provenance test in western Washington, the more southerly high elevation

Table 3—Correlation of height growth with seed weight for 111 families.

	Seed Weight			
	Overall	**Orchard Seed (n = 70)**	**Wild Seed (n = 41)**	**Wild Seed* (n = 33)**
Height 1993	–0.07	–0.13	–0.37[b]	–0.14
Height 1998	–0.17[a]	–0.26[b]	–0.53[c]	–0.34[a]
Height Increment	–0.19[b]	–0.28[b]	–0.51[c]	–0.33[a]

* Correlation excluding Colville and Wenatchee families
[a] significant at 10% level
[b] significant at 5% level
[c] significant at 1% level

sources were dramatically shorter than both northern and inland sources (Richard Sniezko, pers. comm.). Rehfeldt and others (1984) reported that an apparent relationship between seedling height and elevation for populations from this transition zone is derived from the strong correlation of latitude and elevation. Campbell and Sugano (1989) found similar trends across populations, as well as for families from within the transition zone, which they attributed to steep precipitation gradients. They also found, as others have, that most of the variation in western white pine is among individuals within a population, with only small amounts occurring between populations. Similarly, when our data is separated by seed type and forest of origin, for the four groups with more than six female parents represented (table 1), we found that families were significantly different in all cases.

Our correlations of growth with latitude and elevation reflect a similar relationship across populations, especially for the wild seed (table 4), even when the more northerly and easterly populations are excluded (table 5), (so that the remaining families fall within the “transition zone” described above). A plot of seed source elevation vs. latitude

Table 4—Family mean correlations of survival, growth and seed weight with location, by parent and seed source type for 133 Families*.

	Orchard Seed (n=93*)			Wild Seed (n=40*)		
	Lat.	**Long.**	**Elev.**	**Lat.**	**Long.**	**Elev.**
Survival % – F**	–0.06	0.06	–0.06	0.29	0.40[b]	0.07
Height Incr. – F	0.08	0.22[a]	–0.11	0.71[b]	0.76[b]	–0.41
Height Incr. – M**	0.13	0.23[a]	–0.06	N/a	n/a	n/a
1993 Height – F	0.23[a]	0.40[b]	–0.14	0.62[b]	0.64[b]	–0.48[b]
1993 Height – M	0.27[b]	0.36[b]	–0.10	N/a	n/a	n/a
1998 Height – F	0.13	0.28[b]	–0.12	0.75[b]	0.79[b]	–0.46[b]
1998 Height – M	0.18	0.28[b]	–0.07	N/a	n/a	n/a
Seed Weight – F	–0.18	–0.24	–0.12	–0.53[b]	–0.49[b]	0.26
Seed Weight – M	–0.11	–0.15	–0.14	N/a	n/a	n/a

* Latitude, longitude, elevation, and seed weight not available for all seedlots
** F = correlation with female location, M = correlation with male location
[a] significant at 5% level
[b] significant at 1% level

Table 5—Family mean correlations of survival, infection, growth and seed weight with maternal location for wild seed, excluding Wenatchee NF and Colville NF.

	Wild Seed		
	Lat.	**Long.**	**Elev.**
Survival %	0.25	0.55[b]	0.28
Infection %	–0.08	0.13	0.07
Height Increment	0.25	0.40[a]	–0.19
Seed Weight	–0.06	0.01	0.01

[a] significant at 5% level
[b] significant at 1% level

(fig. 4) shows a significant negative relationship for wild seed (r = –0.49, p< 0.01) indicating that the northern sources in this study are generally from lower elevations than southern sources. With the Colville and Wenatchee National Forest sources excluded, the relationship is weaker and still nearly statistically significant (r = –0.35, p = 0.051). The plot also shows that when the more northerly sources are excluded, the remaining families come from a range of latitudes that is much more limited than the orchard seed, and these two groups should not be viewed as paired samples since they represent different parts of the ranges with only partial overlap.

An examination of a plot of the origin of the seedlots (fig. 5) shows that orchard and wild parents cover different ranges in geographic location. There are strong relationships between latitude and longitude for both orchard families (r = 0.79, p<0.01) and wild families (r = 0.92, p<0.01). When the northerly families are excluded, the correlation for the wild families drops dramatically (r = 0.39), although it is still significant (p = 0.02). It is likely that the significant correlations between growth traits and longitude and elevation actually can be explained by differences associated with latitude, and our results suggest that height growth may be associated with latitude, at least within families from the Cascade Range in Oregon and Washington.

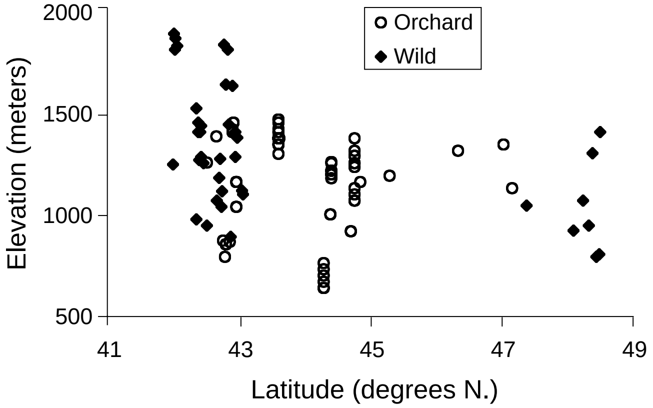


Figure 4—Latitude vs. elevation of female parents by seed type.

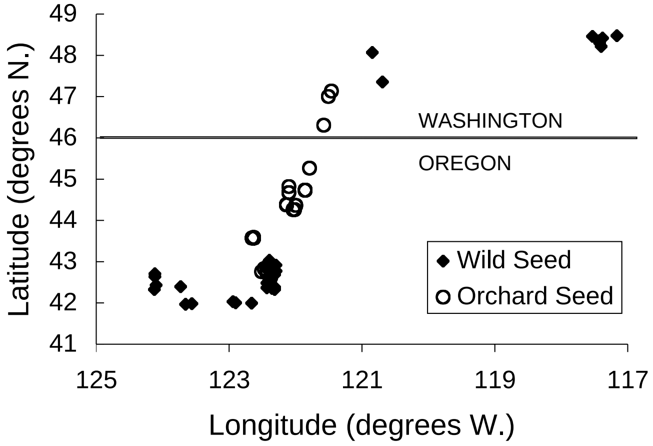


Figure 5—Latitude vs. longitude of female parents of orchard and wild seed families.

Seed Weight Effects—Previous studies have reported that total height for western white pine in the first several years of seedling growth is positively correlated with seed weight (Squillace and Bingham 1958, Squillace and others 1967). Squillace and Bingham (1958) found that seed weight had a variable effect on parent-progeny growth correlations. In our study, HT10o and HTINCRo both had significant but low negative correlations with seed weight (r = –0.26 and –0.28; p = 0.033 and 0.020, respectively, Table 3). The correlation for HT5o was also negative, but weaker and non-significant (r = –0.13; p = 0.273). All three height growth variables for the wild seed had significant negative correlations with seed weight with moderate r-values (r-values ranged from –0.37 to –0.53).

However, using all families for each seed type, seed weight was also negatively correlated with latitude and longitude of the female parent, significantly so for the wild seed. In a stepwise regression of family mean height increment on seed weight, latitude, longitude, and elevation of the female parent for orchard seed, only seed weight was significant in the model (p = 0.023), while for the wild seed, only longitude was significant (p<0.01) in the model. Latitude and longitude of the wild seed are highly correlated (r = 0.922), and the Colville families are more disjunct in longitude than latitude compared to the other wild families (see Forest 21 on fig. 1). When the regression was redone excluding the families from the Wenatchee and Colville National Forests, only latitude was significant in the model (p< 0.01). Although seed weight is correlated with height growth, the correlation is probably a function of maternal origin which is reflected by latitude of the mother tree. Heavier (larger) seed would be expected to produce larger seedlings, therefore the correlations of seed weight and height increment should be positive, as has been reported previously (Squillace and Bingham 1958, Squillace and others 1967). However, the correlation we observed was negative. This would indicate that wild seed from higher latitudes is smaller but the seedlings grow faster. Therefore, we feel that it is likely that the difference in height growth between orchard families and wild families is not due to differences in seed weight, but due to genetic effects associated with the source of origin of the parents.

Conclusions

Despite its desirable growth and adaptive advantages, white pine is underutilized for reforestation or restoration in Oregon and Washington due to potential losses from white pine blister rust and the limited availability of resistant seedlings. Our results from a young (10-year-old) western white pine plantation on a site with low rust-hazard (but high frost-hazard) show moderate to high survival among families, with significant differences between families, and low rust infection through age 10. Even though rust infection levels were low, orchard seedlots showed lower infection than wind-pollinated wild seedlots from resistant parents, as expected. Although growth has initially been slow, observations in summer 2001 by the authors indicate that many trees appear to be entering the rapid phase of growth typical of western white pine. We found strong family differences in height growth that appear to be associated with seed weight and latitude of the parent trees, and also found differences between progeny from orchard and wild trees from similar geographic origins. Families from parents originating from the more northerly latitudes show higher average growth potential than families from more southerly latitudes, although the best families from southerly latitudes are comparable. These findings correspond with other results in the literature, which distinguish three zones for western white pine: (1) A broad zone north from northern Oregon; (2) A southern zone in California; (3) A distinct transition zone in the southern Oregon cascades. Current plans are to follow this planting over time to see if the growth differences that are apparent among sources from different latitudes will remain and/or increase as the trees age during the period of rapid growth, and to see if sources originating furthest from the planting site remain vigorous. In addition, the early results on this site show that with careful selection of appropriate sites and utilization of resistant planting stock, western white pine can be a viable choice for use in reforestation or restoration.

Acknowledgments

The authors would like to acknowledge the work of several past and present employees of the Dorena Genetic Resource Center: Rob Mangold and Jude Danielson for selecting and growing the seedlings, Bob Danchok and Sally Long for taking the measurements on the trees, and all of the Dorena personnel for their assistance in planning, preparation, and planting of the site. Thanks also to Jeremy Kaufman for construction of the map, Angelia Kegley for her careful review of an early draft of this paper and Randy Johnson for his review and suggestions on this manuscript.

References

Bingham, R. T., Squillace, A. E. and Duffield, J. W. 1953. Breeding Blister-Rust-Resistant Western White Pine. J. For. 51: 163-168.

Bingham, R. T., Hoff, R. J., and Steinhoff, R. J. 1972. Genetics of Western White Pine. USDA Forest Serv. Res. Pap. WO-12, 18p. (revised 1974)

Bishaw, B., DeBell, D. S., and Harrington, C. A. 2003. Patterns of Survival, Damage, and Growth for Western White Pine in a 16-year-old Spacing Trial in Western Washington. West. J. Appl. For. 18(1): 35-43.

Bower, R. C. 1987. Early Comparison of an Idaho and a Coastal Source of Western White Pine on Vancouver Island. West. J. Appl. For. 2(1): 20-21.

Bower, R. C. and Yeh, F. C. 1988. Heritability and Gain Calculations for Sic-Year Height of Coastal Western White Pine in British Columbia. Can. J. Plant Sci. 68: 1191-1196.

Burns, R. M., and Honkala, B. H., tech. cords. 1990. Silvics of North America: 1. Conifers. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. vol. 1, 675 p.

Campbell, R. K. and Sugano, A. I. 1989. Seed Zones and Breeding Zones fore White Pine In the Cascade Range of Washington and Oregon. Res. Paper. PNW-RP-407. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 20 p.

Fins, L., Byler, J., Ferguson, D., Harvey, A., Mahalovich, M. F., McDonald, G., Miller, D., Schwandt, J., and Zack, A. 2001. Return of the Giants; Restoring White Pine Ecosystems by Breeding and Aggressive Planting of Blister Rust-Resistant White Pine. University of Idaho, Station Bulletin 72. 20 p.

Goheen, E. 2000. Impact Assessments and Restoration Strategies for Five-Needle Pines in Southwest Oregon. Proceedings of the 48th Western International Forest Disease Work Conference. August 2000. pp. 19-22.

Hadfield, J. S., Goheen, D. J., Filip, G. M., Schmitt, C. L., and Harvey, R. D. 1986. Root Diseases in Oregon and Washington Conifers. R6-FPM-250-86. Portland, OR: U. S. Department of Agriculture, Forest Service, Pacific Northwest Region Forest Pest Management. 27 p.

Hanover, J. W. and Barnes, B. V. 1969. Heritability of Height Growth in Western White Pine Seedlings. Silvae Genet. 18: 80-82.

Harrington, T. C. and Wingfield, M. J. 1998. Diseases and the Ecology of Indigenous and Exotic Pines. In Richardson, D. M. (ed.), Ecology and Biogeography of Pinus. Cambridge University Press. Cambridge, United Kingdom. 527 p.

Hoff, R. J. 1984. Resistance to *Cronartium ribicola* in *Pinus monticola*: Higher Survival of Infected Trees. Res. Note. INT-RN-343. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 6 p.

Hoff, R. J. 1986. Inheritance of the Bark Reaction Resistance Mechanism in *Pinus monticola* Infected by *Cronartium ribicola*. Res. Note. INT-RN-361. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 8 p.

Hoff, R. J. and McDonald, G. I. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: Short shoot fungicidal reaction. Can. J. Bot. 49: 1235-1239.

Hoff, R. J. and McDonald, G. I. 1980. Resistance to *Cronartium ribicola* in *Pinus monticola*: Reduced needle-spot frequency. Can. J. Bot. 58: 574-577.

Kinloch, B. B., Jr., Sniezko, R. A., Barnes, G. D., and Greathouse, T. E. 1999. A Major Gene for Resistance to White Pine Blister Rust in Western White Pine from the Western Cascade Range. Phytopathology 89: 861-867.

McDonald, G. I. 1979. Resistance of Western White Pine to Blister Rust: A Foundation for Integrated Control. Res. Note. INT-RN-252. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 5 p.

McDonald, G. I. and Hoff, R. J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: Early shedding of infected needles. Res. Note. INT-RN-124. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 8 p.

McDonald, G. I. and Hoff, R. J. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: Genetic control of needle-spots-only resistance factors. Can. J. Forest Res. 1, 197-202.

Meagher, M. D. and Hunt, R. S. 1998. Early Height Growth of Western White Pine Provenances in British Columbia Plantations. West. J. Appl. For. 13(2): 47-53.

Parent, D. R. 1998. Western White Pine Tree Improvement Committee 1998 Progress Report. In: Fins, L. and Rust, M. (eds.), Inland Empire Tree Improvement Cooperative, Twenty-second Progress Report. University of Idaho, Moscow, Idaho. 130 p.

Parent, D. R. 1999. Western White Pine Tree Improvement Committee 1999 Progress Report. In: Fins, L. and Rust, M. (eds.), Inland Empire Tree Improvement Cooperative, Twenty-second Progress Report. University of Idaho, Moscow, Idaho. 110 p.

Rehfeldt, G. E. 1979. Ecotypic Differentiation in Populations of *Pinus monticola* in North Idaho – Myth or Reality? M. Nat. 114: 627-636.

Rehfeldt, G. E. and Steinhoff, R. J. 1970. Height Growth in Western White Pine Progenies. Res. Note. INT-RN-123. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 4 p.

Rehfeldt, G. E., Hoff, R. J. and Steinhoff, R. J. 1984. Geographic Patterns of Genetic Variation in *Pinus monticola*. Bot. Gaz. 145(2): 229-239.

SAS Institute Inc. 1989. SAS/STAT® User's Guide, Version6, Fourth Edition, Volume 2, Cary, NC: SAS Institute, Inc., 846 p.

Sniezko, R. A. 1996. Developing Resistance to White Pine Blister Rust in Sugar Pine in Oregon. In: Kinloch Bohun. B., Jr., Melissa Marosy, and May E. Huddleston (eds.) Sugar Pine: Status, Values, and Roles in Ecosystems. Proceedings of a Symposium presented by the California Sugar Pine Management Committee. University of California, Division of Agriculture and Natural Resources, Davis, California. Publication 3362.

Sniezko, R. A., Bower, A. D., and Danielson, J. 2000. A comparison of Early Field Results of White Pine Blister Rust Resistance in Sugar Pine and Western White Pine. Horttechnology Vol. 10 No. 3:519-522. (Proceedings of Ribes – Pines – White Pine Blister Rust Conference, September 1999, Corvallis, Oregon)

Squillace, A. E. and Bingham, R. T. 1954. Breeding for Improved Growth Rate and Timber Quality in Western White Pine. J. For. 52: 656-661.

Squillace, A. E. and Bingham, R. T. 1958. Localized Ecotypic Variation in Western White Pine. For. Sci. 4(1): 20-34.

Squillace, A. E., Bingham, R. T., Namkoong, G, and Robinson, H. F. 1967. Heritability of Juvenile Growth Rate and Expected Gain from Selection in Western White Pine. Silvae Genet. 16(1): 1-6.

Steinhoff, R. J. 1979. Variation in Early Growth of Western White Pine in North Idaho. Res. Pap. INT-RP-222. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 22 p.

Steinhoff, R. J. 1981. Survival and Height Growth of Coastal and Interior Western White Pine Saplings in North Idaho. Res. Note. INT-RN-303. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 3 p.

Steinhoff, R. J., Joyce, D. E. and Fins, L. 1983. Isozyme Variation in *Pinus monticola*. Can. J. For. Res. 13: 1122-1132.

Struckmeyer, B. E. and Riker, A. J. 1951. Wound-Periderm Formation in White-Pine Trees Resistant to Blister Rust. Phytopathology 41: 276-281.

Townsend, A. M., Hanover, J. W. and Barnes, B. V. 1972. Altitudinal Variation in Photosynthesis, Growth, and Monoterpene Composition of Western White Pine (*Pinus monticola* Dougl.) Seedlings. Silvae Genet. 21, 3-4: 133-139.

Development of an In Vitro Technology for White Pine Blister Rust Resistance

Danilo D. Fernando
John N. Owens

Abstract—In spite of the progress made towards isolating blister rust resistant white pines, there is still a threat from the evolution of new pathogenic strains of blister rust in North America and/or introduction of new virulent strains from Asia. Through interspecific hybridization with the most resistant Eurasian white pines, resistance genes may be passed on to North American white pines, and the gene pool for rust resistance in North America diversified. An earlier approach using wide crosses with Eurasian white pines was abandoned because of failure to obtain viable seeds. We believe that wide hybridizations are possible through the removal of the nucellus which is a probable site of incompatibility reactions. This study aims to develop a novel approach to hybridization through in vitro fertilization (IVF). Our work involves co-culture of *Pinus aristata* female gametophytes with *P. monticola* pollen tubes (and vice versa). Female gametophytes were isolated and introduced to pollen tubes grown in culture for 2 to 3 days. Pollen tubes and female gametophytes were then co-cultured for 6 to 10 days. Histological analysis showed that in both types of interspecific crosses, pollen tubes did not only penetrate the female gametophytes through the neck cells of the archegonia, but also release their contents into the egg cytoplasm. The inability to maintain viability of female gametophytes in culture presently precludes successful IVF. Our results on the in vitro interspecific cross between *P. aristata* and *P. strobus* showed the same interaction as the reciprocal cross between *P. aristata* and *P. monticola*.

Key words: in vitro fertilization, *Pinus aristata*, *P. monticola*, *P. strobus*, pollen tube, female gametophyte, interspecific hybridization

Introduction

Blister rust (*Cronartium ribicola*) is one of the most destructive forest pathogens, and it affects all native North American white pines. Infection caused by this fungus results in the formation of large blister-like cankers on branches and the main stem leading to stunted growth and eventually death of trees. This disease has resulted in the significant loss of white pine timber values, but according to Kinloch (2000), the ecological damage may even be worse. Since the unwanted introduction of the fungus to North America in the early 1900s, white pine breeders have been concerned with isolating resistant trees through selection, screening and intraspecific breeding. As a result, blister rust resistant stocks are available (Kinloch and others 1970, Bingham 1983, Kinloch 1992, Blada 1994, Kinloch and others 1999). However, as new pathogenic strains develop and/or new races of wider virulence are reintroduced from Asia (Kinloch and Comstock 1981, MacDonald and others 1984, Kinloch and others 1996, Kinloch and Dupper 1999, Kinloch 2000), the rust problem remains a constant threat. Therefore, it is necessary to develop new strategies that can be incorporated into the current breeding programs to serve as insurance against new or different pathogenic races of blister rust. The need to widen the spectrum of rust resistance is imperative and one such strategy is in vitro fertilization (IVF) coupled with interspecific hybridization and/or genetic transformation (Fernando and others 1998).

The present "resistant" selection process that is underway in North America may not impart long-term resistance. Widening the spectrum of resistance in North American white pines entails interspecific hybridizations with the most resistant Eurasian white pines (Spaulding 1929, Bingham 1972). Interspecific hybridization may not only impart resistance genes, but may also diversify the gene pool for rust resistance. Of the species ranked by Bingham (1972), *Pinus armandii* is considered the most resistant, followed by *P. cembra* and *P. aristata*. These species constitute a repository of resistance genes that seem advisable to exploit in white pine breeding programs. In fact, hybrids have been formed between *P. armandii* and *P. lambertiana* (Stone and Duffield 1950, Heimburger 1972), and *P. cembra* and two of the most susceptible but economically important white pines, *P. monticola* and *P. strobus* (Blada 1994). Unfortunately, *P. armandii* or *P. aristata* crossed with *P. monticola* or *P. strobus* were all unsuccessful (Wright 1959, Patton 1964, Bingham 1972, Bingham 1983). The cross between *P. armandii* and *P. monticola* did not even produce any cone (Wright 1959), and while cones were produced between *P. armandii* and *P. strobus*, no filled seeds developed (Patton 1964).

One of the important features of IVF is its capability to bypass prefertilization incompatibility barriers (Fernando and others 1998), and through IVF, species that do not normally hybridize in nature may be hybridized in culture. The ultimate aim of this project is to develop rust resistant white pines through interspecific hybridization in vitro. Because

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Danilo D. Fernando is with the Faculty of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, 461 Illick Hall, 1 Forestry Drive, Syracuse, NY 13210. E-mail: fernando@esf.edu. Phone: (315) 470-6746. Fax: (315) 470-6934. John N. Owens is with the Centre for Forest Biology, University of Victoria, Victoria, BC V8W 3N5, Canada.

there are no previous works on the culture of reproductive structures of any species of white pine that can be directly used, the current and immediate concerns of this research are basic. What are the nutrient and cultural requirements for growing pollen tubes and female gametophytes of pines in vitro? How long can pollen tubes and female gametophytes remain viable in culture? Will pollen tubes penetrate the archegonia of female gametophytes? Will in vitro fusion occur between two different pine species?

Materials and Methods

Plant Materials

Pollen and seed cones of *P. aristata* were obtained from the University of Victoria, British Columbia, while pollen and seed cones of *P. monticola* were obtained from Saanich Seed Orchard, Saanich, British Columbia. All crosses involving female gametophytes of *P. aristata* and *P. monticola* were done at the Centre for Forest Biology, University of Victoria, Victoria, British Columbia, Canada.

Pollen cones of *P. strobus* were collected from the SUNY-ESF Lafayette Experimental Station, Syracuse, New York, while seed cones were obtained from the SUNY-ESF Heiberg Memorial Forest, Tully, New York. Crosses involving female gametophytes of *P. strobus* were done at the Department of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, Syracuse, New York USA.

Surface Sterilization of Pollen and Seed Cones

Pollen cones of *Pinus aristata*, *P. monticola* and *P. strobus* were collected 2 to 3 days before dehiscence while seed cones were collected at central cell stage (Fernando and others 1997). Pollen and seed cones were surface-sterilized by washing in 70 percent ethanol, sterile distilled water, and 1 percent sodium hypochlorite for 30 seconds each step. They were rinsed three times with sterile distilled water for 10 seconds each time, blotted dry on sterile paper towels, and left in Petri dishes covered with sterile filter paper for 48 to 72 hours at 27°C. Dried sterile pollen grains were collected in sterile vials and stored at 4 °C for short-term or –20 °C for long-term storage.

After surface sterilization of seed cones, ovuliferous scales were separated individually using sterile forceps. Ovules were dissected under a stereomicroscope, and the female gametophytes were mechanically isolated and placed in culture. Representative female gametophytes from each seed cone used in culture were fixed in formalin-acetic-alcohol. These were used to monitor the initial stage of development and also serve as the control.

Media Composition and Co-Culture Conditions

The basal medium contained macro- and micronutrients and vitamins as described by Murashige and Skoog (1962), supplemented with boric acid and calcium nitrate following Brewbaker and Kwack (1963). The working solution was half-strength diluted with deionized distilled water, and supplemented with 15 percent sucrose and 0.4 percent phytagel. The pH was adjusted to 6.0 with KOH. This medium is referred to as MSBK.

Pollen grains were grown on MSBK and after 2 to 3 days, freshly isolated female gametophytes were introduced at the tips of growing pollen tubes. Viability of pollen tubes (table 1) was based on whether they had collapsed or not, while viability of female gametophytes (table 2) was based on whether the central cell had undergone plasmolysis or not (Fernando and others 1997). The co-cultures were incubated in the dark at 23 °C. Several intraspecific and interspecific crosses were done and a total of 1,200 female gametophytes were used (table 3).

Histological Analysis

Pollen grains and tubes were examined at various stages of development by fixing in 4 percent paraformaldehyde in saline phosphate buffer and staining with DAPI (4',6-diamidino-2-phenylindole). The specimens were examined using a Leica DMLB fluorescence microscope. A total of 1,200 female gametophytes were co-cultured with pollen tubes. After the co-cultures were incubated for 6 to 10 days, female gametophytes which when lifted, had firmly attached pollen tubes were fixed in 4 percent glutaraldehyde in phosphate buffer. Specimens were rinsed with phosphate buffer and dehydrated through a graded series of ethanol.

Table 1—Length and longevity of pollen tubes in culture (n = 50).

Species	Days in culture	Length of pollen tubes (µm)	Viability (%)
		Mean (range)	
P. aristata	15	650 (530-850)	100
	20	750 (690-980)	100
	30	920 (840-1090)	98
P. monticola	15	600 (460-800)	100
	20	760 (540-920)	98
	30	800 (630-990)	95
P. strobus	15	580 (340-700)	95
	20	620 (440-880)	88
	30	770 (650-910)	85

Table 2—Viability of female gametophytes in culture (n = 100).

Number of days in culture	Number of viable female gametophytes		
	P. aristata	***P. monticola***	***P. strobus***
2	92	85	70
4	70	61	54
6	58	49	37
8	34	23	12
10	19	12	05

Table 3—Intraspecific and interspecific crosses in vitro (n = 200).

Pollen tubes	Female gametophytes		
	P. aristata	***P. monticola***	***P. strobus***
P. aristata	x	x	x
P. monticola	x	x	-
P. strobus	-	-	x

x indicates intraspecific or interspecific cross; - indicates no cross was made

Specimens were gradually infiltrated with a solution containing hydroxyethyl methacrylate (Technovit 7100 embedding kit, Energy Beam Sciences Inc., MA). Sections (8 to 10 µm) were cut using a JB4 ultramicrotome, mounted on glass slides and stained with Toluidine Blue O. Specimens were examined under a brightfield microscope and images captured using a digital video camera (Optronics, CA).

Results and Discussion

Viability and Longevity of Pollen Tubes and Female Gametophytes

Percentage pollen viability in *P. aristata*, *P. monticola* and *P. strobus* were very high (table 1). Growth of pollen tubes was maintained in vitro for 30 days with at least 85 percent viability (table 1). Of the three species of pine examined, *P. aristata* appears to be the most vigorous because of relatively greater longevity and length of pollen tubes. Our results also show that pollen tubes of all three species continue to elongate under in vitro conditions. This shows that in vitro, there is no stage that corresponds to the resting stage that occurs in vivo (Gifford and Foster 1989). Under our in vitro conditions, the average length of pollen tubes (table 1) attained by all three species of white pines (that is, 830 mm) is much longer than the average length of the nucellus (that is, 700 mm) that the pollen tubes traverse prior to reaching the archegonia in vivo.

The longevity of pollen tubes in culture makes them suitable as targets for genetic transformation. In fact, pollen grains are natural vectors for delivering foreign DNA since they are involved in the normal fertilization process. Transformed pollen grains are being used to artificially pollinate flowers in several plants and these have resulted in the formation and recovery of transgenic progenies (Häggman and others 1997, Aronen and others 1998). This technique is very promising because it avoids the use of elaborate and time-consuming tissue culture steps. In white pines, the protocols for the transformation of pollen grains and tubes have already been optimized for *P. aristata* and *P. monticola* (Fernando and others 2000). There are several broad-spectrum pathogenesis related genes that are available for flowering plants (Shewry and Lucas 1997, Osusky and others 2000, Powell and others 2000), and these need to be tested in white pines.

In culture, the viability of female gametophytes declined very rapidly reaching very low numbers after 10 days in culture (table 2). The decline in the viability of female gametophytes in *P. aristata* appears less drastic when compared to those of *P. monticola* or *P. strobus* (table 2). It has long been known that unlike some other conifers, unpollinated ovules in pine do not develop into maturity. In vivo, development of pine ovules proceeds only in the presence of germinated pollen (McWilliam 1959). This suggests that the presence of developing pollen tubes on the nucellus provides some stimulatory factors that are required for the maturity of the female gametophytes. Apparently, the co-culture of pollen tubes and female gametophytes does not have the same effect. It will be interesting to find out if pollen tube extracts added to the culture medium will improve the response of female gametophytes in culture.

Interactions Between Pollen Tubes and Female Gametophytes

When freshly isolated female gametophytes of *P. monticola* were co-cultured with 2 to 3 day old *P. aristata* pollen tubes (and vice versa), the pollen tubes continued to elongate resulting in the penetration of the female gametophytes. In several instances, pollen tubes of *P. aristata* entered the canal leading to the neck cells of the archegonia in *P. monticola* (fig. 1). This is similar to what has been reported to happen in nature under intraspecific crosses (Owens and Morris 1990). This type of penetration was also observed between *P. monticola* pollen tubes and *P. aristata* female gametophytes. In both types of interspecific crosses, some pollen tubes also penetrated the female gametophytes through the prothallial cells far from the neck cells of the archegonia (fig. 2). Only in one instance was a pollen tube observed to reach the neck cells of viable female gametophytes (fig. 3).

It is interesting to note that in vitro, pollen tubes formed minute projections to penetrate between neck cells (fig. 4), as

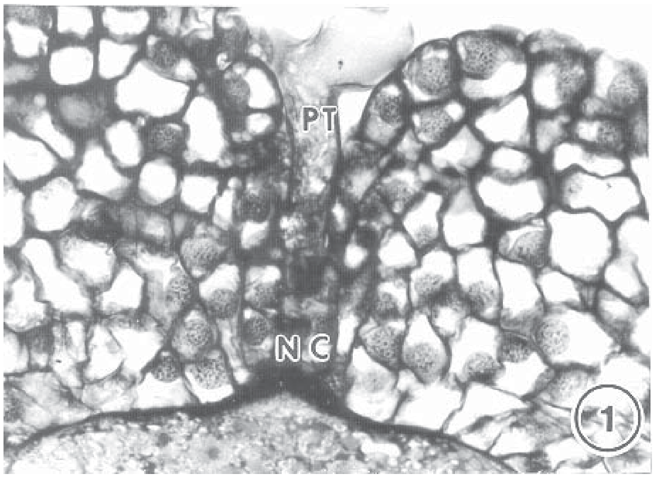


Figure 1—Pollen tube penetrating female gametophyte through neck cells. Figures 1-5 – Acronyms are: EC egg cell, EN egg nucleus, FG female gametophyte, NC neck cells, PC prothallial cells, PT pollen tube, SC starch grains.

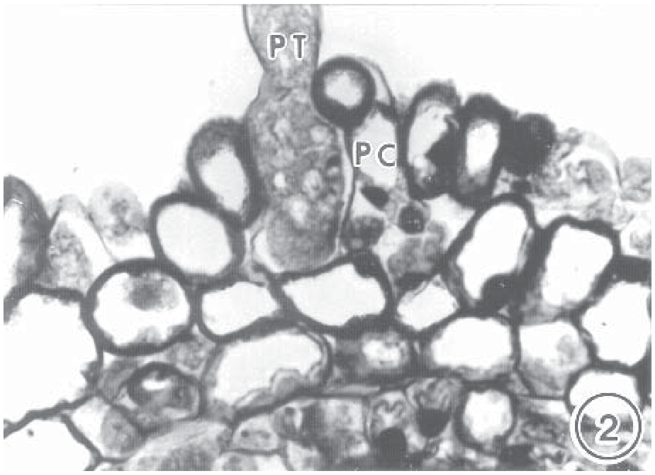


Figure 2—Pollen tube penetrating prothallial cells of female gametophyte.

happens when pollen tubes make contact with the neck cells in vivo (Owens and Morris 1990). In culture, however, formation of minute projections appeared to form not only from the tips of pollen tubes but also from the lateral walls as seen in *P. aristata*.

During co-culture, the elongating pollen tubes could have all passed under or over the female gametophytes, but instead many penetrated the archegonia through the neck cells. This suggests that some sort of cellular recognition do exists under in vitro conditions. Furthermore, some pollen tubes that penetrated the archegonia released their contents into the egg cytoplasm (fig. 5). This suggests not only that in vitro pollen tubes recognize their target destination,

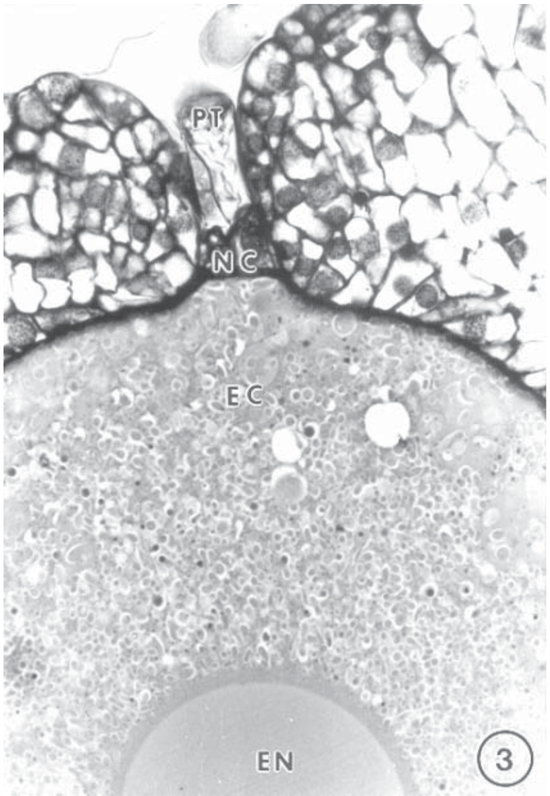


Figure 3—Female gametophyte with pollen tube and unplasmolyzed egg.

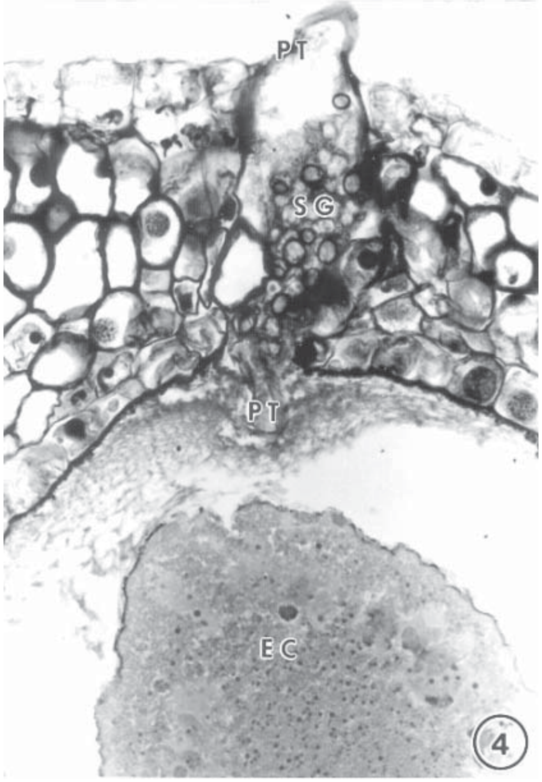


Figure 4—Pollen tube tip inside plasmolyzed egg cell; pollen tube containing starch grains.

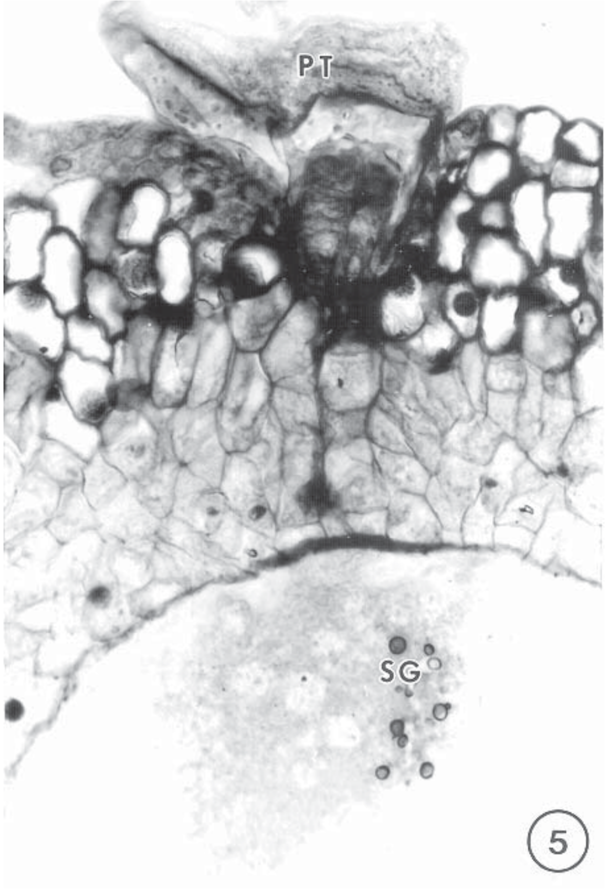


Figure 5—Contents of pollen tube such as starch grains are in the egg cytoplasm.

but they also react in the same way as in vivo. None of the pollen tubes that penetrated the prothallial cells of the female gametophytes released their contents.

Summary

Our results show that our current culture conditions are optimum for growth and development of *P. aristata*, *P. monticola*, and *P. strobus* pollen tubes. The activities of pollen tubes as they penetrate the archegonia of the female gametophytes in culture resemble those that have been reported to occur in vivo. Our results on histological analysis show similar pattern for intraspecific and interspecific crosses (table 3). It is also important to note that the activities of pollen tubes are not hindered by the source of the co-cultured female gametophytes, suggesting that in vitro, no incompatibility reaction is manifested.

Sustaining growth and development of female gametophytes in culture is extremely difficult. Although we have tried different media and supplements without success (unpublished data), there are still countless options to try. Because the culture medium is not optimized for female gametophyte development, no interaction occurred after pollen tube penetration and release of gametes into the egg cytoplasm. Therefore, there is a need to develop a culture medium that is suitable to sustain growth and development of female gametophytes, and at the same time allow the sperm cells that are released in the egg cytoplasm to fuse with the egg nucleus and develop into embryo.

Although in vitro fertilization was not achieved, our results are promising. If we succeed in sustaining the growth of female gametophytes in culture, this IVF technology can offer several novel alternative approaches such as interspecific hybridization and imparting resistance genes into pollen tubes or archegonia followed by IVF. Another benefit of this work applies to all pines and their breeding system. The normal life cycle from pollination to mature embryos takes about 15 months. Through IVF, the time from “pollination” to development of mature embryos could be shortened to 3 to 4 months.

References

Aronen, T.S., Nikkanen, T.O., Häggman, H.M. 1998. Compatibility of different pollination techniques with microprojectile bombardment of Norway spruce and Scots pine pollen. Can J. For. Res. 28:79-86.

Bingham, R.T. 1972. Taxonomy, crossability and relative blister rust resistance of 5-needled white pines, pages 271-280. *In*: Biology of Rust Resistance in Forest Trees, Bingham, R.T., Hoff, R.J. and McDonald, G.I. Miscellaneous Publication No 1221. USDA, Washington DC.

Bingham, R.T. 1983. Blister rust resistant western white pine for the inland empire: the story of the first 25 years of the research and development program. USDA General Tech Rep INT-146.

Blada, I. 1994. Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). Silvae Genet. 43:14-20.

Fernando, D.D., Owens, J.N. and Misra S. 2000. Transient gene expression in pine pollen tubes following particle bombardment. Plant Cell Rep. 19:224-228.

Fernando, D.D., Owens, J.N. and von Aderkas, P. 1998. In vitro fertilization from co-cultured pollen tubes and female gametophytes of Douglas fir (*Pseudotsuga menziesii*). Theor. Appl. Genet. 96:1057-1063.

Fernando, D.D., Owens, J.N., von Aderkas, P. and Takaso, T. 1997. In vitro pollen tube growth and penetration of female gametophyte in Douglas fir (*Pseudotsuga menziesii*). Sex. Plant Reprod. 10:209-216.

Gifford, E.M. and Foster, A.S. 1989. Morphology and Evolution of Vascular Plants. W.H. Freeman Company, NY.

Häggman, H.M., Aronen, T.S. and Nikkanen, T.O. 1997. Gene transfer by particle bombardment to Norway spruce and Scots pine pollen. Can. J. For. Res. 27:928-935.

Heimburger C. 1972. Breeding of white pine for resistance to blister rust at the interspecies level, pages 541-549. *In*: Biology of Rust Resistance in Forest Trees, Bingham, R.T., Hoff, R.J. and McDonald, G.I. Miscellaneous Publication No 1221. USDA, Washington DC.

Kinloch, B.B. Jr. 1992. Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Can. J. Bot. 70:1319-1323.

Kinloch, B.B. Jr. and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. Plant Dis. Rep. 65:604-605.

Kinloch, B.B. Jr. and Dupper, D.E. 1999. Evidence of cytoplasmic inheritance of virulence in *Cronartium ribicola* to major gene resistance in sugar pine. Phytopathology 89:192-196.

Kinloch, B.B. Jr., Davis, D. and Dupper, G.E. 1996. Variation in virulence in *Cronartiumribicola*: What is the threat? pages 133-136. In: Kinloch, B.B. Jr., Marosy, M. and Huddleston, M.E. Proceedings of a Symposium presented by the California Sugar Pine Management Committee: Sugar pine: status, values and roles in ecosystems. Publication No. 3362, University of California, Division of Agriculture and Natural Resources, Davis, California.

Kinloch, B.B. Jr., Parks, G.K. and Fowler, C.W. 1970. White pine blister rust: simply inherited resistance in sugar pine. Science 167:193-195.

Kinloch, B.B. Jr., Sniezko, R.A., Barnes, G.D. and Greathouse, T.E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western cascade range. Phytopathology 89:861-867.

McDonald, G.I., Hansen, E.M., Osterhaus, C.A. and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Dis. 68:800-804.

Osusky, M., Zhou, G.Q., Hancock, R.E.W., Kay, W.W. and Misra, S. 2000. Transgenic potatoes expressing cationic peptides confers broad-spectrum resistance to phytopathogens. Nature Biotech. 18:1162-1166.

Owens, J.N. and Morris, S.J. 1990. Cytoplasmic basis for cytoplasmic inheritance in *Pseudotsuga menziesii*. II. Pollen tube and archegonial development. Amer. J. Bot. 78:1515-1527.

Patton, R.F. 1964. Interspecific hybridization in breeding for white pine blister rust resistance. 367-376.

Powell, W.A., Catranis, C.M. and Maynard, C.A. 2000. Design of self-processing antimicrobial peptides for plant protection. Let. Appl. Microbio. 31:163-168.

Shewry, P.R. and Lucas, J.A. 1997. Plant proteins that confer resistance to pests and pathogens. Adv. Bot. Res. 26:135-192.

Spaulding, P. 1929. White pine blister rust: a comparison of European with North American conditions: USDA Technical Bulletin 87.

Stone, E.C. and Duffield, J.W. 1950. Hybrids of sugar pine by embryo culture. J. Forestry 48:200-201.

Wright, J.W. 1959. Species hybridization in the white pines. Forest Science 5:210-222.

Natural Hybridization between Russian Stone Pine (*Pinus siberica*) and Japanese Stone Pine (*Pinus pumila*)

Sergej N. Goroshkevich

Abstract—A study was conducted on phenology and reproductive characteristics of Siberian stone pine (*Pinus pumila*), Japanese stone pine (*Pinus pumila*), and their putative hybrids in high altitudes. The study demonstrated that the species were not reproductively isolated, and putative hybrids were identified. Seed development and production were very poor in the putative hybrids indicating that introgression is very slowly or not occurring beyond the first filial generation.

Key words: *Pinus siberica*, *Pinus pumila*, stone pines, reproductive biology, natural hybridization

Introduction

In the Baikal region of Siberia, the natural ranges of Siberian stone pine (*Pinus sibirica* Du Tour) and Japanese stone pine (*P. pumila* Regel) overlap. Siberian stone pine grows to become a large tree and occurs in pure stands between 600 and 1,600 m in elevation but becomes increasingly scarce as altitude increases. Japanese stone pine generally grows as a shrub (1 to 4 m high) that can generate adventitious roots. A single individual can often form vast clones, up to 50 m in diameter. Japanese stone pine is an occasional understory species or occurs in pure populations on steep, stony slopes at altitudes between 1,000 and 1.600 m. The occurrence of this species in forest composition increases with altitude. At altitudes of 1,600 to 2,000 m, it forms a thick brushwood. At altitudes of 2,000 to 2,100 m (timberline), Japanese stone pine and dwarfed, sterile Siberian stone pine occur together.

The occurrence of natural hybridization between these species has been the subject of debate since putative hybrids were described 70 years ago (Pozdnjakov 1952; Galazij 1954; Molodjnikov 1975). Although natural hybridization has been refuted in many recent reviews (Bobrov 1978; Lanner 1990; Homentovski 1995), individual trees were recently found north of Khamar-Daban (15 km southwest of Baikal), which were intermediate between the species in a number of traits including: form, growth, needle and shoot structure, and cone color (Goroshkevich 1999). The purpose of this research was to determine where this natural hybridization occurs and to study reproductive characteristics and seed production in these two pine species and their putative hybrids.

Material and Methods

Samples from trees were collected north of Khamar-Daban, 30 to 35 km southwest of Baikal, where Siberian stone and Japanese stone pines can be found growing together. Putative hybrids are occasionally found in intermixed populations. An area of approximately 5 by 5 km in the region of Cherskij peak, Serdze Lake, and the Podkomarnaja riverhead was inspected for putative hybrids. Putative hybrids were distinguished by a combination of characteristics from the parental species: multiple crooks in the stem (Japanese stone pine characteristic) and the violet color of 2-year-old cones (Siberian stone pine characteristic).

Studies on reproductive characteristics were conducted in populations at an altitude of approximately 1,500 m from June 30 to July 10, 1998, according to methodology of Titov (1982) and Nekrasova (1983). At this altitude, Siberian stone pine was common (approximately 200 cone-bearing trees per ha), but Japanese stone pine was less frequently encountered (approximately 10 clones per ha). In development of pollen cones and pollen dispersal, successive stages were distinguished as follows: (1) initiation of pollen shedding, (2) abundant pollen shedding, and (3) residual pollen shedding. In development of the female cone, successive stages of receptivity for pollination were distinguished by changes in the ovuliferous scales as follows: (1) onset of receptivity, (2) optimal receptivity, and (3) declining receptivity.

Cone samples were made from 10 Siberian stone pines, 10 Japanese stone pines, and 10 putative hybrids. Samples of cones (10 per individual) were collected at the late August 1998 from 106 individuals (27 Siberian stone pines, 59 Japanese stone pines, and 20 putative hybrids). Seed quality was studied by X-ray photography. Different characteristics were assessed including: cone length (cm), cone width (cm), average number of scales/cone, average number of initial ovules/cone, ovules/cone lost in the early stages of development (percentage), average number of seeds/cone, poorly developed seeds/cone (percentage), average number of filled seeds/cone, hollow seeds/cone (percentage), average number of seeds/cone with endosperm, seeds/cone with imperfect endosperm (percentage), average number of seeds/cone with

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Sergej N. Goroshkevich is with the Russian Academy of Sciences, Siberian Branch, Filial of the Forest Institute,Academichesky pr., 2, Tomsk, Russia, 634021. Telephone 3822-258680, Fax 3822-258855, e-mail gorosh@forest.tsc.ru.

perfect embryos, seeds/cone without embryos (percentage), average number of seeds /cone with embryo, seeds/cone with differentiated embryos (percentage), average number of seeds/cone with differentiated embryos, seeds/cones with undifferentiated embryos (percentage), and average weight of a single seed with complete endosperm (mg).

Significant differences (p<0.05) among means were determined using an analysis of variance (ANOVA), and mean separation was by Duncan's multiple range test (Steel and Torrie 1980).

Results

The phenology studies indicated that putative hybrids were occasionally found in populations below 1,990 m in elevation (one clone per ha). In contrast, the number of hybrids increased with elevation with a density of two to three clones per ha found above 1,900 m in elevation. The observations on reproductive characteristics showed that timing of reproductive maturation of the two species and putative hybrids practically coincide. Reproductive maturation generally occurred during a 2 to 3 week period, which precludes reproductive isolation of either species, given that hybridization is possible.

The results of cones and seeds morphological analysis are presented in table 1. The seed analysis showed that Japanese stone pine was fertile up timberline, and Siberian stone pine became increasingly infertile above altitude of 1,750 m. Siberian stone pine was significantly different from Japanese stone pine in most of the cone and seed traits studied. Putative hybrids were found to differ from either Siberian pine or both of the parental species in all characteristics, except for the initial number of ovules. Putative hybrids had intermediate values between both parents for cone length, cone diameter, average number of scales per cone, and average weight of seed. For characteristics related to seed quality (with the exception of seed weight), the putative hybrids had significantly higher poorly developed seeds/cone (percentage), hollow seeds/cone (percentage), and seeds/cone without embryos (percentage), and significantly lower seeds/cone with differentiated embryos (percentage), when compared to both parents. Differences of putative hybrids only from Siberian pine were apparent in a number of characteristics.

Discussion

It is known that taxonomically close pine species can be often crossed to form fertile hybrids (Critchfield and Little 1966) and that species with sympatric ranges can form hybrid swarms (Wright 1975). Siberian and Japanese stone pines belong to the same *Pinus* subsection (*Cembrae* Lond.), but their natural distribution overlaps only by 5 to 10 percent. This study shows that no reproductive isolation between the species occurs due to the timing of pollen flow and female cone receptivity, and occasionally morphologically intermediate individuals occur, that is, putative hybrids. Further introgression, however, does not appear to be rapidly occurring, if at all. Production of viable seed in the putative hybrids was proportionally lower than in the parental species. Additionally, the number of seed/cone in the putative hybrids was very low (on average one filled seed per cone), thereby indicating little introgression between these species beyond the first filial generation. Future studies using various combinations of controlled pollinations between Siberian and Japanese stone pines with corresponding genetic analyses could further corroborate the origin of the naturally occurring hybrids and better delineate limitations for introgression between these two species.

Table 1—Cones and seed characteristics in Siberian stone pine, Japanese stone pine, and putative hybrids.

Characteristic	Siberian stone pine	Putative hybrid	Japanese stone pine
Cone length (cm)	5.0a[1]	4.0b	3.0c
Cone diameter (cm)	4.3a	3.0b	2.1c
Average number of scales/cone	73.6a	55.9b	37.6c
Average number of initial ovules/cone	68.3a	57.0ab	35.9b
Ovules/cone lost in the early stages of development (%)	15.5a	32.2b	43.9b
Average seed number/cone	58.3a	38.6b	20.7b
Poorly developed seeds/cone (%)	14.6a	52.0b	27.9a
Average number of filled seeds/cone	49.5a	19.6b	14.1b
Hollow seeds/cone (%)	3.2a	24.6b	5.3a
Average number of seeds/cone with endosperm	48.0a	14.9b	13.5b
Seeds/cone with imperfect endosperm (%)	32.3a	79.2b	63.0b
Average number of seeds/cone with perfect endosperm	32.5a	3.6b	5.0b
Seeds/cone without embryos (%)	0.2a	48.5b	5.9a
Average number of seeds/cone with embryo	32.4a	1.6b	4.7b
Seeds/cone with undifferentiated embryo (%)	5.8a	35.3b	17.8b
Average number of seeds/cone with differentiated embryo	30.5a	1.0b	3.9b
Seeds/cone with differentiated embryo (%)	44.7a	1.8c	10.9b
Average weight of a single seed with complete endosperm (mg)	216.2a	142.4b	72.3c

[1] Significant differences (p<0,05) between means were determined using Duncan's multiple range test; values with different letters within a line differ significantly.

References

Bobrov, E.G. 1978. Forest-creating conifers of the USSR. Nauka. Leningrad. 189 p. (In Russian).

Critchfield, W.B. and Little, E. L., Jr. 1966. Geographic distribution of the pines of the world. USDA Forest Service. Misc. Publ. 991, 97 p.

Galazij, G.I. 1954. Timberline flora in the mountains of Eastern Siberia and their dynamics. Proceedings of Botanical Institute, USSR Academy of Sciences. 3 (9): 210-329. (In Russian).

Goroshkevich, S.N. 1999. On the possibility of natural hybridization between *Pinus sibirica* and *Pinus pumila* in the Baikal Region. Botanicheskij Journal. 84(9): 48-57. (In Russian).

Homentovskij P.A. 1995. Japanese stone pine ecology on Kamchatka. Dalnauka. Vladivostok. 227 p. (In Russian).

Lanner, R.M. 1990. Biology, taxonomy, evolution, and geography of stone pines of the world. Proceedings of the Symposium on whitebark pine ecosystems: ecology and management of a high-mountain-resource; 1989 March 29-31; Bozeman, MT. Ogden, UT: USDA Forest Service Gen. Tech. Rep. INT-270, pp. 14-22.

Molodjnikov, V.N. 1975_ Japanese stone pine of mountain landscapes of Northern Baikal Region. Nauka. Moscow. 203 p. (In Russian).

Nekrasova, T.P. 1983. Pollen and pollination in Siberian conifers trees. Nauka. Novosibirsk. 168 p. (In Russian).

Pozdnjakov, L.K. 1952. Tree form of Japanese stone pine. Botanicheskij jurnal. 37: 688-691. (In Russian).

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill. New York. 633 p.

Titov E.V. Seed and cone development in Siberian stone pine. Lesnaja geobotanica i biologija drevesnyh rastenij. 18: 136-140. (In Russian).

Wright, J.W. 1975. Introduction in forest genetics. Academic Press. New York, San Francisco, London. 470 p.

Estimation of Heritabilities and Clonal Contribution Based on the Flowering Assessment in Two Clone Banks of *Pinus koraiensis* Sieb. *et* Zucc.

Wan-Yong Choi
Kyu-Suk Kang
Sang-Urk Han
Seong-Doo Hur

Abstract—Reproductive characteristics of 161 Korean pine (*Pinus koraiensis* Sieb. *et* Zucc.) clones were surveyed at two clone banks for 3 years. These clone banks were established at Yongin and Chunchon (mid-Korea) in 1983. Characteristics in female and male strobili were spatial (between locations) and temporal (among investigated times) variables. Broad sense heritabilities were found to vary between 0.20 - 0.46 in females and between 0.34 to 0.56 in males. Among 161 clones, 32 clones (20 percent of the total clones) accounted for 42 to 54 percent of clonal contribution in female strobili and 83 to 96 percent in male strobili, suggesting that the clonal contribution for male parents was severely unbalanced compared to that for female parents. The effective population numbers varied depending on time (year), location and sex. The mean values of relative effective population numbers at gamete levels were 0.56 in females and 0.09 in males, respectively, and that value at the clonal level was 0.27 (0.25 at Yongin and 0.29 at Chunchon). The degree of sexual asymmetry (A_s) varied with a range of 0.03 to 0.24 at Chunchon and 0.07 to 0.44 at Yongin. The pattern of gamete production within clones was highly asymmetrical as compared to that of other conifers. This indicates that *P. koraiensis* is extremely low in male gamete production compared to female gamete production.

Key words: *Pinus koraiensis*, strobili, clone bank, clonal contribution, broad sense heritability, effective population number, sexual asymmetry

Introduction

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is a five-needle pine (*Pinus* subgenus *Strobus*) belonging to subsection *Cembrae*. The species has a wide natural distribution in the northeastern part of Eurasia. It usually occurs as a mixed forest stand consisting of various broad-leaved tree species and other conifers. Korean pine has been widely planted as a pure stand accounting for about 30 percent of the yearly planting areas in Korea due to its high-quality timber and edible seeds. A breeding program for this species has been conducted since 1959 and has resulted in the establishment of 98 ha seed orchards (Mirov 1967, Chun 1992, Choi 1993, Wang 2001).

The main goal for seed orchards is large-scale production of genetically improved seeds that maintain genetic diversity to prevent inbreeding depression. Thus, the maintenance of random mating among clones is one of the key elements to successful management of seed orchards (Roberds and others 1991, Chaisursri and El-Kassaby 1993, Matziris 1993, El-Kassaby and Cook 1994, Burczyk and Chalupka 1997, Han and others 1999).

The clonal contribution to seed production in a seed orchard is one of the most important factors; genetic composition of the seed produced is determined by the contributions of each clone. Differences in clonal contribution have been previously reported in several studies and have been attributed to genetic rather than environmental factors (Griffin 1982, Schmidtling 1983, Askew 1988, Brunet and Charlesworth 1995, Kjaer 1996, Han and others 1999, Nikkanen and Ruotsalainen 2000).

To date, numerous studies have been conducted to obtain information related to reproductive processes such as flowering characteristics, clonal contribution, and sexual asymmetry in seed orchards. Clonal contribution within a seed orchard is commonly depicted by a flowering or cone yield curve. In this method, the clones are ranked from high to low in flower production, and cumulative contribution (in percent) is plotted against the proportion of the clones. Additionally, the concept of effective population number has been recently applied to the estimation of clonal contribution (Griffin 1982, Kjaer 1996, Choi and others 1999, Han and others 2001a, 2001b, Kang 2001).

Our major interest in this study is to quantify the reproductive processes using empirical data from two *Pinus koraiensis* clone banks, to survey the differences of clonal contribution by means of flowering assessments, and to monitor the genetic diversity measured by effective population sizes. These include estimating heritability, gamete contribution, and sexual asymmetry.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Wan-Yong Choi, Kyu-Suk Kang, and Sange-Urk Han are with the Tree Breeding Division, Korea Forest Research Institute, 44-3 Omokchun, Suwon 441-350, Republic of Korea. Seong-Doo Hur is with the Sobu Forest Experiment Station, Korea Forest Research Institute, 670-4 Sangmo, Chungjoo 380-940, Republic of Korea. Correspondence should be addressed to e-mail wychoi@foa.go.kr.

Materials and Methods

Reproductive characteristics such as number of male and female strobili were surveyed in the two clone banks of *P. koraiensis.* The two clone banks were established at Chunchon (lat. 37°55', long. 120°46') and Yongin (lat. 37°30', long. 127°20') in 1983. A total of 167 clones were grafted at Chunchon and 180 clones at Yongin with a space of 4mx4m. Reproductive characteristics of 161 clones, which the two clone banks have in common, were investigated for 3 consecutive years (1998 to approximately 2000).

The clone banks were not considered as fully mature populations when the numbers of female and male strobili were counted. Generally, Korean pine begins to show strobili at age of 12 or 15 in natural stands. Grafted clones, however, produce strobili earlier than natural stands. In these clone banks, there is not much difference in height (4 to 5m) and DBH. Five ramets per clone were chosen for assessment in early June. The number of female strobili was counted individually from a whole tree. The total number of male strobili was estimated by multiplying the average number of strobili per branch by the total number of branch bearing male strobili.

Analysis of variance (ANOVA) tests and heritability estimates were conducted based on the data for female and male strobili production. The ANOVA was performed using a logarithmic transformation of the original data to normalize the distribution of variances (Steel and Torrie 1980). SAS program (ver 6.12; SAS Institute Inc., 1996) was used for ANOVA tests and heritability estimation. Broad-sense heritabilities (H^2) were estimated on the basis of individual trees (Schmidtling 1983) as:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}.$$

Parental balance was assessed using a cumulative gamete contribution curve (Griffin 1982). The numbers of female and male strobili were ordered by clone from high to low strobilus production, and the cumulative contribution percentages were plotted against the proportion of the clones (Kang 2000).

The maleness index (Ai) is defined as the proportion of a clone's reproductive success that is transmitted through its pollen (Kang 2000). Maleness index based on strobilus production was estimated as follows:

$$Ai = \frac{ai}{gi + ai}$$

where ai and gi are the proportions of i^{th} clone of which male and female strobili contribute to the whole population. A high maleness index of a clone indicates that the clone is contributing more as a paternal, rather than maternal parent.

The effective population numbers at gamete level (Eq. 1 and Eq. 2) and clonal level (Eq. 3 and Eq. 4) and the sexual asymmetry (Eq. 5) were estimated using Choi and others' (1999) methods as follows:

$$\mu_{♀} = (\sum_{i=1}^{n} \mathrm{xi}_{(♀)}{}^{2})^{-1} \tag{1}$$

$$\mu_{♂} = \{\sum_{i=1}^{n} \mathrm{xi}_{(♂)}{}^{2}\}^{-1} \tag{2}$$

$$\mu_{a} = \tfrac{1}{2}(\mu_{♀} + \mu_{♂}) \tag{3}$$

$$\mu_{b} = \{\sum_{i=1}^{n} (\tfrac{1}{2}(\mathrm{xi}_{(♀)} + \mathrm{xi}_{(♂)}))^{2}\}^{-1} \tag{4}$$

$$A_s = \mu_a / |\mu_b - \mu_a|,\ 0 \le A_s \le 1 \tag{5}$$

where n is the total number of clones, $\mu_{♀}$ is the female effective population number, and $\mathrm{xi}_{(♀)}$ is the proportion of the female strobili of the i^{th} clone to the whole production of females. $\mu_{♂}$ and $\mathrm{xi}_{(♂)}$ in males correspond to those for females. μ_a is the arithmetic mean of the two measures ($\mu_{♀}$ and $\mu_{♂}$) and μ_b is based on the relative frequency of $\mathrm{xi}_{(♀)}$ and $\mathrm{xi}_{(♂)}$. In this study, we used the relative effective population number instead of effective population number for easy comparison with those of other studies.

Results and Discussion

Reproductive Characteristics and Heritability

Large variations in both female and male strobilus production among clones were observed at both Yongin and Chunchon (table 1). The differences of male strobilus production among clones were far more extreme than that of female strobilus production. It seems that this phenomenon is a typical character of Korean pine from our experience of

Table 1—Mean, standard deviation (S.D.) and coefficient variation (C.V.) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000.

	Yongin						Chunchon					
	1998		1999		2000		1998		1999		2000	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Mean	5.3	64	13.6	240	9.1	36	2.2	146	12.5	393	5.8	307
S.D.(±)	4.6	184	12.8	975	9.2	111	2.1	514	9.3	988	5.1	982
C.V.(percent)	87	289	94	406	101	305	97	353	75	251	87	320

orchard management. The average female strobilus productions per clone ranged between 0 and 46.7 at Yongin and between 0.1 and 63.5 at Chunchon. The production of female strobili in Yongin was consistently greater than that in Chunchon, while male strobili production showed an opposite trend. During this study, the production of female and male strobili was most abundant in 1999.

The ANOVA results and broad sense heritabilities for reproductive characteristics are presented in table 2. The number of female and male flowers was significantly different among clones within a clone bank, while those for ramets within a clone did not show any significant differences. These results showed that the reproductive characteristics are under genetic influences rather than environmental influences. Similar results have been reported in other conifers such as *P. taeda* (Byram and others 1986), *P. densiflora* (Han and others 1999), *P. thunbergii* (Han and others 2001b) and *Picea abies* (Nikkanen and Ruotsalainen 2000).

The values of broad sense heritabilities for female strobili ranged from 0.21 to 0.20 in a poor flowering year (1998) and they varied between 0.46 and 0.27 in a good flowering year (1999). Temporally those values for male strobili varied between 0.21 in 1999 and 0.42 in 1998 at Chunchon and 0.20 in 1998 and 0.34 in 2000 at Yongin. The values for males (0.22 to 0.56) were higher than females (0.20 to 0.51) for all years studied. This indicates that the genetic influence determining the reproductive characteristics is stronger in males than in females.

The two-way ANOVA results and estimated heritabilities for reproductive characteristics in the two clone banks are presented in table 3. The differences in the number of female and male strobili among clones were statistically significant for 3 years excluding that of males in 1998 and that of females in 2000. Significant differences in reproductive characteristics between the two locations were observed for females in 1998, and for females and males in 2000. In 1999, the flowering characteristics for both sexes were significantly different among clones. The interaction of clone and location effects was significant in all years, implying that clones should be selectively chosen when production (in other words, seed orchards) and/or breeding populations are established at the different sites.

The heritabilities for female and male strobili in each year showed maximum values of 0.59 and 0.77, respectively. The minimum values for heritabilities were 0.02 for female in 2000 and 0.23 for male in 1998.

Clonal Contribution

We used two types of measures, cumulative contribution curves and relative effective population number, for demonstrating the clonal contribution. The cumulative contribution curves of 161 clones for female and male strobili are presented in figure 1. Thirty-two clones (20 percent of the total clones investigated were at both locations) accounted for 42-54 percent of clonal contribution in female and 83 to

Table 2—Analysis of variance and broad sense heritability (H^2) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000.

		1998		1999		2000	
Location		Female	Male	Female	Male	Female	Male
Yongin	Among clones	0.37**	2.06**	0.69**	3.50**	0.43**	1.23**
	Within clones	0.17	0.49	0.15	0.56	0.25	0.28
	H^2	0.21	0.42	0.46	0.56	0.24	0.45
Chunchon	Among clones	0.46**	5.99**	0.60**	5.47**	0.68**	4.24**
	Within clones	0.12	0.47	0.17	0.92	0.24	0.96
	H^2	0.20	0.51	0.27	0.42	0.22	0.34

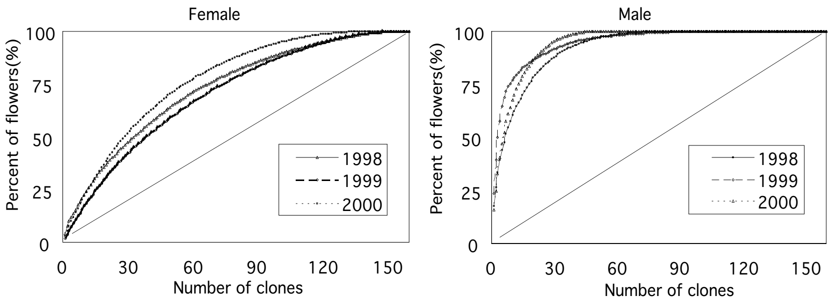
**: Significant at 1 percent level.

Table 3—Two-way ANOVA and broad sense heritabilities—(H^2) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000.

	1998		1999		2000	
	Female	Male	Female	Male	Female	Male
Clone	0.48*	3.40	0.90**	7.03**	0.50	2.92**
Location	33.55**	0.04	0.33	4.94	8.59**	46.72**
Clone x Location	0.31**	3.03**	0.37**	1.34**	0.49**	1.07**
Error	0.13	0.47	0.17	0.79	0.24	0.83
H^2	0.38	0.23	0.59	0.77	0.02	0.52

**,* Significant at 1 percent and 5 percent level, respectively.

Yongin



Chunchon

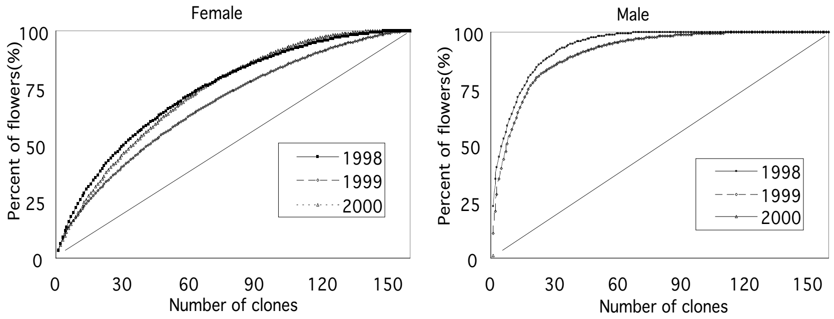


Figure 1—Cumulative female and male strobilus production curves of clones at Yongin and Chunchon during the period of 1998 to 2000.

96 percent in male strobili. The curves for male strobili were severely distorted compared to those for female strobili. Alternately, the clonal contributions of female and male strobili for each year were 49 percent and 89 percent in 1998, 44 percent and 92 percent in 1999, and 54 percent and 96 percent in 2000, respectively at Yongin, while those for Chunchon were 51 percent and 92 percent in 1998, 42 percent and 86 percent in 1999, and 47 percent and 83 percent in 2000, respectively. The biased contribution of a small number of clones to the whole clonal contribution was greater for pollen parents than female parents.

Park and others (1987) reported that 19 percent of the total clones in a *P. koraiensis* clone bank accounted for 63 percent of male strobili production and 58 percent of female strobili production. This study was conducted at Chunchon where our study was also conducted. However, they studied a 4 to 5 year old clone bank. Alternately, Han and others (1997) conducted a similar study in a *P. koraiensis* clone bank at Yongin, our other study site. In that study, they reported that 20 percent of the total clones investigated accounted for 49 to 65 percent of female strobili production, while 8 to 15 percent of the total clones accounted for over 80 percent of male strobili production. The differences in results between the above studies and our study might be due to plantation age. Regardless, these comparisons show that clonal contribution to strobili production is more balanced in female than that in male reproduction.

When compared to other conifers, Korean pine appears to have a more unbalanced clonal contribution in seed production. Han and others (1999) observed that the contribution of 33 percent of 99 *P. densiflora* Ait. clones varied between 46 percent and 70 percent in female and 40 percent and 87 percent in male, and the degree of contribution increased with age. In *P. radiata* D. Don, 23 percent of the total clones accounted for 50 percent of seed production (Griffin 1982). Adams and Kunze (1996) found that 49 percent of the total clones in *Picea mariana* (Mill.) B.S.P. and 43 percent of the clones in *P. glauca* (Moench.) Voss. accounted for a total of 80 percent of seed production.

The relative effective population numbers estimated at gamete and clonal levels are shown in table 4. The relative effective population number for sexes were extremely different with $\mu_{♀} = 0.56$ and $\mu_{♂} = 0.09$. The values of relative effective population number at the gamete level did not differ significantly by year or location. In a *Pinus sylvestris* L. clonal seed orchard at the age of 17-19, Burczyk and Chaluka (1997) found that the effective population number (0.76) in males was only slightly lower than that (0.96) in females. In contrast, Han and others (2001a) observed slightly higher values in males (mean 0.63 with a range of 0.24 - 0.94) than those in females (mean 0.55 with a range of 0.28 - 0.83) in a *P. densiflora* clonal seed orchard.

The values at the clonal level (μ_b) ranged from 0.19 - 0.38. The values of μ_b between the two locations ranged from 0.24 in 1998 to 0.38 (mean: 0.29) in 1999 at Chunchon and from 0.19 in 1999 to 0.28 (0.25) in 2000 at Yongin. Interestingly, the values of μ_b are lower than those of $\mu_{♀}$ and μ_a in all observations. It is generally known that the values of μ_b are

Table 4—Relative effective population number at the gamete level and the clonal level in *Pinus koraiensis* clone banks investigated for 3 consecutive years.

		Yongin			Chunchon		
	N	1998	1999	2000	1998	1999	2000
$\mu_{♀}$	161	0.52 (83.7)[a]	0.62 (99.8)	0.50 (80.5)	0.52 (83.7)	0.64(103.0)	0.57 (91.8)
$\mu_{♂}$	161	0.10 (16.1)	0.06 (9.7)	0.10 (16.2)	0.07 (11.3)	0.14 (22.5)	0.09 (14.5)
μ_a	161	0.31 (49.9)	0.34 (54.7)	0.30 (48.3)	0.30 (48.4)	0.39 (62.8)	0.33 (53.1)
μ_b	161	0.28 (45.1)	0.19 (30.6)	0.28 (45.2)	0.24 (38.6)	0.38 (61.2)	0.25 (40.3)

[a] Effective population number in parenthesis

always larger than those of μ_a and similar to or larger than those for $\mu_{♂}$ and $\mu_{♀}$. For instance, Han and others (2001a) showed that the value for μ_b (0.69) was higher than those for μ_a (0.58) in a *P. densiflora* seed orchard. The reason for the contrary tendency as shown in this study was explained in elsewhere (Choi and others 1999).

Sexual Asymmetry

The degrees of sexual asymmetry between female and male strobili were shown in table 5. The degree of sexual asymmetry (0.03 to approximately 0.24 with a mean of 0.17) for Chunchon was lower than that of Yongin (0.0 to approximately 0.44 with a mean of 0.20). The degree of sexual asymmetry (A_s) was variable depending on time and location.

Table 5—Estimation of the degree of sexual asymmetry (A_s) at *Pinus koraiensis* clone banks for 3 years.

	Yongin			Chunchon		
	1998	1999	2000	1998	1999	2000
$\lvert \mu_a - \mu_b \rvert$	4.84	24.16	4.83	9.66	1.61	12.87
A_s	0.10	0.44	0.07	0.20	0.03	0.24

The degree of sexual asymmetry in this study was higher especially when it was compared to that of *P. densiflora* (Han and others 2001a). In this species, the difference between two types of effective population number at clonal levels μ_a and μ_b were large because a majority of clones did not bear male flowers while most of them bore female flowers, therefore contributing to sexual asymmetry (see also Choi and others 1999). In contrast, most conifer species (*P. densiflora* and *P. thunbergii*) had similar effective population numbers between sexes.

Male index estimates are showed in figure 2. The distribution pattern of maleness indices in the present study deviated from the normal distribution pattern found in other pine trees (Burczyk and Chalupka 1997). Generally, most clones in other pines such as *P. densiflora*, *P. thunbergii* and *P. sylvestris* had maleness index of 0.8 to 0.2. Our study demonstrated a bimodal distribution, with the majority of Korean pine clones maleness indices above 0.8 or below 0.2. For instance, more than 80 percent of clones had values above 0.9 or below 0.1 regardless of year or location. In 2000 at Yongin and in 1998 at Chunchun, more than 95 percent of clones had maleness indices above 0.9 or below 0.1. On the other hand, the sexual balance within clones was highly asymmetrical in *P. koraiensis* as compared to that of other pine species such as *Pinus densiflora*, *P. thunbergii* Parl. and *P. sylvestris* (Han and others 2001a, 2001b, Burczyk and Chalupka 1997). This tendency is due to the extreme

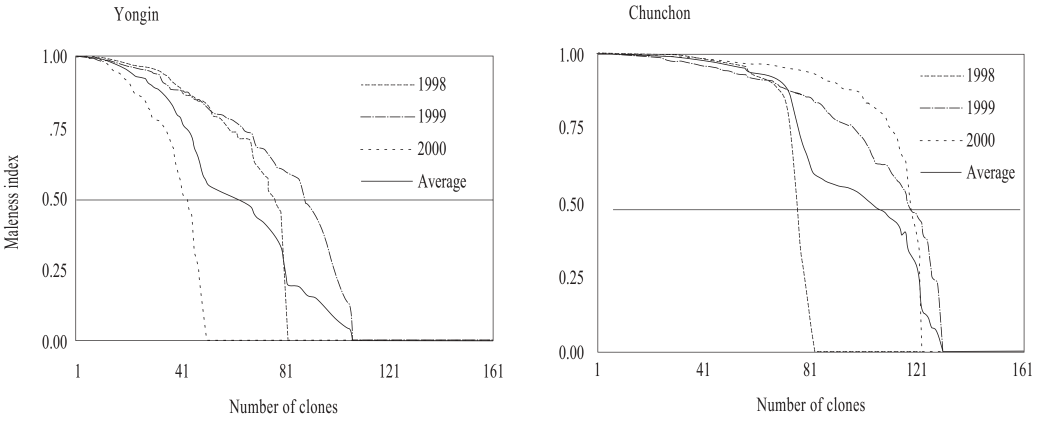


Figure 2—Maleness index curves for two different *P. koraiensis* clone banks estimated during the period of 1998 to 2000.

difference in effective population number between sexes and high degree of sexual asymmetry as already shown above.

Our study indicates potential problems in the seed orchard management of *P. koraiensis*. These problems are: 1) differential fertility variation, 2) inadequate pollen supply, 3) panmictic disequilibria, and 4) parental unbalance. Such problems relate to both the amount of seed produced and the genetic diversity of seed crops (Kang 2001). Thus, some management options, such as supplemental mass pollination, flower stimulation and equal seed harvest, should be considered in the clonal seed orchard of *P. koraiensis*.

References

Adams, G.W. and Kunze, H.A. 1996. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. For. Chron. 72: 475-480.

Askew, G.R. 1988. Estimation of gamete pool compositions in clonal seed orchard. Silvae Genet. 37: 227-232.

Brunet, J. and Charlesworth, D. 1995. Floral sex allocation in sequentially blooming plants. Evolution 49: 70-79.

Burczyk, J. and Chalupka, W. 1997. Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. Ann. Sci. For. 54: 129-144.

Byram, T.D., Lowe, W.J. and McGriff, J.A. 1986. Clonal and annual variation in cone production in loblolly pine seed orchards. For. Sci. 32: 1067-1073.

Chaisursri, K. and El-Kassaby, Y.A. 1993. Estimation of clonal contribution to cone and seed crops in Sitka spruce seed orchard. Ann. Sci. For. 50: 461-467.

Choi, W. 1993. Genetische Strukturen bei der Koreakiefer (*Pinus koraiensis* Sieb. et Zucc.) und ihre Veranderung durch Zuchtung. Gottinger Forstenetische Berichte 15.

Choi, W.Y., Hattemer, H.H. and Chung, H.G. 1999. Estimation of sexual asymmetry based on effective population number by flowering assessment and its application to an observed data from *Pinus densiflora* clonal seed orchard. FRI. J. For. Sci. 61: 33-42.

Chun, L.J. 1992. The broad-leaved Korean pine forest in China. *In* Proc. of International Workshop on Subalpine Stone Pine and Their Environment: the Status of Our Knowledge, 5-11 Sept. 1992. St. Moritz, Switzerland. *Edited* by W.C. Schmidt and F.K. Holtmeier.

El-Kassaby, Y.A. and Cook, C. 1994. Female reproductive energy and reproductive success in a douglas-fir seed orchard and its impact on genetic diversity. Silvae Genet. 43: 243-246.

Griffin, A.R. 1982. Clonal variation in radiata pine seed orchards. !a Flowering phenology. Aust. For. Res. 14: 271-281.

Han, S.U., Choi, W.Y. and Tak, W.S. 1997. Clonal variation of flowering in *Pinus koraiensis* S. et Z. Korean J. Breed. 29(1): 139-144.

Han, S.U., Chang, K.H. and Choi, W.Y. 1999. Clonal and annual variation in flowering in *Pinus densiflora* S. et Z. seed orchard. FRI. J. For. Sci. 62: 17-24.

Han, S.U., Choi, W.Y., Chang, K.H. and Lee, B.S. 2001a. Estimation of effective population numbers and sexual asymmetry based on flowering assessment in clonal seed orchard of *Pinus densiflora*. Korean J. Breed. 33(1): 29-34.

Han, S.U., Choi, W.Y., Chang, K.H., Kim, T.S. and Song, J.H. 2001b. Clonal variation of flowering in *Pinus thunbergii* seed orchard. Jour. Korean For. Soc. 90(6): 717-724.

Kang, K.S. 2000. Clonal and annual variation of flower production and composition of gamete gene pool in a clonal seed orchard of *Pinus densiflora*. Can. J. For. Res. 30(8): 1275-1280.

Kang, K.S. 2001. Genetic gain and gene diversity of seed orchard crops. Ph.D. thesis. SLU-Umeå, Sweden. Acta Universitatis Agriculturae Sueciae, Silvestria 187. 75pp.

Kjær, E.D. 1996. Estimation of effective population number in a *Picea abies* (Karst.) seed orchard based on flower assessment. Scand. J. For. Res. 11: 111-121.

Lloyd, D.G. 1979. Parental strategies of angiosperms. NZ Jour. Botany. 17: 595-606.

Matziris, D. 1993. Variation in cone production in a clonal seed orchard of black pine. Silvae Genet. 42: 136-141.

Mirov, N.T. 1967. The Genus *Pinus*. The Ronald Press Company, New York. p.263

Nikkanen, T. and Ruotsalainen, S. 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34(3): 205-222.

Park, M.H., Lee, S.B., Kim, W.W. and Chang, D.K. 1987. Flowering in a clone bank of 156 clones of *Pinus koraienesis* S. et Z. Res. Rep. Inst. For. Gen. Korea 23: 78-83.

Roberds, J.H., Friedmann, S.T. and El-Kassaby, Y.A. 1991. Effective number of pollen parents in clonal seed orchards. Theor. Appl. Genet. 82: 313-320.

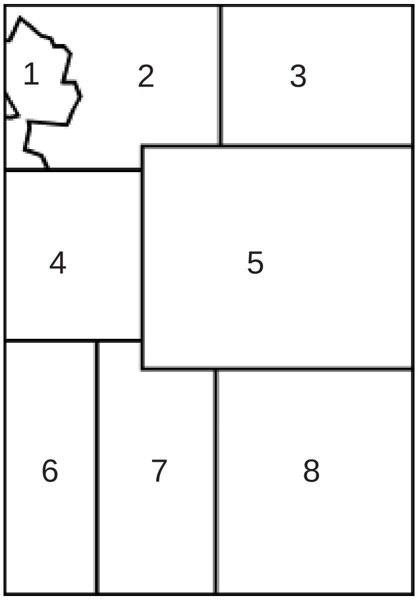
Schmidtling, R.C. 1983. Genetic variation in fruitfulness in a loblolly pine (*Pinus taeda* L.) seed orchard. Silvae Genet. 32: 76-80.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill Book Co., N.Y. 633pp.

Wang, F. 2001. An overview of ecological studies on natural forest vegetations dominated by Korean Pine (*Pinus koraiensis*) in China. *In* Proceedings of the Korea-China-Russia Joint Symposium on Korean Pine. p.8-14.

Part III: Genetic Diversity and Conservation

Collage by R. Berdeen



1. Underside of *Ribes bracteosum* leaf exhibiting blister rust infection.
2. Dorena crew members laying out *Ribes* leaves in preparation for inoculation.
3. Pine seedlings under racks of *Ribes* leaves during inoculation.
4. Inoculation chamber at 100% RH with mist system engaged.
5. Infected pine seedling exhibiting numerous needle lesions.
6. Infected pine seedling with several stem cankers.
7. Infected pine seedling exhibiting
    a) a needle lesion
    b) an incipient stem canker at a needle fascicle
    c) a bark reaction
8. Frames of pine seedlings showing a high rate of mortality due to blister rust infection.

Collage by: R. Berdeen

Whitebark Pine Genetic Restoration Program for the Intermountain West (United States)

M.F. Mahalovich
G. A. Dickerson

Abstract—A strategy to restore whitebark pine communities is presented that emphasizes genetic resistance to white pine blister rust (*Cronartium ribicola* Fisch.) and mountain pine beetle (*Dendroctonus ponderosae* Hopkins), in combination with an active tree planting program. Early and active intervention may prevent listing of whitebark pine under the Endangered Species Act and further aid in the successful recovery of the grizzly bear (*Ursus arctos horribilis*). The restoration program initiated in 2001 includes a multi-State effort (Idaho, Montana, Oregon, Nevada, Wyoming, and Washington) designating permanent leave trees, emphasizing clean trees in high blister rust areas or areas with a high incidence of mountain pine beetle, or areas where both conditions are present. Cone collections from these trees will provide an immediate seed source for fire restoration, reforestation, *ex situ* genetic conservation, and seedlings to be screened for blister rust resistance. Pollen will be collected for genetic conservation and to advance blister rust resistance in seed and breeding orchards. Data generated from the rust screenings will identify whitebark pine seed sources that provide high levels of blister rust resistance and provide information needed to refine seed transfer guidelines. Leave trees elevated to elite-tree status, as identified by their rust-resistant progeny in the rust screenings, will serve as a seed source for operational collections and seed trees for natural regeneration. Survivors from the blister rust screening will be planted in clone banks for genetic conservation purposes, to serve as donors for future seed orchard establishment, and to facilitate selective breeding for blister rust resistance.

Key words: white pine blister rust resistance, fire restoration, genetic conservation, seed transfer guidelines.

Introduction

Whitebark pine, a keystone species in upper and subalpine ecosystems, provides a food source for grizzly bear, Clark's nutcracker (*Nucifraga columbiana*), and red squirrels (*Tamiasciurus hudsonicus*). It is also a foundation species for protecting watersheds as it tolerates harsh, windswept sites that other conifers cannot, the shade of its canopy regulates snowmelt runoff and soil erosion, and its roots stabilize rocky and poorly developed soils (Tomback and Kendall 2001).

The native pathogen, limber pine dwarf mistletoe (*Arceuthobium cyanocarpum* (A. Nelson ex Rydberg) Coulter & Nelson) and the exotic pathogen, white pine blister rust, are contributing to the overall decline of the species. The parasitism of dwarf mistletoe impacts cone and seed production, reducing the reproduction potential of whitebark pine in severely infested stands (Taylor and Mathiason 1999). White pine blister rust rapidly kills small trees, impeding successful regeneration. Blister rust infections in larger trees can persist a long time and are frequently found in the upper crown, reducing a tree's cone-bearing potential. Whitebark pine trees that survive blister rust infections are further threatened by mountain pine beetle attacks.

Wildfire occurrence aids in the preparation of a seed bed for natural regeneration. Fire suppression has reduced the role of fire in regeneration of pure whitebark pine stands and has allowed successional replacement of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and Engelmann spruce (*Picea engelmanni* Parry ex Engelm.) in mixed-conifer stands. Careful control is needed to reintroduce fire into high elevation ecosystems. Uncontrolled wildfire can destroy young whitebark pine regeneration and kill trees of cone-bearing age, which will limit the food supply for dependent wildlife and cause loss of future seed sources for restoration purposes.

High elevation ecosystems are at high risk because one or two species of white pines are usually dominant (McDonald and Hoff 2001). Because the loss of mature whitebark pine is occurring so rapidly, often in the absence of successful regeneration, there has been a pronounced loss of whitebark pine cover type. When only thinning and prescribed fire are utilized to promote vigorous stands of western white pine (*Pinus monticola* Dougl. ex D. Don), this has led to increased blister rust infection levels by opening up stands and encouraging *Ribes* spp. establishment (Schwandt and others 1994). Successful natural regeneration is dependent upon sufficient blister rust resistant seed available on site. This is due to the unique seed dispersal and seed caching by Clark's nutcrackers (Tomback and Schuster 1994) and red squirrels.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Mary F. Mahalovich is a Geneticist, USDA Forest Service, Northern, Rocky Mountain, Southwestern, and Intermountain Regions, Forestry Sciences Lab, 1221 S. Main Street, Moscow, ID, USA 83843. Office phone (208) 883-2350, Fax (208) 883-2318, e-mail mmahalovich@fs.fed.us. Gary A. Dickerson is Acting Budget Director, USDA Forest Service, Northern Region, P.O. Box 7669, 200 E Broadway, Missoula, MT, USA 59807. Office phone (406) 329-3352, Fax (406) 329-3132, e-mail gdickerson@fs.fed.us.

The 2000 fire season burned 929,000 ha on USDA National Forest System lands. Much of the fire devastation occurred in high elevation ecosystems, resulting in the destruction of both diseased and healthy whitebark pine trees.

Emergency National Fire Plan funding was made available in 2001 to initiate a landscape-level approach to restoring whitebark pine over the next 5 years on National Forest System lands in Idaho, Montana, Nevada, and Wyoming. Adjacent National Forests in Washington and Oregon were invited to participate. Glacier, Grand Teton, and Yellowstone National Parks, facing similar management challenges and stringent restoration policies (Kendall 1994), were also invited to participate. The scope of the program is based on cooperators whose landholdings are high elevation sites typically found in Federal ownership. The multi-State, multiagency collaboration forged in this endeavor provides a unified front to increase the likelihood of favorable outcomes in our restoration efforts, and a synergy that has been difficult to achieve by any one administrative unit or special project in the past.

Project Goals

The short-term goals over the next 5-year period are: (1) operational cone collections for planting burned areas, and (2) plus-tree identification and individual-tree cone collections for rust screenings and genetic conservation. These activities will facilitate identification of whitebark pine populations at most risk due to blister rust (more than 70 percent infection), which may require additional intervention to stabilize their survival. Field personnel will also become more familiar with the distribution of whitebark pine, which will provide land managers current information on the species distribution (Little 1971) and associated blister rust infection levels and mountain pine beetle infestations across the landscape. These data will also be used to adjust the number of plus-trees needed per zone and to develop a database for a seed transfer expert system.

Over the long-term, seedlings from the plus-tree selections will reveal patterns of genetic variation in survival, blister rust resistance, and early growth in rust screening trials. Data obtained from the rust screenings will help identify the presence or absence of various blister rust resistance mechanisms (Mahalovich and Eramian 1995) and their relative frequency among populations. The performance of the rust-resistant progeny will also be used to rank the original plus-trees. Those with high rankings (elite trees) will be identified as scion and pollen donors for seed orchard and clone bank establishment.

Implementation Plan

Cone Collections for Fire Rehabilitation

National Forests and Parks with immediate restoration needs should use the current seed zone boundaries to estimate their seed needs (fig. 1). There are no elevational restrictions on seed transfer within a seed zone. When blister rust infection levels vary within a zone, seeds collected for immediate rehabilitation efforts should not be moved from areas with low (less than 49 percent) to moderate (50 to 70 percent) infection levels to planting sites with higher infection levels (more than 70 percent). Seeds collected from phenotypically resistant trees in areas with high infection levels are suitable for planting on sites with low, moderate or high infection levels (Mahalovich and Hoff 2000).

Operational cone collections should be from no fewer than 20 individuals separated by 67 m within a zone to ensure a broad genetic base in the seed lot. This bulked seed lot collected from similar rust infection sites is referred to as a tree-seed zone or bulked collection.

Additional improvement in insect and disease resistance and growth can be achieved by collecting from above-average stands with more than 50 reproductively mature trees per 0.5 ha, emphasizing collections from a minimum of the 20 best trees. This bulked seed lot is referred to as a seed collection stand.

Moreover, communities with high blister rust infection or mountain pine beetle infestations, with at least 50 clean, reproductively mature trees per 0.5 ha, could be cultivated as a seed production area. This concept offers even more improvement, by first selecting an above-average stand, followed by removal of undesirable trees with insect and disease problems and poor growth and form, improving the genetic base of both the seed and pollen parents. These potential seed production areas will provide the most promising seed source for immediate cone collections until a grafted seed orchard of proven rust-resistant donors can be established and cultured for cone production.

Identifying Phenotypically Superior Individuals

An effective restoration strategy in whitebark pine includes components related to patterns of genetic variation, particularly to blister rust. Restoration efforts may be hampered if the assumption is made that whitebark pine and western white pine have a similar genetic response to blister rust. One key difference is that percent infection is higher in whitebark than western white pine (Bingham 1972, Hoff and others 1994, McDonald and Hoff 2001). Until more information becomes available on the biology and genetics of whitebark pine and blister rust in the Inland West, the best model to develop blister rust improvement in whitebark pine is the western white pine protocol (Mahalovich and Eramian 1995). Several modifications have recently emerged regarding the western white pine protocol and in the recommended breeding plan to develop resistance in whitebark pine put forth by Hoff and others (1994). The revised protocol follows.

Plus-tree selections (that is, designation of permanent leave-trees) are based on existing seed zones (fig. 1). Assignments within zones facilitates broad sampling among National Forests and Parks, emphasizing broadly adaptable populations for blister rust resistance development and isolated populations supporting unique gene frequencies or adapted gene complexes for gene conservation. If the target seed orchard size is 30 unrelated individuals, sufficient candidate trees must be identified within a zone to assure finding several genes for blister rust resistance in the rust screenings.

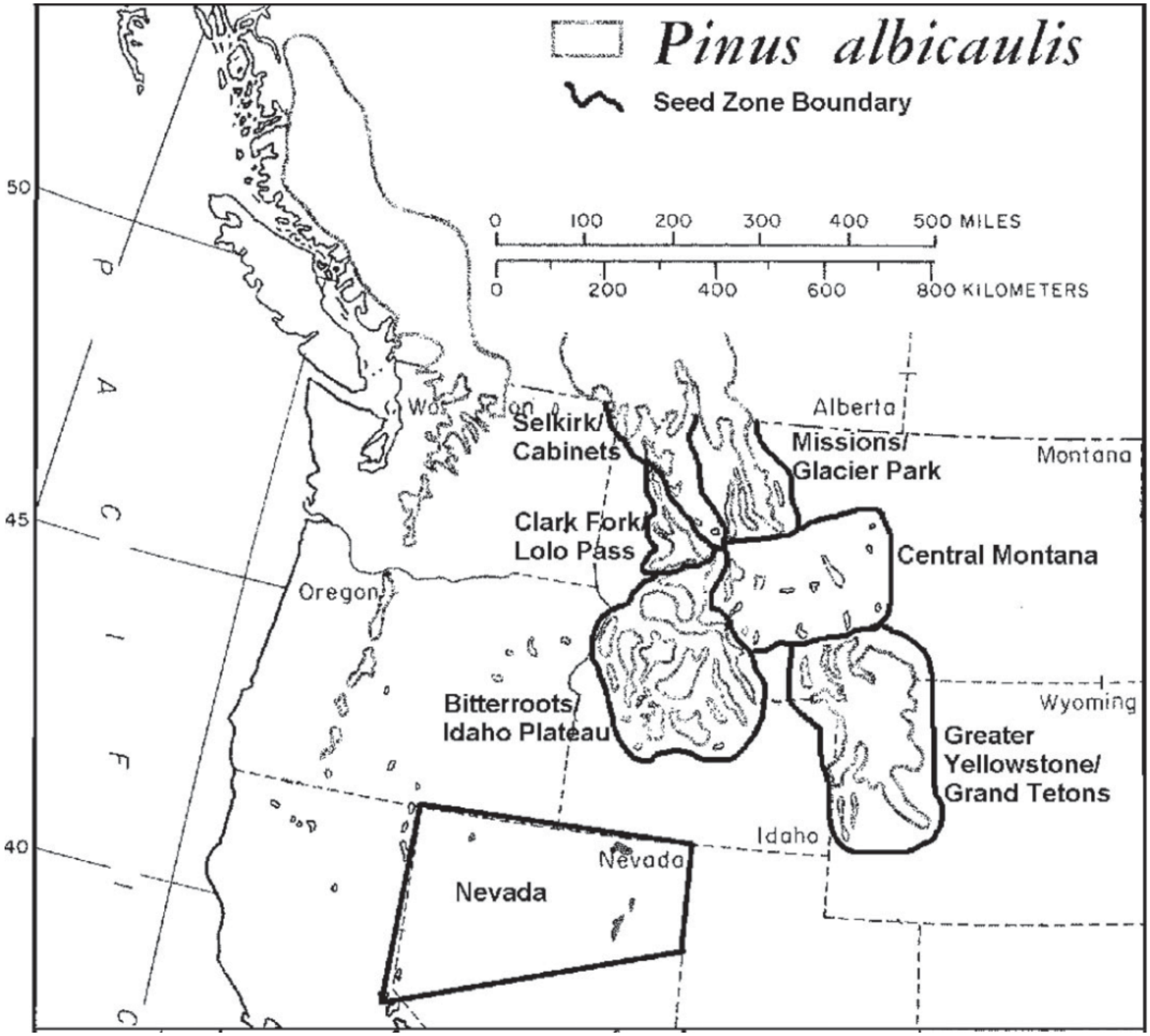


Figure 1—Whitebark pine seed zones for the Intermountain West, USA.

Approximately 100 plus-trees are assigned in each seed zone relative to the number of hectares of whitebark pine occurring on National Forests and Parks (Little 1971). The state of Nevada is comprised primarily of isolated populations with an expectation of 50 plus-trees for that zone. The outlier populations in northeastern Oregon are typically considered as part of the western range of whitebark pine (McCaughey and Schmidt 2001); however, these populations are also in proximity to the Bitterroots/Idaho Plateau seed zone boundary (fig. 1). Until more information becomes available on these populations, progeny from northeastern Oregon should be evaluated in rust screenings alongside progeny from both the Bitterroots/Idaho Plateau and the Nevada seed zones.

The total base population across all zones is 650 trees. The base population may seem small as compared to the 3,100 plus-trees in the western white pine tree improvement program (Mahalovich and Eramian 1995). The effective population size in western white pine is actually less than 3,100 plus-trees, as field validation has shown some trees separated by as little as 10 m will increase the probability that they are related. The goal is to have a moderate number of trees per zone to assure finding several genes for resistance. Problems in too small a population size within a zone may arise if 30 rust-resistant elite trees are not identified in a rust screening.

The western white pine program required 900 sound seeds per plus-tree; 300 to be set aside for gene conservation and the remaining to be sown to provide 144, 2-year-old container seedlings for rust screenings (Mahalovich and Eramian 1995). For whitebark pine, field units have been asked to collect 1,800 wind-pollinated seed per tree, 300 for gene conservation, and the remaining for rust screenings. Wire cages are recommended to protect the cones from bird and squirrel predation to achieve the target number of seeds per tree. The wire cages should be installed during June, on branches bearing second-year conelets. The increased number of seeds per tree are needed to compensate for low germination rates from sowing seedlots that have been in extended cold storage from the early 1990s (Burr and others 2001). Efforts are under way with the USDA Forest Service National Tree Seed Laboratory to study the special germination and seed storage requirements of whitebark pine, to make a seed bank a more promising gene conservation tool in the future.

Whitebark Pine Plus-Tree Selection Criteria

Stand-Level Selection Criteria—The stand selection criteria were relaxed for whitebark pine, emphasizing blister rust infection levels instead of mortality levels (table 1). If average mortality levels were followed, as was recommended for western white pine, almost no whitebark pine stands would qualify for plus-tree selections. Mortality levels can reach upwards of 90 percent or higher in whitebark pine stands in the Selkirk-Cabinet seed zone. Where field units do not support stands of whitebark pine (for example, more than 50 trees per 0.5 ha) and have dispersed trees in mixed-conifer settings, field personnel should move forward to the individual-tree selection criteria.

The average infection level for the target stand is determined by carefully counting both live and dead cankers on a representative sample of 100 living or dead trees. Presence or absence of cankers (bole and branch) from the 100-tree survey is used to determine the overall stand infection level. Actual counts should be made for main-bole cankers, whereas branch cankers can be estimated and grouped in the following categories: 0=no cankers present, 1 to 9 cankers, 10 to 20, 21 to 40, 41 to 75, 76 to 150, 150+ cankers. The combined total of main bole cankers and estimated branch cankers is equal to the number of cankers per tree. The average number of cankers per tree for the 100-tree survey then yields the stand average. When rust infection is heavy (some 90 percent), allowances are made for the possible presence of difficult-to-see or undetectable cankers (for example, flagging, dead tops, dead branches, and animal damage with extensive sap on the main bole are assumed to be due to a canker).

Each area should be more than 25 years of age and the average tree height around 10 to 35 m. This will increase the likelihood that the stand will have had at least 25 years of exposure to blister rust, be of cone bearing age, be producing pollen, and be climbable. A moderately open stand density is desirable so the target plus-trees are easy to examine from the ground, have persistent branches at ground level to facilitate climbing, and have full crowns for better cone-bearing potential (Hoff and McDonald 1980).

When rust infection levels are low (less than 50 percent) and whitebark pine grows in either a mixed- or pure-stand setting, field units should proportionally balance the number

Table 1—Whitebark pine plus-tree selection criteria.

Stand level criteria	Individual-tree level
Vigorous and representative of the species	Dominant or co-dominant trees
Habitat type where species normally occurs	Minimum of 100 [1] m between selected trees to avoid relatedness
Provide a broad sample of both the geography and range of elevations	Free of insects and diseases
Overall composition has a high proportion of living or dead whitebark pine, well represented throughout the stand	Have a history or the potential to bear cones
Uniformly and heavily infected with blister rust (10 or more cankers per tree on the average)	Be within 100 to 200 m from the nearest road or trail
Confirmed blister rust infection of 90 percent or higher in uniform stands	No more than three of the best candidates in any given stand
Stands with 50 to 90 percent rust infection, limit selected trees to no more than five cankers	No squirrel cache cone collections

[1] Spacing between plus-trees (100 m) differs from spacing requirements in operational cone collections (67 m).

of selections between the two stand types. Likewise, if field units have both concentrated stands and sparsely distributed whitebark pine, plus-tree collections should be proportionally balanced based on the number of hectares occurring in both types of tree densities.

Individual-Tree Selection Criteria—Each plus-tree should be relatively free of blister rust when compared to the overall infection level in the stand. Allowable infection levels for each plus-tree (table 2) are modeled after Hoff and McDonald (1977, 1980). The presence or absence of cankers is determined by examining each tree both from the ground with binoculars and by climbing the tree and examining each individual whorl. Though desirable, based on the preliminary field reports, accurate canker counts are difficult in whitebark pine because of the high levels of infection, sap weeping from cankers and animal damage (chewing), as compared to western white pine.

Three growth forms are acceptable in whitebark pine: single-stem, erect; multiple-stem, erect; and krummholz. Dominant or co-dominant trees are preferable, but the multiple-stem, erect or krummholz categories may lend themselves more to the intermediate or suppressed crown classes. In contrast to western white pine, the acceptable growth form is the single-stem, erect form, or the timber archetype in the dominant or co-dominant crown class.

Each tree should be free of insects, particularly mountain pine beetle, and other diseases such as limber pine dwarf mistletoe, as these characteristics are likely inherited and passed onto their progeny. Squirrel-cache cone collections should be avoided because of unknown parentage and because seeds have come in contact with forest litter and soils, increasing the likelihood of seed-borne fungi *Fusarium* spp., *Sirococcus strobilinus*, and the snow bank or cold fungus, *Calocypha fulgens* (Kolotelo and others 2001, Hoff and Hagle 1990).

Each tree should be within 100 to 200 m from the nearest road or trail, unless intervening vegetation is sparse enough so that longer lines of sight are possible, to facilitate caging of branches to protect cone crops from bird predation and for ease of relocation. When plus-trees are easily accessible by road or trail, the possibility exists to use cherry pickers or man-lifts to collect cones from the upper portion of the crown.

Care should be taken to avoid collections from limber pine, when whitebark and limber pine are intermixed on the same national forest or park. The operational cone collection guidelines for whitebark pine (Mahalovich and Hoff 2000) provide additional information on how to distinguish the two species by cone morphology, strobilus color, and pollen catkin color.

Table 2—Acceptable canker limits for individual plus-trees based on stand averages.

Stand average (cankers/tree)	Plus-tree limits
10 to 20	No cankers
21 to 40	1 canker
41 to 75	2 cankers
76 to 150	3 cankers
151+	4 or 5 cankers

Blister Rust Screening Trials

A rust screening will let us know how successful our restoration efforts may be by identifying the amount of genetic variation present in survival and disease resistance and by quantifying how much of that variation occurs among or within stands.

The progeny of 200 plus-trees can be reliably handled in a rust screening, allowing approximately two seed zones to be tested at a time. A bulked check lot of untested seed from existing whitebark pine seed lots will need to be constructed upfront, to facilitate comparisons among the plus-trees. Rust screening scheduling will depend on how quickly each field unit completes its plus-tree selections within a zone. The goal is to sow a rust screening trial by 2005.

Modifications in the composition of aeciospore samples are recommended as a conservative course of action for inoculating *Ribes* spp. in the rust screening trials. Low levels of genetic differentiation exist among samples of *C. ribicola* collected from eastern white pine (*Pinus strobus* L.) in eastern North America (Et-touil and others 1999) and among *C. ribicola* samples collected from western white pine in western North America (Kinloch and others 1998). Little is known however, about specific races of blister rust in the Inland West in western white or whitebark pine. One exception is the identification of yellow and red-spotting races occurring on western white pine (McDonald 1978), with one type not necessarily more virulent than the other. *Ribes* spp. leaves used in the inoculations should be treated with aeciospores collected from cankers on whitebark pine, in the event there are different rust populations in whitebark and western white pine communities. Aeciospores will be collected 1 to 2 years prior to rust screening from a representative sample across all seed zones. State-to-state plant inspection regulations may prohibit the transfer of spore collections across state lines, so further modifications in the rust screening protocol may be warranted in the future.

This conservative approach is also appropriate when considering the alternate host, because a different mix of *Ribes* spp. occurs in whitebark pine communities (for example, *Ribes lacustre*, *R. viscosissimum*, and *R. montigenum)* than in western white pine (for example, *R. cereum*, *R. nigrum* and *R. hudsonianum* var. *petiolare)*. A Ribes garden for whitebark pine inoculations was established at Lone Mountain Tree Improvement Area, Idaho Panhandle National Forests in 2000.

Hoff and others (1994) recommended inoculating 2-year old whitebark pine seedlings. Due to the slower growth rates of whitebark pine as compared to western white pine, these rust screenings will use 3-year old container seedlings in order to have enough top shoot and secondary needles to be challenged with inoculum.

During the inoculation procedure, basidiospores will be delivered at a target rate of 3,500 spores per cm^2. Previous rust screenings of whitebark pine using a rate recommended for western white pine have shown a delivery of 6,000 spores per cm^2 to be too high, killing most of the seedlings in a given block (Mahalovich unpublished data).

Four rust inspections will be performed in each trial. The first and second inspections will occur 9 months and 12 months, respectively, after inoculation. The third and fourth inspections will occur during September in subsequent years. Overall, the four rust inspections span a 3-year period. Data collected during each inspection will be the same as data acquisition for western white pine trials (Mahalovich and Eramian 1995).

Last, to minimize cross-contamination of susceptible seedlings and inoculated *Ribes* spp. leaves, and the possible introduction of virulent rust races between species, a recommended quarantine procedure is to avoid inoculating western white and whitebark pine seedlings in the same calendar year at the same location (Coeur d'Alene Nursery, Coeur d'Alene, Idaho).

Data Applications

Refine Seed Transfer Guidelines—Seed transfer (Mahalovich and Hoff 2000) is currently based on seed zones (fig. 1) driven by major mountain ranges and existing knowledge of blister rust infection levels in populations of whitebark pine (Hoff and others 1994). A better approach to seed transfer is to develop guidelines based on phenological and blister rust resistance data. Early genetic studies using isozymes point to low levels of genetic variation among and within-stands of whitebark pine (Lanner 1982, Jorgensen and Hamrick 1997, Bruederle and others 1998). Richardson (2001) examined uniparentally inherited mitochondrial (mt)DNA and chloroplast cp(DNA) microsatellites (cpSSRs) to examine population genetic structure from 38 coastal and interior populations of whitebark pine. Analysis of Molecular Variance (AMOVA) groups based on an exact test suggested four zones among Inland West populations: Sierra Nevada Mountains, Yellowstone, central Idaho, and northern Idaho. Data obtained from the sites sampled for plus-trees (blister rust infection levels) and the rust screening trials will validate whether the existing seed zones could be combined into four zones, determine where the geographic boundaries should be drawn, and provide a model for predicting safe seed transfer for individual seed lots using a seed transfer expert system. Zone boundaries will be revised before proceeding with the establishment of seed orchards and clone banks.

Seed Orchard Establishment and Design—Each plus-tree will be ranked based on the performance of its progeny in the rust screening trials using the same evaluation criteria established in western white pine (Mahalovich and Eramian 1995). Preliminary rust screenings have shown whitebark pine seedlings to exhibit rust resistance responses much like the other five-needle pines but at different frequencies (Hoff and others 1980, Hoff and Hagle 1990). The higher-ranking parent trees will be revisited to collect scion for establishing production seed orchards within each zone. Sowing and growing of rootstock will be coordinated with the completion of each rust screening. Until these orchards reach reproductive maturity, the rankings of the plus-trees can be matched to their native stands to identify promising cone collecting areas (seed collection stand or seed production area) not previously identified during 2001 through 2005, to meet more immediate seed needs for resistant planting stock.

Data collected from the rust screenings will also be used to facilitate seed orchard design and seed deployment strategies by resistance mechanism(s). This strategy of using patterns of genetic variation and deploying more than one resistance mechanism on any given hectare makes it unlikely a new, more virulent race of rust will develop in planted stock (Mahalovich and Eramian 1995).

Pollen can be a limiting factor in immature pine orchards, when the goal is to obtain enough sound seed from a broad genetic base as quickly as possible. A practical application of collecting whitebark pine pollen will be supplemental mass pollination in the grafted seed orchard(s) to promote an earlier cone crop rather than relying on wind pollination. Unlike long-term storage of whitebark pine seed, there are no major pollen viability problems over the long-term with *Pinus* spp., as long as the pollen is properly extracted and stored.

Additional Gene Conservation Measures—Pollen will also be collected to establish a pollen bank as part of the *ex situ* strategy and to advance blister rust resistance in seed and breeding orchards.

The surviving progeny in each rust screening will be used to establish clone banks. Though not in our life times, these clone banks could serve as an operational cone collection site if they are designed by zone, concentrating the better performers in the interior core to enhance gain and in grouping trees by resistance mechanism, as is done in the Phase II western white pine seed orchards (Mahalovich and Eramian 1995).

Last, this information can be cross-referenced with field inventories to prioritize those communities that are good candidates to stabilize their numbers by active intervention involving prescribed fire to promote natural regeneration and by removal of encroaching species such as subalpine fir, lodgepole pine and Engelmann spruce.

Summary

This restoration strategy highlights the need to incorporate genetic considerations into a comprehensive strategy to restore whitebark pine. It emphasizes the biology and genecology of the host species, with a modest emphasis on the biology and ecology of the rust. The amount of gain achieved in blister rust and mountain pine beetle resistance will be determined by how many cones are collected from presumably rust-free and insect-free trees in areas with a high frequency of blister rust and insect populations. Meaningful levels of genetic variation are needed in adaptive traits (for example, survival, growth, insect and disease resistance) to develop seed transfer guidelines and improved planting stock.

Acknowledgments

We thank Robert L. Schrenk and Michael J. Paterni for their leadership and support for a landscape approach to whitebark pine restoration and the many silviculturists, wildlife biologists, pathologists, and fire fighters on National Forest System and National Park lands involved in cone and spore collections. We also thank Drs. Sue Hagle, Mee-Sook Kim, Ned Klopfenstein, Gerald E. Rehfeldt, and John Schwandt, and Ms. Maridel Merritt for their comments on an earlier version of this manuscript. This work is funded in part by USDA Forest Service Title IV Wildland Fire Emergency, Rehabilitation and Restoration Appropriations.

References

Bingham, R.T. 1972. Taxonomy, crossability, and relative blister rust resistance of 5-needled white pines. In: Bingham, R.T., Hoff, R.J. McDonald, G.I., eds. Biology of rust resistance in forest trees, USDA Forest Service Misc. Pub. 1221, Washington, DC, pp 271-280.

Bruederle, L.P., D.F. Tomback, K.K. Kelly, Hardwick, R.C. 1998. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (Pinaceae). Can. J. Bot. 76:83-90.

Burr, K.E., Eramian, A., Eggleston, K. 2001. Growing whitebark pine seedlings for restoration. *In* Whitebark pine communities, Island Press, Washington, D.C., pp 325-345.

Et-touil, K. Bernier, L., Beaulieu, Bérubé, J.A., Hopkin, A., Hamelin, R.C. 1999. Genetic structure of *Cronartium ribicola* populations in eastern Canada. Phytopathology 89:915-919.

Hoff, R.J., Bingham, R.T., McDonald, G.I. 1980. Relative blister rust resistance of white pines. European Jounral of Forest Pathology. 10:307-316.

Hoff, R.J., Hagle, S. 1990. Disease of whitebark pine with special emphasis on white pine blister rust. *In* Proc.—Symposium on Whitebark Pine Ecosystems: Ecology and Management of a High-Mountain Resource, March 29-31, 1989, Bozeman, MT, USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GTR-270, Ogden, Utah, pp 179-190.

Hoff, R.J., Hagle, S.K., Krebill, R.G. 1994. Genetic consequences and research challenges of blister rust in whitebark pine forests. *In* Proc. International Workshop on Subalpine Stone Pines and Their Environment: the Status of Our Knowledge, September 5-11, 1992, St. Moritz, Switzerland, USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GTR-309, Ogden, Utah, pp 118-135.

Hoff, R.J., McDonald, G.I. 1980. Improving rust-resistant strains of Inland Western white pine. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah, Research Paper INT-245, 13 p.

Hoff, R.J., McDonald, G.I. 1977. Selecting western white pine leave-trees. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah, Research Note INT-218, 2p.

Jorgenson, S.M., Hamrick, J.L. 1997. Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. Can. J. For. Res. 27:1574-1585.

Kendall, K. 1994. Whitebark pine conservation in North American National Parks. *In* Proc. International Workshop on Subalpine Stone Pines and Their Environment: the Status of Our Knowledge, September 5-11, 1992, St. Moritz, Switzerland, USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GTR-309, Ogden, Utah, pp 302-305.

Kinloch,B.B., Jr., Westfall, R.D., White, E.E., Gitzendanner, M.A., Dupper, G.E., Foord, B.M, Hodgskiss, P.D. 1998. Genetics of *Cronartium ribicola*. IV. Population structure in western North America. Can. J. Bot. 6:91-98.

Kolotelo, D., Van Steenis, E., Peterson, M., Bennett, R., Trotter, D., Dennis, J. 2001. Seed Handling Guidebook. B.C. Ministry of Forests, Tree Improvement Branch, 106 pp.

Lanner, R.M. 1982. Adaptation of whitebark pine for seed dispersal by Clark's nutcracker. Can. J. For. Res. 12: 391-402.

Little, E.L. 1971. Atlas of United States Trees. Volume 1. Conifers and important hardwoods. USDA Forest Service Misc. Pub. No. 1146, Washington, DC, p 43-W.

Mahalovich, M.F., Hoff, R.J. 2000. Whitebark pine operational cone collection instructions and seed transfer guidelines. Nutcracker Notes No. 11, pp 10-13.

Mahalovich, M.F., Eramian, A. 1995. Breeding, seed orchard, and restoration plan for the development of blister rust resistant white pine for the northern Rockies. USDA Forest Service Northern Region and Inland Empire Tree Improvement Cooperative, 60 pp.

McCaughey, W.W., Schmidt, W.C. 2001. Taxonomy, distribution, and history. *In* Whitebark pine communities, Island Press, Washington, D.C., pp 29-40.

McDonald, G.I. 1978. Segregation of "red" and "yellow" needle lesion types among monoaeciospore lines of *Cronartium ribicola*. Can. J. Genet. Cytol. 20:313-324.

McDonald, G.I., Hoff, R.J. 2001. Blister rust: an introduced plague. *In* Whitebark pine communities, Island Press, Washington, D.C., pp 193-220.

Richardson, B.A. 2001. Gene flow and genetic structure of whitebark pine (*Pinus albicaulis*): inferences into bird-dispersed seed movement and biogeography. Unpublished Master's Thesis, University of Idaho, College of Forest Resources, Moscow, ID, 55pp.

Schwandt, J.W., Marsden, M.A., McDonald, G.I. 1994. Pruning and thinning effects on white pine survival and volume in northern Idaho. *In* Proc. Interior Cedar-Hemlock-White Pine Forests: Ecology and Management, March 2-4, 1993, Spokane, WA. *Edited* by Baumgartner, D.M., J.E. Lotan, and J.R. Tonn,Washington State University, Department of Natural Resources, Cooperative Extension, pp. 167-172.

Taylor, J.E., Mathiason, R.L. 1999. Limber pine dwarf mistletoe. USDA Forest Service, Forest Insect and Disease Leaflet No. 171, Northern Region, Missoula, MT.

Tomback, D.F., Kendall, K.C. 2001. Biodiversity losses: the downward spiral. *In* Whitebark pine communities, Island Press, Washington, D.C., pp 243-262.

Tomback, D.F., Schuster, W.S.F. 1994. Genetic population structure and growth form distribution in bird-dispersed pines. *In* Proc. International Workshop on Subalpine Stone Pines and Their Environment: the Status of Our Knowledge, September 5-11, 1992, St. Moritz, Switzerland, USDA Forest Service, Intermountain Research Station, Gen. Tech. Rep. INT-GTR-309, Ogden, Utah, pp. 43-50.

Diversity and Conservation of Genetic Resources of an Endangered Five-Needle Pine Species, *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima

Sei-ichi Kanetani
Takayuki Kawahara
Ayako Kanazashi
Hiroshi Yoshimaru

Abstract—*Pinus armandii* var. *amamiana* is endemic to two small islands in the southern region of Japan and listed as a vulnerable species. The large size and high wood quality of the species have caused extensive harvesting, resulting in small population size and isolated solitary trees. Genetic diversity of the *P. armandii* complex was studied using allozyme analyses. Genetic distances between (1) *P. armandii* var. *amamiana* and *P. armandii* var. *armandii* and (2) *P. armandii* var. *amamiana* and *P. armandii* var. *mastersiana* were 0.488 and 0.238, respectively. These genetic differences were comparable with congeneric species level (0.4) and much greater than conspecific population level (less than 0.1) that occur in *Pinus* species in general. No differences in diversity were recognized between populations of *P. armandii* var. *amamiana* from each island. The impact of human activity on the endangered status of *P. armandii* var. *amamiana* in Tane-ga-shima Island was demonstrated by inspecting historical records, starting in the 16th century. A strategy for conservation of *P. armandii* var. *amamiana* was discussed in consideration of sparse distribution, pollen flow, and the effects of pine wilt disease, caused by *Bursaphelenchus xylophilus*.

Key words: *Pinus armandii* var. *amamiana*, endangered species, genetic diversity, pollen flow, conservation strategy

Introduction

Pinus armandii Franch. var. *amamiana* (Koidz.) Hatusima, is an endangered pine species endemic to Tane-ga-shima and Yaku-shima Islands, southern Japan (Yahara and others 1987). The species is closely related to *P. armandii* var. *armandii* that is distributed in the western part of continental China and *P. armandii* var. *mastersiana* Hayata from the highlands of Taiwan. The wood of *P. armandii* var. *amamiana* was traditionally used for making fishing canoes and also used in house construction (Kanetani and others 2001). Consequently, large numbers of *P. armandii* var. *amamiana* trees have been harvested and populations have dwindled on both islands. Currently, the estimated number of surviving *P. armandii* var. *amamiana* trees in natural populations are 100 and 1,000 to 1,500 on Tane-ga-shima and Yaku-shima Islands, respectively (Yamamoto and Akasi 1994).

In recent years, the number of *P. armandii* var. *amamiana* trees has rapidly declined, with dead trees frequently observed (Hayashi 1988; Yamamoto and Akashi 1994; Kanetani and others 2002). Several factors are responsible for the recent decline, including inbreeding depression (Hayashi 1988; Kanazashi and others 1998), reduced natural regeneration (Chigira 1995; Kanetani and others 1998), and pine wilt disease (Hayashi 1988; Yamamoto and Akashi 1994; Nakamura and others 2002). *Pinus armandii* var. *amamiana* has been classified as an "Endangered" species in the *Japanese Red List*, a compilation of endangered Japanese species, due to the rapid decrease in population size and the isolation of small populations (Environment Agency of Japan 2000).

In order to establish an *in situ* conservation scheme for an endangered species, it is important to collect information on the species in natural habitats, such as decline and genetic variation (compare Primack 1995; Meffe and Carroll 1997). In this study, we clarified the genetic diversity and phylogenetic relationship of *P. armandii* var. *amamiana* with other *P. armandii* varities, researched the historical distribution of the species on Tane-ga-shima Island, and propose a strategy to conserve the genetic resources of this species.

Materials and Methods

Study Site

Tane-ga-shima and Yaku-shima Islands are located about 60 km south of Kyushu Island in southern Japan (fig.1). These islands are approximately 20 km apart. Tane-ga-shima Island is relatively flat (elevation to 282 m), 58 km long (north to south), and 10 km wide (west to east). In contrast, Yaku-shima Island is round with a diameter of about 30 km and dominated by a series of peaks over 1,800 m in height. About 21 percent of the island has been registered as a World Natural Heritage Area in 1993.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Sei-ichi Kanetani, Ayako Kanazashi, and Hiroshi Yoshimaru are with the Ecological Genetics Laboratory, Department of Forest Genetics, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan. Phone: +81- 298-73-3211 ext. 445; FAX: +81- 298-74-3720; e-mail: kanekane@ffpri.affrc.go.jp. Takayuki Kawahara is with the Hokkaido Research Center, Forestry and Forest Products Research Institute, Japan.

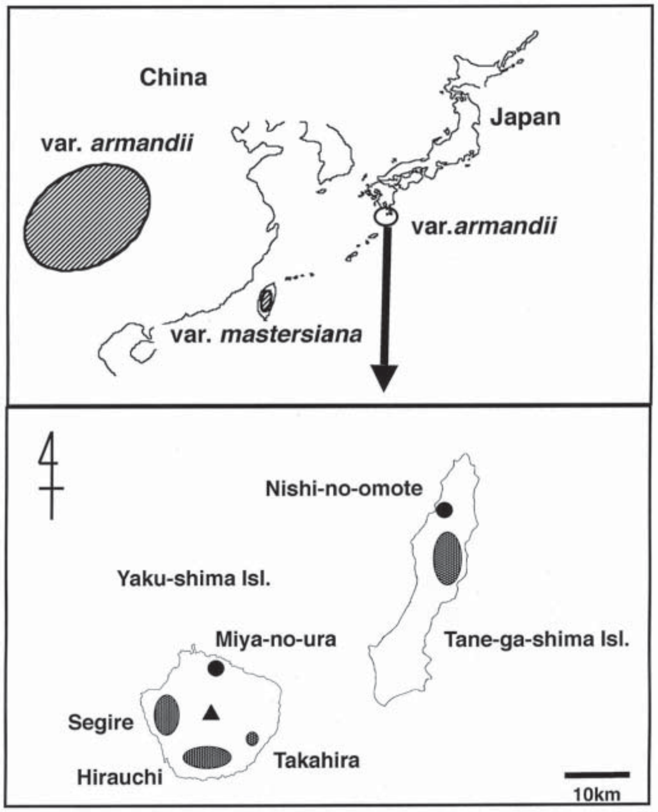


Figure 1—Distribution of *Pinus armandii* vars. *amamiana*, *armandii* and *mastersiana* populations.

Surviving *P. armandii* var. *amamiana* trees are located in the center of Tane-ga-shima Island, while three local populations of this species are distributed from 300 to 800 m elevation on Yaku-shima Island (Yamamoto and Akashi 1994; Kanetani and others 1997).

Genetic Variation

We collected 88 samples of *P. armandii* var. *amamiana* from three populations, Segire, Takahira and Hirauchi, on Yaku-shima Island, and 17 samples from Tane-ga-shima Island. The trees from Tane-ga-shima Island were treated as one population because most trees are solitary or occur in small groups. We collected 24 samples of *P. armandii* var. *armandii* from one natural population near Dali, Yunnan, China, and 12 samples of *P. armandii* var. *mastersiana* from seedlings originating from a population near Musha, Taiwan.

Crude extracts from inner bark of twigs were prepared according to Yahara and others (1989) for allozyme analyses. The starch gel electrophoretic system with tris-borate buffer (#8 in Soltis and others 1983) was used for allozyme analysis on mannose phosphate isomerase (*MPI*; 1), phosphoglucoisomerase (*PGI*; 2) and triose-phosphate isomerase (*TPI*; 2). The numbers in parentheses refer to the numbers of allozyme loci employed in the following analyses. Another starch gel electrophretic system with histidine and citric acid buffer (Cardy and others 1981) was used for isocitrate dehydrogenase (*IDH*; 1), malate dehydrogenase (*MDH*; 2), 6-phosphogluconate dehydrogenase (*6PGD*; 1), phosphoglucomutase (*PGM*; 2), shikimate dehydrogenase (*SkDH*; 1) and UDP glucose pyrophosphorylase (*UGP*; 1). Acrylamide gel electrophoresis (Tsumura and others 1990) were used for asparatate aminotransferase (*AAT*; 2), amylase (*AMY*; 1), esterase (*EST*; 1), glutamate dehydrogenase (*GDH*; 1) and leucine aminopeptidase (*LAP*; 2).

Calculations of Genetic Parameters

Three kinds of genetic diversity (*P*, rate of polymorphic loci; *A*, mean number of alleles; *He*, expected mean heterozygosity) and fixation index for the populations of *P. armandii* var. *amamiana* were calculated. For calculations of Nei's genetic distance and genetic identity (Nei 1972), the Segire population was excluded, since the number of examined loci was different from those of the other populations. F-tests were used to detect significant differences.

Historical Distribution of *P. armandii* var. *amamiana* on Tane-ga-shima Island

We surveyed historical records owned by Kagoshima Prefectural Museum of Culture, Reimei-kan for all descriptions on surviving number and size of "Goyo-matsu (5-needle-pine; var. *amamiana*)" and the number of "Maruki-bune" (canoes) constructed.

Results

Genetic Variation

The three populations on Yaku-shima Island showed values of 0.069-0.131 (mean: 0.100) for *He*, while the *He* value for the Tane-ga-shima population was 0.112 (table 1). Although the population of surviving trees in Tane-ga-shima Island is much less than populations on Yaku-shima Island, it contains the nearly same amount of genetic diversity. The Fixation Index (*F*) in the Tane-ga-shima population, 0.198 (not significant), is larger than those of three populations in Yaku-shima Island, 0.012-0.074 (mean: 0.051).

Genetic identity and Nei's standard genetic distance among the populations of *P. armandii* vars. *amamiana*, *armandii*, and *mastersiana* were shown in table 2. Mean genetic distance among *P. armandii* var. *amamiana* populations was ranged from 0.003 to 0.029 (mean: 0.017). Genetic distances between *P. armandii* var. *amamiana* vs. var. *mastersiana*, *P. armandii* var. *amamiana* vs. var. *armandii* and *P. armandii* var. *armandii* vs. var. *mastersiana* were 0.460-0.510 (mean: 0.488), 0.220-0.250 (mean: 0.238) and 0.137, respectively.

Historical Distribution on Tane-ga-shima Island

In Tane-ga-shima Island, harvest of large *P. armandii* var. *amamiana* populations was regulated by the Shimadzu local government from the 16th through the 19th century (Kanetani and others 2001). Descriptions of the number and stem girth of *P. armandii* var. *amamiana* trees were found from 1685 to 1782. In particularly, 428 *P. armandii* var. *amamiana* trees with stem girth above 150 cm were recorded in 1755. Table 3 is a summary of the number of

Table 1—Genetic diversity and fixation index of *Pinus armandii* var. *amamiana* populations.

Population	Number of loci	P^a	A^b	He^c	F^d
Segire (Yaku-shima Island)	13	0.31	1.31	0.069	0.067^{ns}
Takahira (Yaku-shima Island)	20	0.50	1.60	0.131	0.074^{ns}
Hirauchi (Yaku-shima Island)	20	0.40	1.45	0.101	0.012^{ns}
Tane-ga-shima Island	20	0.45	1.55	0.112	0.198^{ns}

a: rate of polymorphic loci
b: mean number of alleles per locus
c: mean heterozygosity
d: fixation index
ns: not significantly different from zero

Table 2—Genetic identity (upper triangle) and Nei's standard genetic distance (lower triangle) among *Pinus armandii* varieties.

Population (variety)	1	2	3	4	5
1. Takahira (var. *amamiana*)		.980	.998	.631	.803
2. Hirauchi (var. *amamiana*)	.020		.971	.600	.779
3. Tane-ga-shima Island (var. *amamiana*)	.003	.029		.610	.784
4. Dali, Yunnan, China (var. *armandii*)	.460	.510	.495		.872
5. Musha, Taiwan (var. *mastersiana*)	.220	.250	.244	.137	

Table 3—Historical record of stem girth and number of trees of *Pinus armandii* var. *amamiana* growing on Tane-ga-shima Island.

Year	Stem girth (cm)	Number of trees
1685	—	247
1748	210 - 420	355
1755	210 - 420	218
	150 - 180	210
1782	—	28

trees and stem girth of *P. armandii* var. *amamiana* found in the historical record.

It is known large numbers of *P. armandii* var. *amamiana* were harvested for making fishing canoes and house construction during late 19th and early 20th century (Kanetani and others 2001). In 1918, 455 canoes were made probably from *P. armandii* var. *amamiana*. Canoes were used for fishing until twenty years ago in Tane-ga-shima Island.

Discussion

The genetic diversity level of *P. armandii* var. *amamiana* (*He*: 0.069-0.131) is a little lower than the mean value of the genus *Pinus* (*He*: 0.136) (Hamrick and others 1992), in which Hamrick and Godt (1996) recognized wide variation of genetic diversity and population structure. In comparison, *Pinus torrayana*, an endemic species in California with an extremely restricted distribution, has a low genetic diversity of 0.017 (Ledig and Conkle 1983). *P. armandii* var. *amamiana* also has a limited distribution, but maintains a more or less high genetic diversity level. In Tane-ga-shima Island, the trees are separated each other but still retain a level of genetic diversity as populations on Yaku-shima Island. This indicates that the serious decrease of tree number in Tane-ga-shima Island occurred in the near past. Fixation indices are almost zero in Yaku-shima Island populations and 0.198 in Tane-ga-shima Island. This value shows that adult *P. armandii* var. *amamiana* trees in Yaku-shima Island have been produced from random mating. The high value in Tane-ga-shima Island may be explained by the Wahlund effect.

The genetic difference among the three conspecific taxa of *P. armandii* is large. Hamrick and Godt (1996) reviewed genetic heterogeneity of pine populations and introduced some species with disjunct distributions that have high gene diversity levels. The genetic difference of the varieties in *P. armandii* is at the congeneric species level (0.4) and much greater than conspecific population level (less than 0.1) according to Gottlieb (1977, 1981) and Crawford (1983). Therefore genetic conservation on *P. armandii* should be conducted at least for varieties *amamiana*, *armandii* and *mastersiana*, respectively.

A bibliographical study demonstrated the destructive cuttings of *P. armandii* var. *amamiana* in Tane-ga-shima Island until the early 20th century. The species was preferred for making canoes because of a greater amount of resin than in other tree species in Tane-ga-shima Island and only large trees, 2.7m - 6.3 m were used. The historical record indicates that 400 trees were preserved about 250 years ago (table 3). In 1918, however, harvesting must have increased, as 455 canoes made of *P. armandii* var. *amamiana*, were counted. Recently, we have discovered that a few *P. armandii* var. *amamiana* trees were harvested for use in quarries (Kanetani and others 2001). Therefore, the human impact on this species is still continuing.

As a consequent of human impacts, the reproductive potential of the surviving trees on Tane-ga-shima Island may be limited. Kanazashi and others (1998) and Nakashima and Kanazashi (2000) compared cone yields from artificial pollinations to natural (open) pollinations on several isolated trees on Tane-ga-shima Island. The percentage of filled seeds per cone from natural pollinations (15.4 to 37.7 percent) was less than artificial cross-pollinations, which ranged from 66.8 to 97.3 percent. However, there was no significant difference (F-test) in the percentage of filled seeds after artificial self-pollination (34.3 percent) and after open-pollination (34.6 percent). This suggests that natural pollination among Tane-ga-shima Island trees could be primarily self-pollination, which will promote inbreeding depression.

P. armandii var. *amamiana* are now threatened by pine wilt disease, an epidemic disease of the genus *Pinus* in Japan caused by the nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Kiyohara and Tokushige 1971; Kishi 1995). This disease has been inferred to be a major mortality factor of *P. armandii* var. *amamiana* in natural populations (Hayashi 1988; Yamamoto and Akashi 1994). Recently, the nematode's presence was confirmed through detection in dead *P. armandii* var. *amamiana* trees in Tane-ga-shima Island (Nakamura and others 2002). Pine wilt disease, therefore, is a significant threat to the continued existence of natural populations of *P. armandii* var. *amamiana*.

The serious decline of *P. armandii* var. *amamiana* necessitates the formation of a strategy for genetic conservation. Protection and management should be required for *in situ* populations on both islands, and demise from pine wilt disease should be carefully monitored. The small, diffuse population on Tane-ga-shima Island requires artificial cross pollinations for restoration of seed fertility and successful reproduction. Additionally, the establishment of *ex situ* plantations containing grafts of mature trees of *P. armandii* var. *amamiana* (for *ex situ* conservation) is needed to ensure conservation of genetic diversity of Tane-ga-shima Island populations.

Acknowledgments

We are very grateful to K. Nakashima for his valuable contributions to our research. Special thanks are due to the Yaku-shima Management Office of the Environment Agency, the Yaku-shima Forest Environment Conservation Center and the Kagoshima Forest Office of the Forest Agency for permission to conduct this study. We express our gratitude to H. Iwatsubo, M. Nao, Y. Sameshima, M. Okumura, Y, Furuichi, K. Tokunaga and Y. Oguchi for their kind help with bibliographical and field studies.

References

Cardy, B.J., Sturber, C.W. and Goodman, M.N. 1981. Technique s for starch gel electrophoresis of enzyme from maize (*Zea mays*), revised. Institute of Statistic Mimeograph Series No. 1317. North Carolina State University, Raleigh.

Chigira, O. 1995 Seed fertility of *Pinus armandii* Franch. var. *amamiana* Hatusima, one of the vulnerable species in Japan. Trans. Jpn. For. Soc. 106: 303-304. (in Japanese)

Crawford, D. J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. *In* Isozymes in Plant Genetics and Breeding.Part A. Elsevier, Amsterdam, pp. 257-287.

Environment Agency of Japan. 2000. Threatened Wildlife of Japan-Red Data Book 2nd ed.- Volume 8, Vascular Plants. Japan Wildlife Research Center, Tokyo, Japan, pp.271. (in Japanese)

Gottlieb, L. D. 1977. Electrophoretic evidence and plant systematics. Ann. Missouri Bot. Gard. 64: 161-180.

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7: 1-46.

Hamrick, J.L., Godt, M.J.W. 1996. Conservation genetics of endemic plant species. *In* Conservation Genetics. Chaman & Hall, New York, pp. 281-304.

Hamrick, J. L., Godt, M.J.W. and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95-124.

Hayashi, S. 1988. Protection and preservation of *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima (*P. amamiana* Koidz.). For. Tree Breed. 147: 11-13. (in Japanese)

Kanazashi, A., Nakashima, K. and Kawahara, T. 1998. A study for genetic resources conservation of *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima. For. Tree Breed. 188: 24-28. (in Japanese)

Kanetani, S., Akiba, M., Nakamura, K., Gyokusen, K. and Saito, A. 2002. The process of decline of an endangered tree species, *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima, on the southern slope of Mt. Hasa-dake in Yaku-shima Island. J. For. Res. 6: 307-310.

Kanetani,S., Gyokusen,K., Ito,S., and Saito,A. 1997. The distribution pattern of *Pinus armandii* Franch. var. *amamiana* Hatusima around Mt. Hasa-dake in Yaku-shima island. J. Jpn .For. Soc. 79: 160-163. (in Japanese)

Kanetani, S., Gyokusen, K., Saito, A. and Yoshimaru, H. 2001. The distribution of an endangered tree species, *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima, in Tane-ga-shima Island. For. Tree Breed. special issue: 34-37. (in Japanese)

Kanetani, S., Hosoyamada, S., Gyokusen, K., and Saito, A. 1998. Seed dispersal of *Pinus armandii* Franch. var. *amamiana* Hatusima at Terayama Station for Education and Research on Nature, Faculty of Education, Kagoshima University. Bull. Fac. of Educ., Kagoshima Univ. Nat. Sci. 49: 95-104. (in Japanese with English summary)

Kishi, Y. 1995. The pine wood nematode and the Japanese pine sawyer. Thomas Co. Ltd., Tokyo, pp. 1-302.

Kiyohara, T. and Tokushige, Y. 1971. Inoculation experiments of a nematode, *Bursaphelenchus* sp. onto pine trees. J. Jpn. For. Soc. 53: 210-218. (in Japanese with English summary)

Ledig, F.T. and Conkle, M.T. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine, (*Pinus torreyana* Parry ex Larr). Evolution 37: 79-85.

Meffe, G.K. and Carroll, C.R. 1997. Principles of Conservation Biology. Sinauer Associates, Sunderland, U.S.A., pp. 1-729.

Nakamura, K., Akiba, M., and Kanetani, S. 2002. Pine wilt disease as promising causal agent of the mass mortality of *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima in the field. Eco. Res. 16: 795-801.

Nakashima, K. and Kanazashi, A. 2000. Inbreeding owing to isolation restricts regeneration in a vulnerable species growing on isolated small islands in Japan. *In* Proc. of XXI IUFRO World Congress 2000 Kuala Lumpur, vol.2: 42.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106: 283-292.

Primack, R.B. 1995. A Primer of Conservation Biology. Sinauer Associates, Sunderland, USA., pp. 1-277.

Soltis, D.E., Haufler, C.H., Carrow, D.C. and Gastony, G.J. 1983. Starch gel electorophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Amer. Fern J. 73: 9-27.

Tsumura, Y., Tomaru, N., Suyama, Y., Naim, M. and Ohba, K. 1990. Laboratory manual of isozyme analysis. Bull. Tsukuba Univ. For. 6: 63-95.

Yahara, T., Kawahara, T., Crawford, D.J., Ito, M. and Watanabe, K. 1989. Extensive gene duplications in diploid Eupatorium (Asteraceae). Amer. J. Bot. 76(8): 1247-1253.

Yahara, T., Ohba, H., Murata, J., and Iwatsuki, K. 1987. Taxonomic review of vascular plants endemic to Yakushima Island, Japan. J. Fac. Sci. Univ. Tokyo III. 14: 69-119.

Yamamoto, C. and Akasi, T. 1994. Preliminary report on distribution and conservation of a rare tree species of *Pinus armandii* Franch. var. *amamiana* (Koidz.) Hatusima Trans. 105th Ann. Meet. Jpn. For. Soc.: 750. (in Japanese)

Genetic Diversity and Mating System of Korean Pine in Russia

Vladimir Potenko

Abstract—Based on the analysis of 26 allozyme loci, levels of genetic variation were ascertained in 25 natural populations of Korean pine. On average, 58.0 percent of the loci were polymorphic; the number of alleles per locus was 1.92; the expected heterozygosity was 0.182, and the observed heterozygosity was 0.180. On average, the heterozygote deficiency was characteristic of Korean pine populations (F_{IS}=0.013). The most diversity was found within populations (F_{ST}=0.018). Genetic distances between populations were small (on average, D_N=0.003). Level of gene flow was 10.98 migrants per generation. Multilocus outcrossing estimates ranged from 0.751 to 1.031, indicating mating system differences. Results of this study lead to the assumption that the genetic structure of Korean pine populations is under the influence of a complex combination of microevolution factors, including genetic drift, gene flow and natural selection.

Key words: Korean pine, allozymes, genetic variation, differentiation, gene flow, mating system.

Introduction

The Korean pine, *Pinus koraiensis* Sieb. & Zucc., occurs in natural and artificial stands in Russia, China, Korea, and Japan. In the Russian Far East, *P. koraiensis* is distributed in the Primorski Territory, in the southern part of Khabarovsk Territory, in the Jewish Autonomous Region and at the southeast end of the Amur Territory (fig. 1). Usually, *P. koraiensis* grows in mixed stands with broadleaf tree species. The Korean pine-broadleaf forests occupy low and middle altitude zones growing in a wide range of relief and soil conditions. In the south Sikhote Alin mountain range they occur up to 900 m above sea level, while in the north, Korean pine reaches only to 500 m (Usenko 1969). Selective harvesting and fires have repeatedly stressed most of the forests. At present, clear cuttings of broad-leaved Korean pine mixed forests are illegal. However, the harvest of the broad-leaved Korean pine forests is occurring without authorization because of demand for pine and hardwood timber. For this reason, the broad-leaved Korean pine forestlands are decreasing (Koryakin and Romanova 1996). Thus there is a need to emphasize conservation of Korean pine genetic resources.

Knowledge of the level and distribution of genetic variation, both within and among populations, facilitates the conservation of gene resources (Brown 1978; Millar and Libby 1991). Recently, the results of genetic variation studies of Korean pine populations in the Russian Far East have been reported (Krutovskii and others 1995; Potenko and Velikov 1998) and South Korea (Kim and others 1994). Differences in levels of genetic variation were observed within and among the populations in different parts of Korean pine's natural range (Potenko and Velikov 1998). Greater variation was found in South Korean populations, with less variation occurring in the northwestern part of the natural range in Russia. Additionally, the measurements of mating systems showed a high proportion of outbred progeny in an earlier study of three Korean pine populations (Politov and Krutovskii 1994; Krutovskii and others 1995).

The primary objectives of this study were to analyze the genetic diversity and mating system of Korean pine throughout the natural range in Russia and to describe geographical patterns of genetic variation.

Materials and Methods

Seeds for electrophoresis were collected in 25 native populations from 43° to 51° latitude north (fig. 2). In 17 populations the collection of seeds was performed on individual trees. The remaining eight populations were represented by bulked seed lots that were collected from native populations by state forest farms for artificial reforestation (table 1). More details about seed samples and characteristics of the sampled populations can be found elsewhere (Potenko and Velikov 1998, 2001).

Six megagametophytes and ten embryos per tree were subjected to horizontal starch gel electrophoresis. Details of laboratory procedures are described in Potenko and Velikov (1998). Seed tissues were analyzed for 15 enzyme systems: aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), aconitase (ACO), diaphorase (DIA), fluorescent esterase (Fl-EST), formate dehydrogenase (FDH), glutamate dehydrogenase (GDH), glutamate pyruvate transaminase (GPT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), shikimate dehydrogenase (SkDH) and sorbitol dehydrogenase (SDH). In total, 26 loci were scored for genetic variation analysis (Aat-1, Aat-2, Aat-3, Adh-1, Adh-2, Aco, Gdh, Dia-1, Dia-3, Idh, Lap-1, Lap-2, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Gpt, Sdh, Fl-Est, Fdh, Pgm-1, Pgm-2, 6-Pgd-1, 6-Pgd-2, Skdh-1 and Skdh-2). For mating system analysis, four loci (Aat-3, Dia-1, Pgm-1 and Skdh-1) were used.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Vladimir Potenko is head of the Department of Genetics and Breeding, Breeding and Seed Growing Forestry Center, Nagornaya 12, Sosnovka 680555, Khabarovsk Territory, Russia. Tel./fax: +7-4212-922-445, e-mail: forestry@mail.kht.ru

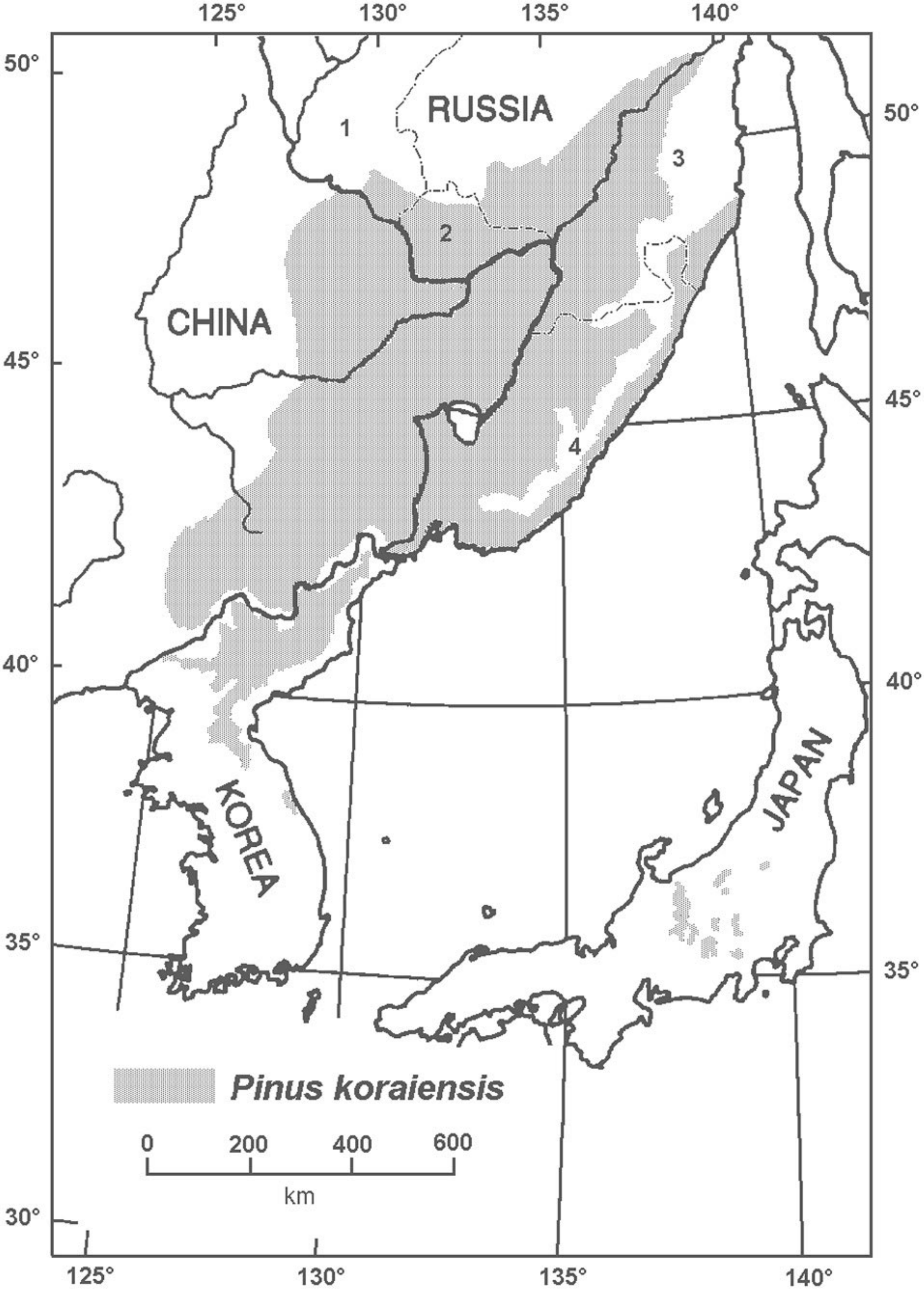


Figure 1—Distribution of Korean pine (modified from Schmidt 1994). Nos: 1 - Amur Territory; 2 - Jewish Autonomus Region; 3 - Khabarovsk Territory; 4 - Primorski Territory.

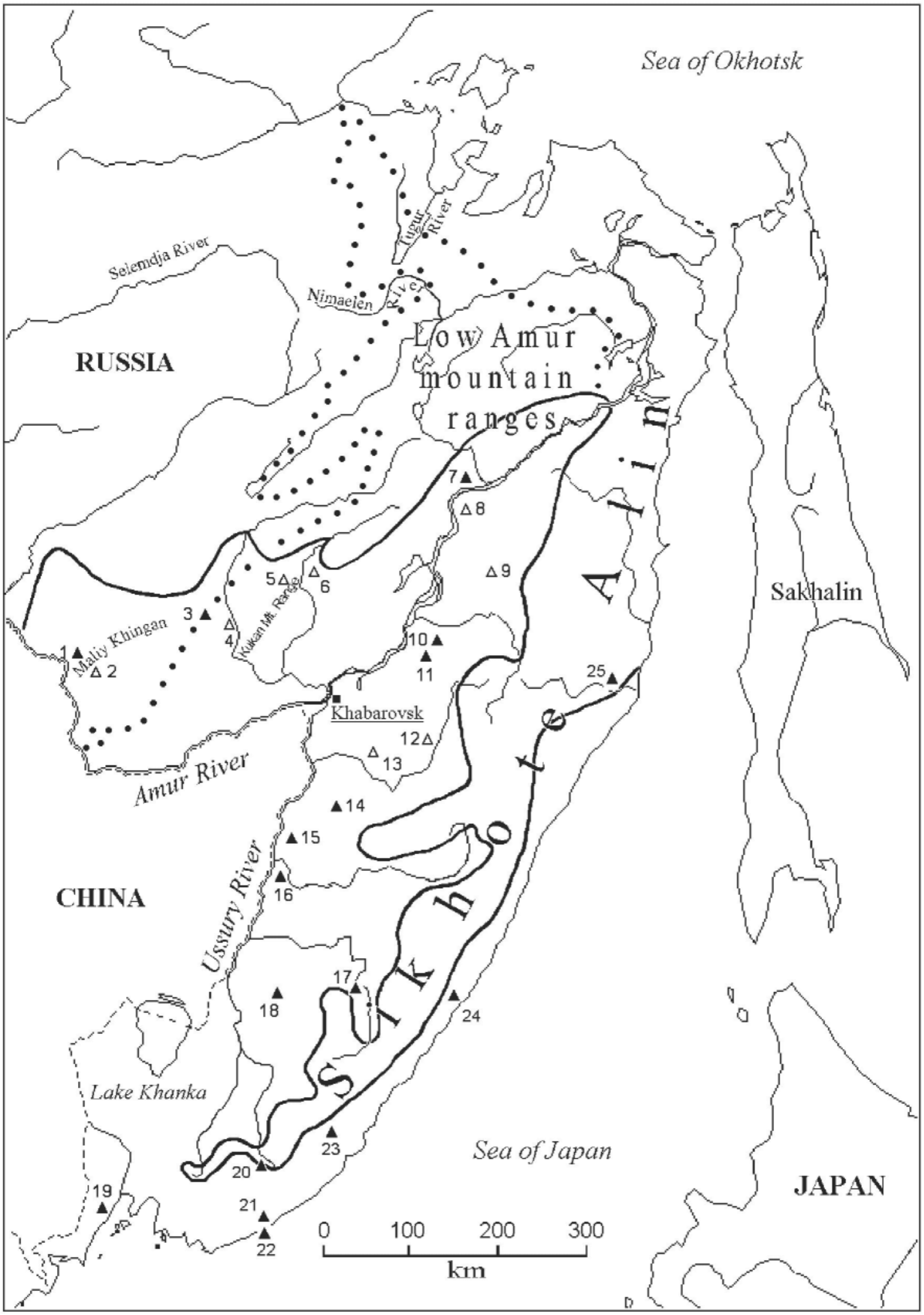


Figure 2—Location of the sampled populations: ▲ - seed collection was conducted from individual tree; Δ - seed lot was sampled. Nos: population numbers shown in Table 1. Solid line: limit of distribution of *P. koraiensis* in Russia. Dotted line: northern limit of the Korean pine-broadleaf and Korean pine-spruce-larch mixed forests in the Holocene climate optimum (modified from Korotkii and others 1997).

Table 1—Genetic variability at 26 loci in 25 populations of *P. koraiensis* (standard errors in parentheses).

Population	Number of trees	Mean No. of alleles per locus, A	Percentage of polymorphic loci		Mean heterozygosity	
			P_{95}	P_{99}	Observed, H_o	Expected, H_e
1. Obluchie	21	1.85	46.2	61.5	0.170	0.171
2. Sutara[a]	70[b]	1.69	42.3	57.7	-	0.169
3. Dogordon	52	1.92	50.0	53.8	0.164	0.171
4. Kukan[a]	70[b]	1.65	46.2	50.0	-	0.170
5. Niran[a]	70[b]	1.88	46.2	61.5	-	0.165
6. Selgon[a]	70[b]	1.96	42.3	53.8	-	0.154
7. Galichnoe	72	2.12	57.7	69.2	0.193	0.204
8. Pivan[a]	70[b]	1.85	53.8	53.8	-	0.194
9. Innokentievka[a]	70[b]	1.77	46.2	53.8	-	0.174
10. Burga	53	1.92	50.0	53.8	0.174	0.187
11. Mulcha	62	2.08	46.2	57.7	0.191	0.189
12. Sukpay[a]	70[b]	1.96	50.0	61.5	-	0.181
13. Obor[a]	70[b]	2.00	53.8	61.5	-	0.183
14. Medvezhy	49	1.88	53.8	57.7	0.180	0.194
15. Boicovo	24	1.81	46.2	53.8	0.164	0.181
16. Pokrovka	73	2.19	50.0	61.5	0.188	0.193
17. Mel'nichnoe	73	2.15	46.2	57.7	0.180	0.180
18. Malinovo	50	2.08	53.8	69.2	0.189	0.218
19. Kedrovaya Pad'	38	1.85	50.0	61.5	0.186	0.187
20. Arkhipovka	51	2.08	50.0	61.5	0.199	0.197
21. Kievka	68	1.85	50.0	53.9	0.172	0.178
22. Petrov's Island	21	1.73	42.3	50.0	0.177	0.161
23. Ustinovka	63	2.00	50.0	61.5	0.193	0.187
24. Ternei	61	1.85	46.2	53.9	0.192	0.190
25. Lesnoi	58	1.92	50.0	57.7	0.156	0.163
Mean for populations Nos. 1-6 Maliy Khingan - Kukan mountain ranges		1.83	45.5	56.4	0.167	0.167
		(0.13)	(2.9)	(4.6)	(0.004)	(0.007)
Mean for populations Nos. 7-25 Sikhote Alin - Low Amur mountain ranges		1.95	49.8	58.5	0.182	0.186
		(0.14)	(3.7)	(5.2)	(0.012)	(0.013)
Mean		1.92	48.8	58.0	0.180	0.182
		(0.14)	(3.9)	(5.1)	(0.012)	(0.015)

[a] Bulked seed lot analyzed. Total weight of any seed lot was 500 kg.
[b] Number of analyzed seeds per seed lot.

Allele frequencies were analyzed using the BIOSYS-1 computer program (Swofford and Selander 1989). For each population, mean number of alleles per locus (A), percentage of polymorphic loci ($P_{0.95}$ and $P_{0.99}$) and expected heterozygosity (H_e) were computed. In addition, Nei's genetic distances (D_N) were calculated (Nei 1978).

For assaying the population genetic structure, the fixation indices (F_{IS}, F_{IT} and F_{ST}) were used (Nei 1977). F_{IS} and F_{IT} measure the deviation of genotype frequencies from Hardy-Weinberg proportions in the populations and in the total population respectively, whereas F_{ST} measures the degree of genetic differentiation among populations. The F_{ST} values were used to calculate interpopulational gene flow (Nm) as follows: $F_{ST}=1/(4Nma+1)$, where $a=(n/n-1)^2$, and n is the number of populations (Govindaraju 1989).

The expected fixation index at inbreeding equilibrium was computed as $F_e=(1-t_m)/(1+t_m)$, where t_m is the multilocus outcrossing rate (Allard and others 1968).

Single locus (t_s) and multilocus (t_m) estimates of the proportion of progeny resulting from outcrossing in a population were determined using the MLT computer program (Ritland 1990). Maternal genotypes, assessed from megagametophyte segregations, were taken into account. The confidence intervals of the outcrossing rates were estimated after 100 boot-straps. At Dia-1, the 4th allele Dia-$1^{0.60}$ with the lowest frequency was combined with the allele Dia-$1^{1.37}$ having the nearest frequency because the computer program can only process a maximum of three alleles per locus. Both t_s and t_m estimates are based on the mixed mating model, which assumes (1) that each viable offspring is the result of a random outcross (with probability t) or a self-fertilization (with probability s=1-t), (2) that the probability of an offspring being an outcross is independent of the genotype of the maternal parent, (3) that outcross pollen pool allele frequencies are homogeneous over space and over time, and (4) that there is no selection between pollination and the time that seeds or seedlings are sampled (Shaw and others 1981). Multilocus estimations require the additional assumption of independence among loci in the outcross pollen pool. Conkle (1981), Politov and others (1989) and Goncharenko and others (1998) showed that the gene arrangement is highly conservative in the pines and found no linkage among loci Aat-3, Dia-1, Pgm-1 and Skdh-1.

Results and Discussion

Genetic Diversity and Differentiation

Parameters of genetic variation (table 1) were calculated on the basis of allele frequencies of 26 loci. In Korean pine populations, the mean number of alleles per locus ranged from 1.65 to 2.19, with an average of 1.92. The proportion of polymorphic loci ($P_{0.99}$) ranged from 50.0 to 69.2 percent, with an average of 58.0 percent. The observed heterozygosity was from 0.156 to 0.199, with an average of 0.180. The expected heterozygosity ranged from 0.154 to 0.218, with an average of 0.182. The genetic variation was lower than in Korean pine populations of South Korea (on average, P_{99}=69.0, A=2.0, H_0=0.200, H_e=0.208; Kim and others 1994). The results seem to support the hypothesis that Korean pine expanded to the far eastern region of Russia from the south in the Holocene. The studies of fossil conifer pollen (Korotkii and others 1997) indicate that 18,000 to 20,000 years ago the vegetation of Sikhote Alin was similar to that of the contemporary northwest coast of the Sea of Okhotsk. After climatic cooling, the Korean pine appeared among mountain vegetation approximately 9,500 years ago, in the Holocene period, and in the middle Holocene the northern border of its area had spread to the Selemdja, Tugur and Nimaelen rivers. As can be seen, the range of *P. koraiensis* was previously much wider than at present (fig. 2). Southward decline of Korean pine occurred because of the cooler climate periods in the middle and late Holocene, resulting in the expansion of taiga boreal forests with *Picea*, *Abies*, and *Larix* species.

Natural populations of Korean pine in Russia contain an appreciable amount of genetic variation comparable to the mean value for the genus *Pinus* (on average, P_{99}=52.0, H_0=0.159, H_e=0.159; Goncharenko and others 1989). The average values for genetic variation of P. *koraiensis* are intermediate among pine species of subsection *Cembrae*. In particular, the values of expected heterozygosity for these species are: for *P. cembra* – 0.109 and 0.118, *P. sibirica* – 0.158 and 0.169, and *P. pumila* – 0.249 and 0.271. These values are in agreement with those of Politov and Krutovskii (1994) and Goncharenko and Silin (1997). A higher heterozygosity level (H_e=0.204) was also found in the only population of *P. albicaulis* studied (Politov and Krutovskii 1994).

Geographical patterns of the distribution of expected heterozygosity (fig. 3) and mean number of alleles per locus (fig. 4) show that Korean pine has a small number of centers of genetic variation. The largest of them is situated in the south of Sikhote Alin. Two small centers are located at the northwestern limit of natural range of Korean pine and the middle part of Sikhote Alin. As the Sikhote Alin and Low Amur mountain range populations have appreciable levels of genetic variation, this may serve as confirmation of the hypothesis of the long-term existence of Korean pine within these areas. In that region, the mean expected heterozygosity was higher than in populations of the Maliy Khingan - Kukan mountain ranges (table 1).

In the coastal region, the peripheral population Lesnoi possesses a lower heterozygosity. This can be explained by genetic drift due to the founder effect of populating a territory by a small number of individuals in recent history, possibly the result of the northward migration of the Korean pine during the Holocene along the narrow coastline (fig. 1, 2). Lower estimates of genetic variation in the Petrov's Island population can also be attributed to a founder effect of Korean pine colonizing the island. Apparently the population was established 9,500 to 9,800 years ago, during the Holocene period, when Korean pine appeared as a member of the mountain vegetation complex of Sikhote-Alin (Golubeva and Karaulova 1983; Korotkii and others 1997). At present, the Petrov's Island area encompasses 36 hectares, on which grow a few hundred Korean pine trees. Heterozygosity decrease has also been found in peripheral populations of other conifers, including *Pinus contorta* Dougl. ex Loud. (Yeh and Layton 1979), *Pinus rigida* Mill. (Guries and Ledig 1982), *Picea abies* (L.) Karst. (Bergmann and Gregorius 1979) and *Picea rubens* Sarg. (Hawley and DeHayes 1994). More intense selection in marginal environments, genetic drift, greater inbreeding in small populations, or migration from different glacial refugia explained the heterozygosity differences between central and peripheral populations in several studies (Yeh and Layton 1979; Guries and Ledig 1982; Hawley and DeHayes 1994).

Positive F_{IS} and F_{IT} values indicate that a deficiency of heterozygotes is typical for *P. koraiensis* populations and for the whole species (table 2). The deficiency of heterozygotes was also found in the south Korean populations, where the F_{IS} and F_{IT} values were 0.007 and 0.066, respectively (Kim and others 1994). For pines, this deficiency was attributed to mating among closely adjacent individuals within a stand, partial self-pollination, pooling of individuals (during sampling) from different family groups within populations, and selection against heterozygotes (Guries and Ledig 1982; Dancik and Yeh 1983; Kim and others 1994; Politov and Krutovskii 1994; Changtragoon and Finkeldey 1995; Lee and others 1998).

The mean F_{ST} value (F_{ST}=0.018) was lower than the mean G_{ST} estimate for genus *Pinus* (G_{ST}=0.065; Hamrick and others 1992). The value indicates that 1.8 percent of the genetic variation is distributed among the Korean pine populations; in other words, the majority of the variation resides within populations and any prominent differentiation processes are absent between populations.

The estimates of Nm, averaged over all populations per locus, were well above 1.0 (ranged from 5.00 at Skdh-2 and Mdh-3 to 26.51 at Pgm-2 and 6-Pgd-2) with a mean of 10.98 migrants per generation (table 2). The gene exchange exceeded those of *P. koraiensis* in South Korea (Nm=3.987; Kim and others 1994) and most of the coniferous tree species (Govindaraju 1989; Goncharenko and Silin 1997). Animal dispersing of Korean pine seeds may explain the large values of Nm. Tomback and Schuster (1994) noted that dispersal of pine seeds by nutcrackers, *Nucifraga* (Corvidae), which occurs routinely over large distances, might result in higher

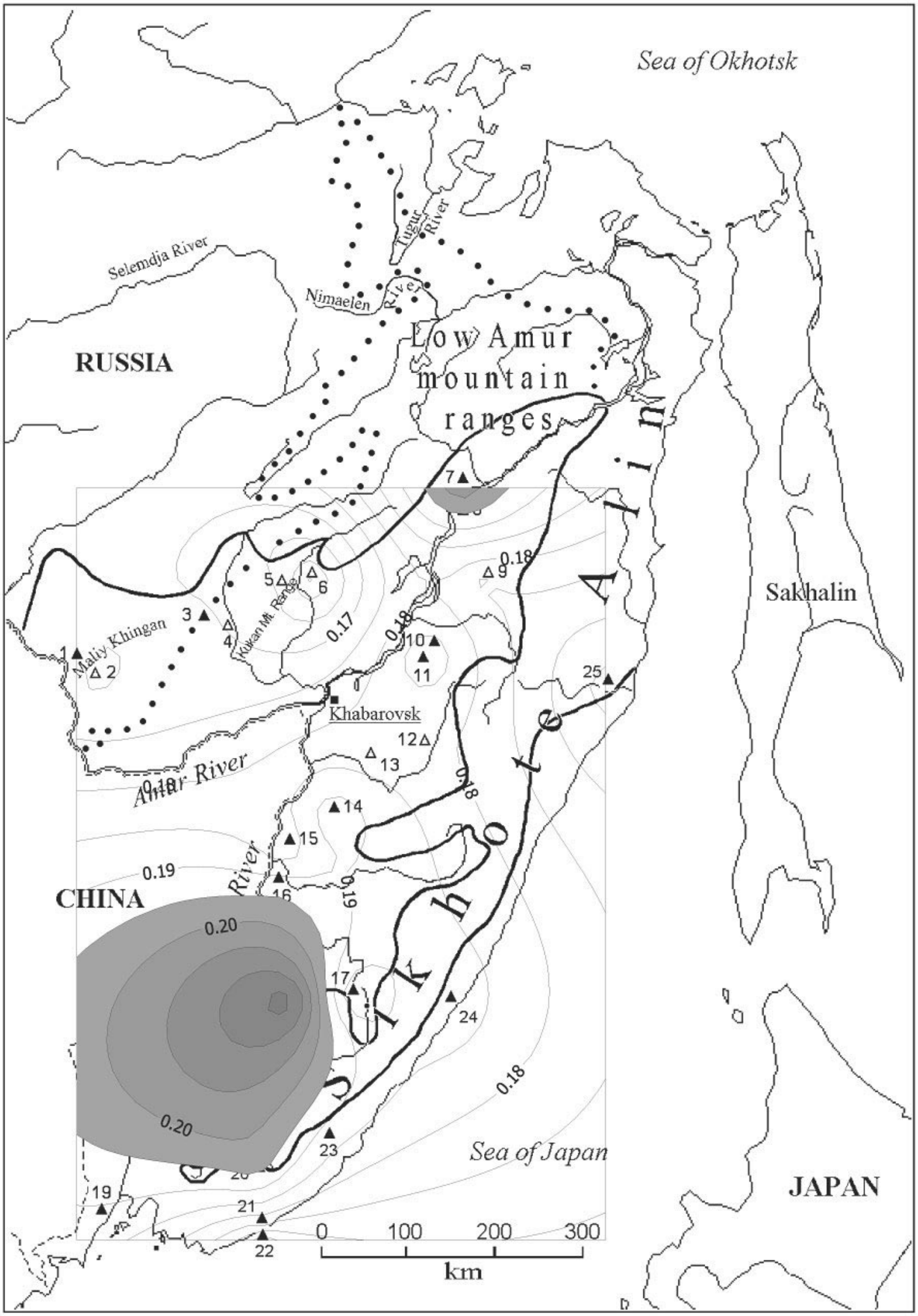


Figure 3—Geographical patterns of the distribution of expected heterozygosity. Grey scale gradient show the most variable parts of Korean pine natural range. Five levels of expected heterozygosity (0.195 to 0.200, 0.200 to 0.205, 0.205 to 0.210, 0.210 to 0.215, and above 0.215) are indicated.

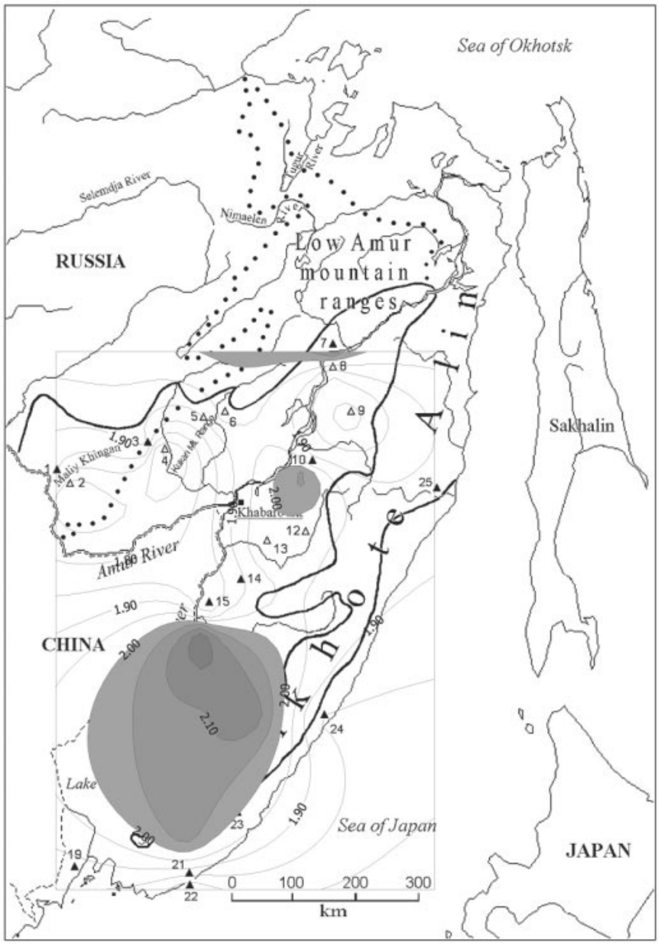


Figure 4—Geographical patterns of the distribution of mean number of alleles per locus. Grey scale gradient shows the most variable parts of Korean pine natural range. Four levels of mean number of alleles per locus (2.00 to 2.05, 2.05 to 2.10, 2.10 to 2.15, and above 2.15) are indicated.

Table 2—Deviation of genotype frequencies from Hardy-Weinberg proportions in individual populations (F_{IS}) and the total population (F_{IT}), degree of genetic differentiation among populations (F_{ST}), and degree of interpopulation gene flow (Nm).

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
Aat-3	–0.019	0.004	0.023	6.80
Adh-1	–0.005	0.015	0.020	7.84
Adh-2	–0.009	0.012	0.022	7.11
Gdh	0.052	0.076	0.025	6.24
Lap-1	–0.051	–0.027	0.023	6.80
Lap-2	0.069	0.085	0.017	9.25
Pgm-1	0.025	0.039	0.014	11.27
Pgm-2	0.001	0.008	0.006	26.51
Skdh-1	0.009	0.025	0.016	9.84
Skdh-2	0.051	0.080	0.031	5.00
Mdh-2	–0.015	–0.004	0.011	14.39
Mdh-3	0.008	0.039	0.031	5.00
Mdh-4	0.115	0.133	0.020	7.84
6-Pgd-1	0.050	0.067	0.018	8.73
6-Pgd-2	0.001	0.008	0.006	26.51
Dia-1	0.002	0.022	0.020	7.84
Dia-3	0.018	0.043	0.026	5.99
Fl-Est	–0.008	0.007	0.015	10.51
Idh	0.002	0.011	0.009	17.62
Sdh	–0.001	0.009	0.009	17.62
Fdh	0.016	0.030	0.014	11.27
Aco	–0.002	0.011	0.013	12.15
Gpt	–0.013	0.002	0.015	10.51
Mean for 23 loci	0.013	0.030	0.018	10.98

Table 3—Estimations of single locus (t_s) and multilocus (t_m) outcrossing rates, fixation index (F_{IS}) and expected inbreeding coefficient (F_e) based on data from 4 polymorphic loci (standard errors in parentheses).

Population	t_s	t_m	F_{IS}	F_e
Obluchie	0.885 (0.044)	0.901 (0.041)	0.118	0.052
Galichnoe	1.018 (0.044)	1.001 (0.048)	0.069	0.000
Boicovo	1.042 (0.050)	1.031 (0.049)	0.029	-0.015
Malinovo	0.896 (0.043)	0.906 (0.038)	0.084	0.049
Ustinovka	0.863 (0.053)	0.861 (0.046)	-0.031	0.075
Kedrovaya Pad’	0.958 (0.050)	0.986 (0.043)	0.081	0.007
Petrov‘s Island	0.763 (0.061)	0.751 (0.057)	-0.105	0.142
Kievka	0.923 (0.053)	0.912 (0.053)	0.113	0.046
Ternei	0.851 (0.061)	0.852 (0.057)	-0.054	0.080
Lesnoi	0.884 (0.046)	0.888 (0.042)	0.009	0.059

levels of gene flow between pine populations than from seed dispersal by wind.

Unbiased Nei’s genetic distance values between the 25 populations of *P. koraiensis* were low, averaging 0.003. Low estimates of Nei’s genetic distances confirm the close genetic relationship between investigated populations and indicate a widespread gene flow between populations.

Mating System Analysis

Single locus estimates of outcrossing ranged from 0.763 to 1.042, and multilocus estimates were from 0.751 to 1.031 (table 3). The lowest value t_m was found in the Petrov’s Island population and the highest in the Boicovo population. Negligible differences were found between single locus and multilocus estimates of outcrossing in any population.

The mean multi-locus value of outcrossing in this study (t_m=0.909) was lower than that for the three populations (t_m=0.974) studied earlier by Politov and Krutovskii (1994) but typical for most coniferous forest tree species (Muona 1990; Adams and Birkes 1991; Mitton 1992). The lowest value t_m, on Petrov’s Island, can be attributed to both selfing and mating among related individuals, supporting the hypothesis that the populating of the island was by a limited number of migrants. The low estimates of the Ustinovka, Ternei and Lesnoi populations can be attributed to partial mating among related individuals due to the founder effect. Although t_m was low in these populations, the estimates of F_{IS} were either negative or slightly positive, thus indicating an excess of heterozygotes or practically a Hardy-Weinberg equilibrium. Any one of these estimates is much lower than those expected under inbreeding equilibrium, given the levels of t_m (table 3). The relationship between multilocus estimates of outcrossing (t_m) and fixation index (F_{IS}) shown in figure 5 suggests that the excess of heterozygotes in Korean pine populations is due to “pseudo-overdominance” as result of inbreeding depression (Ledig 1986), rather than selection in favor of heterozygotes (overdominance) as concluded by Politov and Krutovskii (1994) and Krutovskii and others (1995). These results showed that in Korean pine populations, the selection against inbred progeny appears when outcrossing rate is below 0.9 (fig. 5).

It is suggested that a contrary direction of selection occurs in populations with a deficit of heterozygotes and high outcrossing rates; that is, selection against heterozygotes. High outcrossing is probably maintained by a high migration rate between Korean pine populations (Nm=10.98). However, the validity of the suggested selection against heterozygotes in Korean pine needs to be field-proven by making biparental crosses. Selection against hybrid forms of plants due to outbreeding depression is found in crosses between distant plants of *Delphinium nelsoni* (Price and Waser 1979) and *Lotus scoparius* (Montalvo and Ellstrand 2001).

Possible microsite differentiation of allele frequencies that would upwardly bias the fixation index cannot be excluded as an explanation of a deficit of heterozygotes in some Korean pine populations. If different subpopulations sustain different alleles, the allele frequencies will be maintained at a high level in the whole population (Brown 1979). This phenomenon explains the high level of heterozygosity in populations with a positive fixation index, although reliable conclusions can only be made after an investigation of the genetic parameters of subpopulations.

Thus the coastal Korean pine populations we sampled exhibit different levels of genetic variation and outcrossing. Results of this study lead to the conclusion that the genetic structure of the Korean pine populations is under the influence of a complex combination of microevolution factors, genetic drift, gene flow and natural selection.

Acknowledgments

I thank Andrew Velikov for helpful assistance in the laboratory analysis, Howard Kriebel for improvement of English, tables, and figures, and an anonymous reviewer for helpful comments on the manuscript.

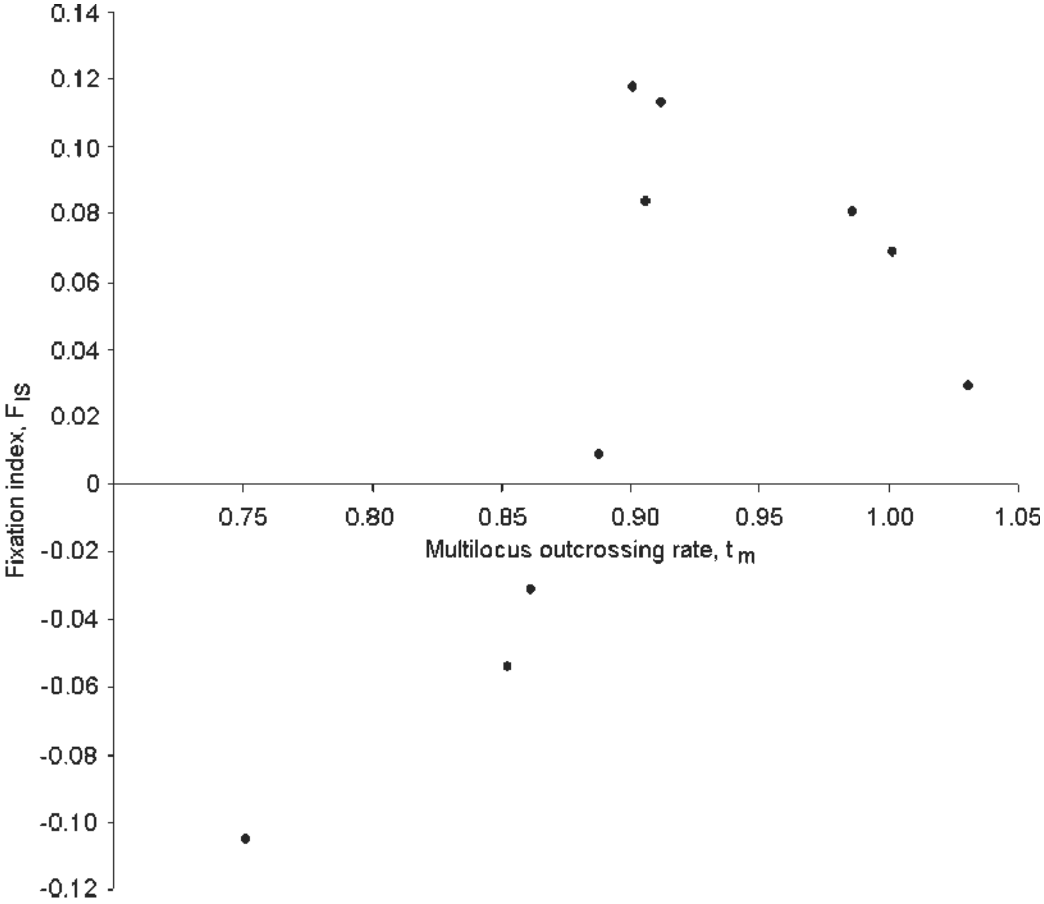


Figure 5—The relationship between multilocus estimates of outcrossing (t_m) and fixation index (F_{IS}).

References

Adams, W.T. and Birkes, D.S. 1991. Estimating mating patterns in forest tree populations. *In* Biochemical markers in the population genetics of forest trees. SPB Academic Publishing bv, The Hague, The Netherlands. pp. 157-172.

Allard, R.W., Jain, S.K. and Workman, P.L. 1968. The genetics of inbreeding populations. Adv. Genet. 14: 55-131.

Bergmann, F. and Gregorius, H.R. 1979. Comparison of the genetic diversities of various populations of Norway spruce (*Picea abies*). *In* Proc. Conf. Biochem. Genet. For. Trees. *Edited* by D. Rudin Dep. For. Genet. Plant Physiol., Swed. Univer. Agr. Sci., Umea, Sweden, pp. 99-102.

Brown, A.H.D. 1978. Isozymes, plant population genetic structure and genetic conservation. Theor. Appl. Genet. 52: 145-157.

Brown, A.H.D. 1979. Enzyme polymorphism in plant populations. Theor. Popul. Biol. 15: 1-42.

Changtragoon, S. and Finkeldey, R. 1995. Patterns of genetic variation and characterization of the mating system of *Pinus merkusii* in Thailand. Forest Genetics 2: 87-97.

Conkle, M.T. 1981. Isozyme variation and linkage in six conifer species. *In* Proc. of Symposium on Isozymes of North Am. For. Trees and For. Insects. *Edited* by M.T. Conkle. Berkeley, California, pp. 11-17.

Dancik, B.P. and Yeh, F.C. 1983. Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*P. banksiana*) in Alberta. Can. J. Genet. Cytol. 25: 57-64.

Golubeva, L.V. and Karaulova, L.P. 1983. Vegetation and climatostratigraphy of Pleistocene and Holocene of the USSR Far East South. Nauka, Moscow, 143 pp.

Goncharenko, G.G. and Silin, A.E. 1997. Population and evolutionary genetics of pine in Eastern Europe and Siberia. Technalohija, Minsk, 191 pp.

Goncharenko, G.G., Padutov, V.E. and Potenko, V.V. 1989. Guide to conifer species research by isozyme methods. Byelorussian For. Res. Inst., Gomel. 163 pp.

Goncharenko, G.G., Padutov, A.E. and Khotyljova, L.V. 1998. Genetic mapping of allozyme loci in four two-needle pine species of Europe. Forest Genetics 5: 103-118.

Govindaraju, D.R. 1989. Estimates of gene flow in forest trees. Biol. J. Linn. Society 37: 345-357.

Guries, R.P. and Ledig, F.T. 1982. Genetic diversity and population structure in Pitch pine (*Pinus rigida* Mill.). Evolution 36: 387-402.

Hamrick, J.L., Godt, M.J.W. and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95-124.

Hawley, G.J. and DeHayes, D.H. 1994. Genetic diversity and population structure of red spruce (*Picea rubens*). Can. J. Bot. 72: 1778-1786.

Kim, Z.-S., Lee, S.-W., Lim, J.-H., Hwang, J.-W. and Kwon, K.-W. 1994. Genetic diversity and structure of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Korea. Forest Genetics 1: 41-49.

Korotkii, A.M., Grebennikova, T.A., Pushkar, V.S., Razjigaeva, N.G., Volkov, V.G., Ganzey, L.A., Mokhova, L.M., Bazarova, V.B. and Makarova, T.R. 1997. Climatic changes in the Russian Far East during late Pleistocene-Holocene. Vestnik FEBRAS 3: 121-143.

Koryakin, V.N. and Romanova N.V. 1996. The Far East cedar stand condition and promising direction of Korean pine regeneration. *In* Proc. Int. Conf.: Korean pine-broadleaved forests of the Far East, 30 Sept. – 6 Oct. 1986, Khabarovsk, Russian Federation. *Edited* by P.W. Owston, W.E. Schlosser, D.F. Efremov and C.L. Miner. Far East For. Res. Inst., Khabarovsk. pp. 179-180.

Krutovskii, K.V., Politov, D.V. and Altukhov, Yu.P. 1995. Isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pine species (Subsection *Cembrae*, section *Strobi*, subgenus *Strobus*). *In* Population genetics and genetic conservation of forest trees. SPB Academic Publishing, Amsterdam, The Netherlands. pp. 279-304.

Ledig, F.T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. *In* Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland Massachusetts. pp. 77-104.

Lee, S.W., Choi, W.Y., Norbu, L. and Pradhan, R. 1998. Genetic diversity and structure of blue pine (*Pinus wallichiana* Jackson) in Bhutan. For. Ecol. and Management 105: 45-53.

Millar, C.I. and Libby, W.J. 1991. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. *In* Genetics and conservation of rare plants. Oxford Univ. Press. pp. 149-170.

Mitton, J.B. 1992. The dynamic mating systems of conifers. New Forests 6: 197-216.

Montalvo, A. M. and Ellstrand, N. C. 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (*Fabaceae*). Am. J. Bot. 88: 258-269.

Muona, O. 1990. Population genetics in forest tree improvement. *In* Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates, inc., Sunderland, M.A. pp. 282-298.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. 41: 225-233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Politov, D.V. and Krutovskii, K.V. 1994. Allozyme polymorphism, heterozygosity, and mating system of stone pines. *In* Proc. International Workshop on Subalpine Stone Pines and their Environment: The Status of Our Knowledge, 5-11 Sept. 1992, St. Moritz, Switzerland. USDA Forest Service: Intermountain Research Station. Gen. Tech. Rep. INT-GTR-309. pp. 36-42.

Politov, D.V., Krutovskii, K.V. and Altukhov, Y.P. 1989. Genetic variability in Siberian stone pine, *Pinus sibirica* Du Tour. III. Linkage relationships among isozymes loci. Genetika (Moscow) 25: 1606-1618.

Potenko, V.V. and Velikov, A.V. 1998. Genetic diversity and differentiation of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Russia. Silvae Genetica 47: 202-208.

Potenko, V.V. and Velikov, A.V. 2001. Allozyme variation and mating system of coastal populations of *Pinus koraiensis* Sieb. et Zucc. in Russia. Silvae Genetica 50: 117-122.

Price, M.V. and Waser, N.M. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature 277: 294-297.

Ritland, K. 1990. A series of FORTRAN computer programs for estimating plant mating system. J. Heredity 81: 235-237.* OK

Schmidt, W.C. 1994. Distribution of stone pines. *In* Proc. International Workshop on Subalpine Stone Pines and Their Environment: The Status of Our Knowledge, 5-11 Sept. 1992, St. Moritz, Switzerland. USDA Forest Service: Intermountain Research Station. Gen. Tech. Rep. INT-GTR-309. p. 1-6.

Shaw, D.V., Kahler, A.L. and Allard, R.W. 1981. A multilocus estimator of mating system parameters in plant populations. Proc. Nat. Acad. Sci. (USA) 78: 1298-1302.

Swofford, D.L. and Selander, R.B. 1989. BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, IL.

Tomback, D. F. and Schuster, W. S. F. 1994. Genetic population structure and growth form distribution in bird-dispersed pines. *In* Proc. International Workshop on Subalpine Stone Pines and their Environment: The Status of Our Knowledge, 5-11 Sept. 1992, St. Moritz, Switzerland. USDA Forest Service: Intermountain Research Station. Gen. Tech. Rep. INT-GTR-309. pp. 43-50.

Usenko, N. V. 1969. Trees, shrubs and lianas of the Far East. Far East For. Res. Inst., Khabarovsk. 415 p.

Yeh, F.C. and Layton, C. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* spp. *latifolia*. Can. J. Genet. Cytol. 21: 487-503.

Part IV: White Pine Blister Rust Resistance

***Pinus albicaulis* (whitebark pine)**

Photo credits: Left-J. Barnes. Right (top to bottom): R. Shoal,
B. Danchok, J. Schwandt, J. Barnes

Variation in *Cronartium ribicola* Field Resistance Among 13 *Pinus monticola* and 12 *P. lambertiana* Families: Early Results from Happy Camp

R.A. Sniezko
A.D. Bower
A.J. Kegley

Abstract—In 1995 seed from 13 *Pinus monticola* (western white pine) and 12 *P. lambertiana* (sugar pine) parents previously included in short-term blister rust testing at Dorena Genetic Resource Center (DGRC), Cottage Grove, OR, were sown to establish field trials. The parents were chosen to represent a wide array of resistance responses shown in earlier artificial inoculation trials. Percentage stem symptoms (cankers and bark reactions) in 1999 and 2000 at the 1996 Happy Camp (HC) trial are reported here. Sugar pine families have a higher percentage of trees with stem symptoms (SS percent) than do western white pine families. In addition to a greater susceptibility to infection overall, the major gene resistance present in several of these sugar pine families (conferred by Cr1) is ineffective due to the relatively high frequency of a virulent (vcr1) strain of rust at this site. Western white pine families varied from 10.4 to 83.3 SS percent and sugar pine from 73.5 to 91.8 SS percent. The susceptible control lot for western white pine showed a much greater percentage of trees with stem symptoms (83.3 percent) than any other western white pine seedlot. The two western white pine families with known major gene resistance (Cr2) were among the families with lowest infection at this site. The resistance mechanism of the family with the second lowest level of stem symptoms is unknown. Moderately strong and significant positive correlations ($r>0.69$, $p<0.05$) existed between SS percent at HC and SS percent following artificial inoculation at DGRC. The correlation between needle lesion frequency in DGRC screening and stem symptoms observed at HC was positive but non-significant ($r>0.5$, $p>0.1$ for sugar pine and western white pine).

Key words: *Pinus monticola*, *Pinus lambertiana*, blister rust, Cr1.

Introduction

In Oregon and Washington, the USDA Forest Service (Region 6) began selecting and screening parent trees of western white pine (*Pinus monticola* Dougl. ex D. Don.) and sugar pine (*P. lambertiana* Dougl.) for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch) in the late 1950s. Over 9500 parent trees of these two species have been selected for rust resistance in natural stands, but variation in rust hazard by site influences the expected efficacy of field selection. Progeny of these field selections have been screened for resistance at Dorena Genetic Resource Center (DGRC) (Sniezko 1996, Kegley and Sniezko this proceedings). Selections from rust screening have been used to establish seed orchards in many of the breeding zones, and resistant seed is now available for some zones.

Operational screening at DGRC generally involves a single inoculation using two-year old seedlings and the subsequent assessment of seedlings and assignment into resistance categories. Primary categories of response in seedlings are lack of visible stem infections versus presence of visible stem symptoms. Seedlings screened at DGRC are assigned to these two categories and are also assessed for other resistance responses (Sniezko 1996, Kegley and Sniezko this proceedings). Some of the mechanisms preventing stem infection include: hyper-sensitive reactions (HR) in the needles conditioned by major genes in sugar pine (Cr1) and western white pine (Cr2) (Kinloch and Comstock 1981, Kinloch and others 1999, Kinloch and Dupper 2002), and two mechanisms hypothesized to be controlled by single recessive genes: (a) fungicidal reaction in the short shoot (Hoff and McDonald 1971, McDonald and Hoff 1971), and (b) premature shed of secondary needles (McDonald and Hoff 1970, McDonald and Hoff 1971). Resistance mechanisms that may reduce the number of stem infections or the severity of these infections include: (a) bark reaction (Hoff 1986, Kinloch and Davis 1996), (b) reduced needle lesion frequency (Hoff and McDonald 1980a, Meagher and Hunt 1996) and (c) tolerance (Hoff and McDonald 1980b). These mechanisms have been at the core of the Region 6 selection program for three decades; however, for both sugar pine and western white pine there has been little or no formal field validation of the test results from the artificial inoculation and screening at DGRC or tracking of individual family rust resistance over time.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, Oregon, 97424, USA. Phone (541) 767-5716, Fax (541) 767-5709, E-mail: rsniezko@fs.fed.us; A.D. Bower's current address: Forest Sciences Department, University of British Columbia, Vancouver, B.C. V6T 1Z4 Canada.

Field trials were established in 1996 and 1997 with seedlings from 13 western white pine and 12 sugar pine families in the first of a series of validation plantings. These 25 seedling families were planted at three test sites, two in Oregon and one in California. The first and largest was planted at Happy Camp, California in 1996. This site was also the first one to show moderate levels of rust infection. The Happy Camp site on the Klamath National Forest has been the principal field test site for blister rust resistance evaluation for the Forest Service's Region 5 (California) sugar pine program since 1962 (Kinloch and Byler 1981). This test site has a high frequency of a strain of blister rust virulent to the major gene resistance conferred by the Cr1 gene in sugar pine (Kinloch and Comstock 1981). Since Cr1 is neutralized on sugar pine at this site, it is possible to observe resistance(s) that would be masked by Cr1 at other sites. The main purpose of these field trials is to validate the effectiveness and durability of the putative mechanisms of resistance to blister rust.

This paper examines results five years after planting and assesses species and family differences in percentage of trees with stem symptoms (SS percent). We also report on the correlations between field results (for SS percent) and results from operational screening of seedlings at DGRC (SS percent and needle lesion frequency).

Materials and Methods

Twenty-five seedlots (13 western white pine and 12 sugar pine) were selected from relatively recent blister rust screening trials ("runs") at Dorena Genetic Resource Center (DGRC). Two of the western white pine seedlots are full-sib families from crosses made among resistant parents in DGRC orchards, and one of the western white pine seedlots is a wind-pollinated collection from a seed orchard at DGRC. The remaining 22 families are open-pollinated from select trees in natural stands. The families selected for these field tests cover a wide range of geographic areas in Oregon and Washington as well as an array of resistance responses observed in five years of assessments following artificial inoculation at DGRC. Families that displayed little or no resistance in a previous DGRC test were included as low resistance controls.

Seed was sown in containers in spring 1995, and seedlings were planted at Happy Camp (HC) in a randomized complete block design in spring 1996 after budbreak. Twelve blocks were established with generally four trees per family per block although a few families had fewer trees per block, and an occasional container held two seedlings that were then planted together. *Ribes sanguineum* Pursh, an alternate host to *C. ribicola*, was inter-planted among the pines to help ensure uniform exposure to the rust.

Height as well as number and type of stem symptoms (SS) were assessed in June 1999 and July 2000 at Happy Camp. For the main analysis in this paper, a tree was recorded as having SS if there was any sign or symptom of rust infection on the bole or branches, including small orange discoloration at the base of infected needles (the initial signs of stem infection), normal cankers or bark reactions in either 1999 or 2000. Percentage of trees with stem symptoms (SS percent) was tabulated by family plot and used for analyses. Analyses of variance were performed using SAS Proc GLM (SAS Institute 1999) to assess differences between species and families within species for SS percent.

Pearson's correlations between family mean SS percent at Happy Camp and family mean SS percent and Needle Lesion class (NLclass) at DGRC were calculated using SAS Proc CORR (SAS Institute 1999). The correlations use results from the 1995 sowing for the 19 families tested in that year, and a second set of correlations uses the 1995 test data plus results for the six families tested in other screening trials (table 1). NLclass is a family and trial-specific value based upon number of needle lesions or "spots" on all secondary needles on each seedling (Table 2). NLclass values range from 0 to 4. Generally, the scale is set up to have approximately 25 percent of the seedlings in each needle lesion class from 1 to 4; seedlings in needle lesion class 0 have no spots.

Results

Sugar pine had a higher percentage of trees with stem symptoms (SS percent) than western white pine at the HC site (fig. 1). Highly significant differences ($p<0.0001$) for SS percent existed between species and among western white pine families ($p<0.0001$) but not among sugar pine families ($p=0.58$). Except for the susceptible western white pine (control) family, there was no overlap in SS percent among the 12 sugar pine families and the 13 western white pine families (fig. 1). Most of the stem symptoms are from infection in autumn 1997.

Overall SS percent (SS observed in 1999 and/or 2000) was 85.4 percent for sugar pine and 43.2 percent for western white pine (fig. 2). Mean SS percent for western white pine was 35.2 percent in the 1999 and 30.1 percent in the 2000 (fig. 2). Ten of the 13 western white pine families had lower SS percent in 2000 than in 1999. SS percent in sugar pine increased from 70.8 percent in 1999 to 77.4 percent in 2000 (fig. 2). Only one sugar pine family had a lower SS percent in 2000 than in 1999, and two families had the same SS percent in 1999 and 2000. There was a relatively narrow range in SS percent among sugar pine families (73.5 to 91.8 percent) but a very wide range among western white pine families (10.4 to 83.3 percent).

Of two western white pine families notable for their very low SS percent (table 1 and Fig. 1), Family #22 is known to have major gene resistance (from Cr2); the resistance mechanism for the other family (#18) is unknown, but it is not Cr2. The low resistance western white pine control (#16) had the highest SS percent (table 1).

Strong and significant correlations exist between family mean SS percent at Happy Camp and those from 1995 artificial screening at DGRC for both sugar pine ($r = 0.73$, $n=9$, $p=0.024$) and western white pine ($r = 0.70$, $n=10$, $p=0.026$) (also see fig. 3a and 3b). There were positive but non-significant correlations between needle lesion frequency (NLclass) in DGRC screening and SS percent at Happy Camp for both species (fig. 4a and 4b). Even western white pine family #25, which was outstanding for NLclass in several tests at DGRC, is only average for SS percent at HC (table 1).

Table 1—Summary results for percent stem symptoms, needle lesion class, and major gene resistance from Dorena rust-screening and Happy Camp, California field planting for 12 sugar pine (SP) and 13 western white pine (WWP) families.

Field ID	Female parent	Male parent	Species	MGR assessment results[b]	Test year[c]	Needle lesion class[d]	% Stem symptoms	
							Dorena	Happy Camp[f]
1[a]	02176-040	wind	SP	—	1992	3.25	100.00	89.58
2	10045-689	wind	SP	—	1995	2.53	100.00	91.83
3	11052-570	wind	SP	not yet tested	1992	1.75	48.33	87.76
4	11054-370	wind	SP	—	1992	1.93	100.00	80.56
5	11054-419	wind	SP	Cr1 (~52%)	1995	1.64	32.20	81.63
6	11054-581	wind	SP	non-Cr1	1995	2.49	100.00	82.22
7	11054-776	wind	SP	—	1995	3.30	85.19	88.89
8	11054-903	wind	SP	Cr1 (~21%)	1995	2.00	43.10	73.47
9	18032-608	wind	SP	—	1995	1.46	92.59	85.71
10	18033-431	wind	SP	—	1995	2.30	96.30	87.50
11	18034-404	wind	SP	—	1995	3.02	94.92	87.50
12	20045-001	wind	SP	non-Cr1	1995	3.54	96.61	89.80
13	11053-552	wind	WWP	—	1995	1.48	88.33	38.10
14	03023-509	wind	WWP	—	1995	2.32	100.00	50.00
15	03024-510	wind	WWP	non-Cr2	1995	1.75	70.00	32.65
16[a]	03024-532	wind	WWP	non-Cr2	1995	3.33	98.33	83.33
17	03024-793	wind	WWP	—	1995	2.43	91.38	53.19
18	05081-003	wind	WWP	non-Cr2	1995	1.92	40.00	12.25
19	06023-521	wind	WWP	not yet tested	1995	1.73	74.51	60.00
20	18034-140	wind	WWP	non-Cr2	1995	2.82	96.67	40.39
21	1803.5-150	wind	WWP	non-Cr2	1995	1.95	90.00	57.45
22	15045-816 x 15045-841	wind	WWP	Cr2 (97%)	1989	2.53	17.02[e]	10.42
23	15045-823	15045-840	WWP	Cr2 (~75%)	1988	2.62	42.50[e]	33.33
24	21105-052	wind	WWP	non-Cr2	1995	2.78	89.83	44.44
25	18033-708	18033-703	WWP	non-Cr2	1988	0.43	23.33	45.83

[a] Susceptible control family based on performance in a single artificial inoculation trial at DGRC

[b] Results from separate inoculation to score hypersensitive reaction (HR) on needles and classify families as to presence or absence of major gene resistance. Percentage seedlings exhibiting HR indicated in parentheses. Preliminary information indicates the female parent of Family 22 (Orchard Accession # 023220) may be homozygous dominant for HR.

[c] Test year refers to the year in which the family was sown at Dorena; inoculation occurred the following year.

[d] Family mean needle lesion class at DGRC based on the number of lesions on all secondary needles approximately 9 months after artificial inoculation with *C. ribicola.*

[e] In many years the mixture of spores used for inoculation at DGRC contained an unknown frequency of a strain of rust virulent to Cr2 in western white pine.

[f] Overall percent stem symptoms (present in 1999 and/or 2000) at Happy Camp.

Table 2—Number of needle lesions in each class by test year and trial at Dorena Genetic Resource Center.

Test year	Trial	Species	Needle lesion class[a]				
			0	1	2	3	4
1988	4	western white pine	0	1	2-3	4-6	7+
1989	4	western white pine	0	1-3	4-9	10-19	20+
1992	4	sugar pine	0	1-10	11-26	27-50	51+
1995	1	western white pine	0	1-3	4-9	10-20	21+
1995	2	sugar pine	0	1-3	4-9	10-27	28+

[a] Each seedling is evaluated for number of needle lesions (“spots”) present on secondary needles and is assigned to a “needle lesion class.” Needle lesion classes are based on counts of number of spots on seedlings in monitoring plots and are trial specific. Family mean needle lesion class is the average of all living seedlings in a family.

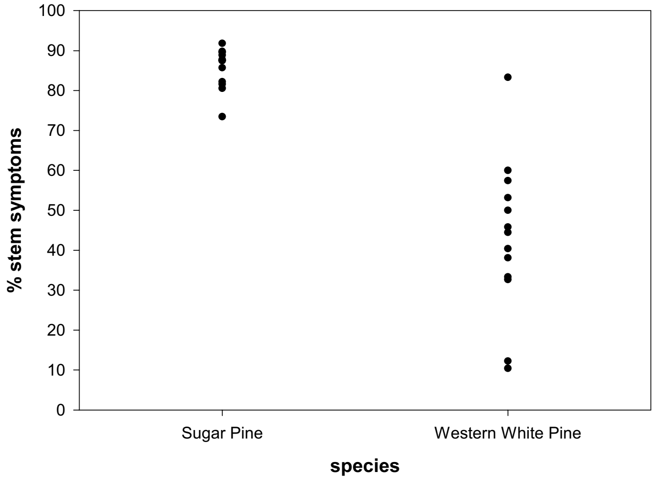


Figure 1—Range of means for overall percent stem symptoms at Happy Camp for 12 sugar pine and 13 western white pine families.

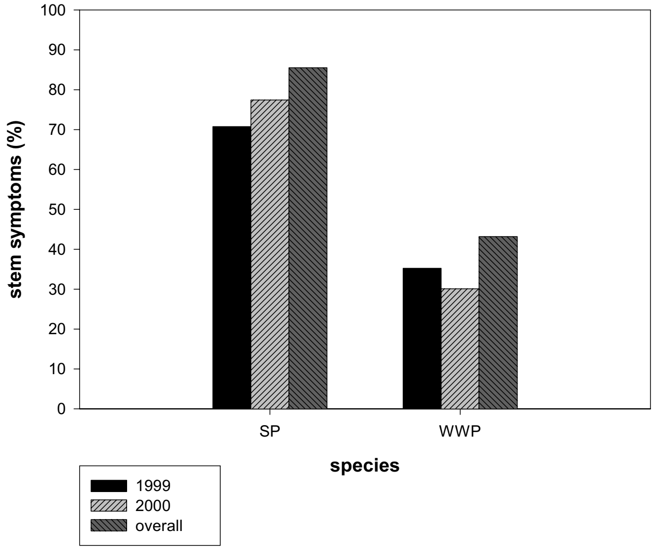


Figure 2—Species means for 1999, 2000, and overall (present in 1999 or 2000) percent stem symptoms for 12 sugar pine and 13 western white pine families outplanted at Happy Camp, California.

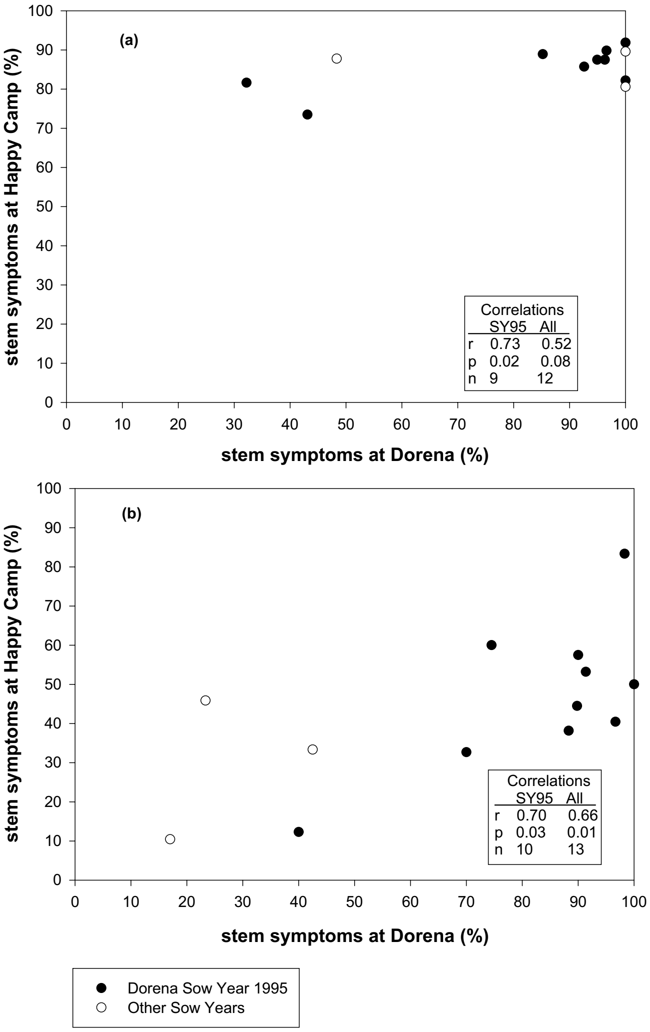


Figure 3—Percent stem symptoms at Happy Camp vs. percent stem symptoms at Dorena for (a) 12 sugar pine families and (b) 13 western white pine families.

Discussion and Summary

The results clearly show that after five years at HC, sugar pine is significantly more susceptible to blister rust than western white pine. In a summary of previous studies involving 16 species of five-needle pines, western white pine appeared to be slightly less susceptible than sugar pine (Bingham 1972). In 1999 at HC, sugar pine had 2.7 times as many stem infections as western white pine (953 vs. 356 total stem infections, Sniezko and others 2000).

Current results from HC demonstrate that SS percent at this site corresponds fairly well to SS percent at DGRC. However, at the current level of infection, there appears to be little or no differentiation between families showing different levels of needle lesion frequency at DGRC and SS percent in the field for either western white pine or sugar pine. Needle lesion frequency may be more associated with number of stem infections rather than presence or absence of stem infections. It is still too early to discern the relationships of other resistance traits at DGRC and in the field.

In examining SS percent by individual year, it was noted that from the 1999 to the 2000 assessment there was a slight increase (6.7 percent) for sugar pine but a decrease (5.2 percent) for western white pine, and that the total for either year was more than 8 percent lower than the overall SS percent (SS present in 1999 or 2000) (fig. 2). Close re-examination of a few trees in spring 2001 showed some small, fading stem symptoms that could have easily been missed and will probably not be visible at all within a year or two.

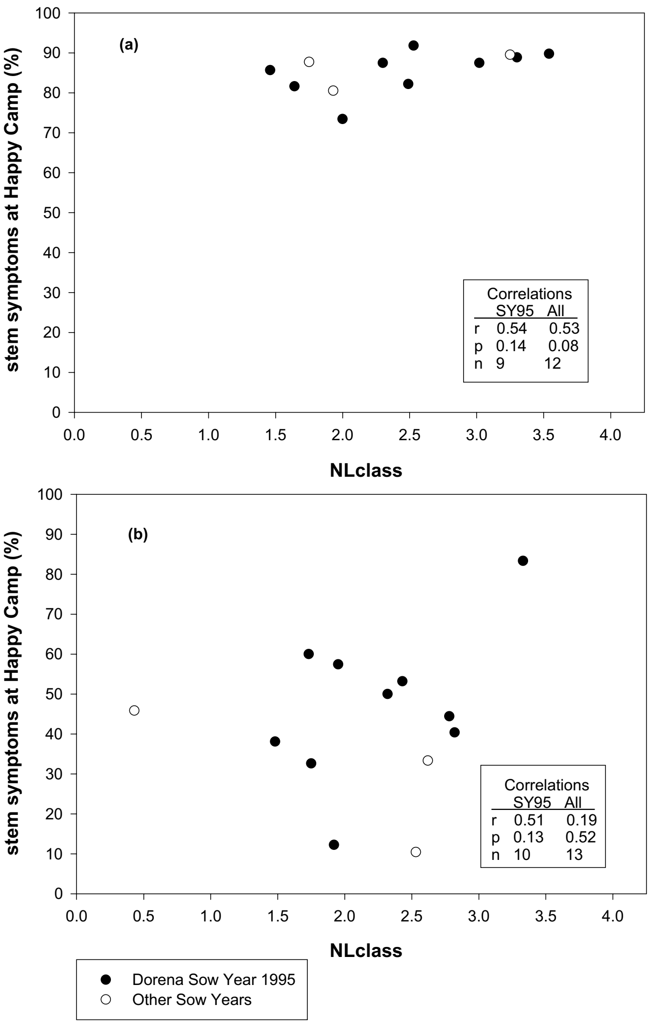


Figure 4—Percent stem symptoms at Happy Camp vs. needle lesion class (NLclass) at Dorena for (a) 12 sugar pine families and (b) 13 western white pine families (see table 2 in text for needle lesion classes).

Although the percentage of sugar pine infected at HC is already high, there is little mortality as of July 2000. On this site the sugar pine families with HR show high levels of infection because of the high frequency of the vcr1 gene (virulent to Cr1) in the rust population at HC; these two families would be expected to show much lower infection at most sites. The lack of significant differences in SS percent among families in sugar pine may not be a true measure of field resistance; future assessments will determine whether differences in bark reaction, mortality or tolerance exist among families.

One of the biggest expected changes over the next ten years would be the performance of the two western white pine families with Cr2 (Families 22 and 23) if a strain of rust with specific virulence to Cr2 (vcr2) became prominent at this site. The virulent strain is known to be present in a small adjacent test established in 1971 (Kinloch, personal communication). Previously, vcr2 had only been documented in a localized area in Oregon (Kinloch and others 1999, McDonald and others 1984), but a recent survey has demonstrated its presence in varying frequency in parts of western Oregon and at Happy Camp, CA (Sniezko and others 2001).

The performance of the Cr2 families in this test demonstrates that there may be important geographic differences in blister rust resistance. The resistance conferred by the Cr2 gene in western white pine appears to be limited geographically to California, Oregon, and southern Washington (Kinloch and others 2003), but there may also be other types of resistances that are geographically restricted and not noted in earlier findings (see discussion below). Elucidation of geographically restricted resistance mechanisms would aid breeding efforts and establishing deployment strategies for resistant seed. At present, operational rust screening at DGRC and elsewhere only discerns categories of phenotypic expression, but it is possible that several mechanisms may have only minor differences in their gross physical expression. For example, only in the mid-1990s has DGRC incorporated an operational screening procedure that separates a major gene for resistance (hypersensitive reaction in the needles, see Kinloch and others 1999, Kinloch and Dupper 2002 for details) from other resistance mechanisms in western white pine that also lead to canker-free seedlings after inoculation such as needle shed or short shoot (Hoff and McDonald 1971, McDonald and Hoff 1970, Sniezko and Kegley 2003).

The low level SS percent in Family #18, an open-pollinated, non-Cr2 western white pine family, might be due to some combination of needle shed or short shoot mechanisms (both purportedly due to single recessive genes). If this were true, the frequency of these genes in natural stands would have to be high and there is no evidence of this in testing at DGRC; the great majority of families show greater than 90 percent infection in screening trials at DGRC. This could also be a previously undefined mechanism (mechanism 'X'), characterized by low incidence of stem symptoms at DGRC in testing (35-60 percent SS, relative to 90-100 percent for most other open-pollinated families) and a negative result for presence of Cr2 resistance in a separate test. Family #18 at HC fits these parameters. The relatively low SS percent for this open-pollinated family at DGRC suggests the involvement of a single major gene, but the very low SS percent at HC suggests a more complicated scenario. Some differences in performance of families in short-term screening and in the field are not unusual. In a summary of studies in other plant species Keller and others (2000) note that the resistance phenotype may vary between tests performed under controlled conditions versus field conditions, or between seedlings and adult plants. From operational screening of thousands of western white pine parents at DGRC it appears that a very low frequency of parents with this type of resistance (low SS percent and non-Cr2) is present in much of Oregon and Washington (unpublished data).

Artificial inoculations and short term screening at DGRC provides a potentially more time- and cost-efficient method of evaluating progeny of thousands of parent trees for an array of resistance mechanisms than costly, long-term field trials. However, field tests are essential for validating effectiveness of the various resistance responses characterized on young seedlings following artificial inoculation. A wide array of rust races can be included in artificial screening, whereas field testing at any one site would generally rely on local populations of the pathogen, which may vary from year to year. However, due to the limitations of a single inoculation on very young seedlings, resistance mechanisms that manifest themselves more clearly on older, larger trees in the field may not be identified in operational screening (such as ontogenetic resistance (Kinloch and Davis 1996); low canker frequency (Sniezko and others this proceedings)), thus field plantings serve a complementary function. Correspondence between results from short-term testing at DGRC and long-term field testing may be dependent upon factors influenced by the environment, the rust population, and the nature of the families and resistance mechanisms under test.

Results from these field tests will allow confirmation of the field effectiveness of resistance responses observed on seedlings following artificial inoculation, as well as provide demonstrations to land managers hoping to use western white pine or sugar pine in restoration or reforestation plantings. The plantings will also serve as monitors to changes in virulence of the rust, and they may help detect resistance mechanisms or other events not apparent in short-term screening. For example, from recent observations in fall 2001, some cankers appear inactive but do not fit the classic pattern of bark reactions.

It may also be very useful to establish some joint field tests among the blister rust programs in Oregon, Washington, Canada, Idaho, and California using a small number of families selected for specific resistance responses in these different locations. Such plantings may help discern the presence of geographically limited mechanisms or the influence of environment and local rust populations on host resistance.

Acknowledgments

The authors would like to thank the Region 5 Genetics Program for use of the Happy Camp test site; Dean Davis and Deems Burton for monitoring the site and advice on assessments; Bro Kinloch and Bob Westfall for early discussions at the trial site; Jude Danielson for help in family selections from the operational program; Bob Danchok and Sally Long for leading the assessment efforts; all the staff and technicians at DGRC whose work and support greatly facilitated this planting; Paul Zambino, Bro Kinloch, Safiya Samman, and Rich Hunt for constructive comments on a earlier version of this manuscript; financial support from the USDA Forest Service's Region 6 Forest Health and Genetics programs in establishing this planting.

References

Bingham, R.T. 1972. Taxonomy, crossability, and relative blister rust resistance of 5-needled white pines, p. 271-280. In: R.T. Bingham, R.J. Hoff, and G.I. McDonald (eds) Biology of rust resistance in forest trees: Proceedings of a NATO-IUFRO Advanced Study Institute. USDA Forest Service Misc. Publ. 1221.

Hoff, R.J. 1986. Inheritance of Bark Reaction Resistance Mechanism in *Pinus monticola* by *Cronartium ribicola*. USDA Forest Service, Res. Note INT-361. 8pp.

Hoff, R.J. and McDonald, G.I. 1971. Resistance to *Cronartium ribicola* in *Pinus monticol*a: short shoot fungicidal reaction. Can. J. Bot. 49:1235-1239.

Hoff, R.J. and McDonald, G.I. 1980a. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. Can. J. Bot. 58:574-577.

Hoff, R.J. and McDonald, G.I. 1980b. Improving rust-resistant strains of inland western white pine. USDA Forest Service, Res. Pap. INT-245. 13p.

Keller, Beat, Feuillet, Catherine, and Messmer, Monika. 2000. Genetics of Disease Resistance: Basic Concepts and Application in Resistance Breeding. In: A. Slusarenko, R.S.S. Fraser, and L.C. van Loon, eds. Mechanisms of Resistance to Plant Diseases. Kluwer Academic Publishers, The Netherlands: 101-160.

Kinloch, Bohun B. and Byler, James W. 1981. Relative effectiveness and stability of different resistance mechanisms to white pine blister rust in sugar pine. Phytopathology 71:386-391.

Kinloch, B.B., Jr. 2000. [Personal communication]. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Kinloch, B. B., Jr. and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. Plant Dis. 65:604-605.

Kinloch, B.B., Jr. and Davis, D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Res. Publ. 3362.

Kinloch, B.B. and Dupper, G. 2002. Genetic specificity in the white pine-blister rust pathosystem, Phytopathology 92(3): 278-280.

Kinloch, Bohun B., Sniezko, Richard A., Barnes, Gerald D., and Greathouse, Tom E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. Phytopathology 89:861-867.

Kinloch, B.B., Jr., Sniezko, R.A., and Dupper, G.E. 2003. Origin and distribution of Cr2, a gene for resistance to white pine blister rust in natural populations of western white pine. Phytopathology 93:691-694.

McDonald, G.I. and Hoff, R.J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: early shedding of infected needles. USDA Forest Service, Res. Note INT 124. 8 pp.

McDonald, G.I. and Hoff, R.J. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: genetic control of needle-spots-only resistance factors. Can. J. For. Res. 1:197-202.

McDonald, G.I., Hansen, E.M., Osterhaus, C.A., and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicol*a from the Cascade Mountains of Oregon. Plant Disease 68: 800-804.

Meagher, M.D. and Hunt, R.S. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. Silvae Genet. 45(2-3):75-81.

SAS Institute Inc. 1999. SAS OnlineDoc, Version 8. Cary, NC: SAS Institute Inc.

Sniezko, R.S. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Natural Res. Publ. 3362.

Sniezko, R.A., Bower, A., and Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance in sugar pine and western white pine. HortTechnology 10(3): 519-522.

Sniezko, Richard A., Kinloch, Bohun, and Dupper, Gayle. 2001.Geographic distribution of 'Champion Mine' strain of white pine blister rust (*Cronartium ribicola*) in the Pacific Northwest. 2001 National Forest Health Monitoring meeting, Las Vegas, NV. Poster presentation. [Online]. Available: http://www.na.fs.fed.us/spfo/fhm/posters/posters01/geo.pdf [2002, June 22].

Sniezko, Richard A. and Angelia Kegley. 2003. Blister rust resistance of five-needle pines in Oregon and Washington. Proceedings of the Second IUFRO Rusts of Forest Trees Working Party Conference. 19-23 August 2002. Yangling, China. Forest Research 16 (Suppl.): 101-112.

Variation in Blister Rust Resistance Among 226 *Pinus monticola* and 217 *P. lambertiana* Seedling Families in the Pacific Northwest

A.J. Kegley
R.A. Sniezko

Abstract—*Pinus monticola* and *P. lambertiana* families sown in 1989 and 1994 were inoculated with *Cronartium ribicola* after two growing seasons. Development of rust symptoms and mortality were followed for five years. During this period, 96 to 99 percent of the seedlings became infected, and 91 to 99 percent of the seedlings developed stem symptoms in the four screening trials (family means varied from 30 to 100 percent). Survival of infected seedlings 5 years after inoculation varied from 1.6 to 13.1 percent for the four trials; family means varied from 0 to 54.8 percent. The frequency and level of resistance responses in half-sib progeny of phenotypic selections from natural stands were generally low, but some families had survival comparable to the full-sib checklots. In both species the families with the highest levels of survival generally had higher than average levels of several resistance responses, including percentage of seedlings without stem symptoms, delayed appearance of stem symptoms, bark reaction, and survival with stem symptoms. Levels of bark reaction were generally low in the four trials (3.5 to 12.3 percent of the trees), with half-sib families varying from 0 to 59.9 percent. Many trees with bark reaction also had normal cankers and died by the end of the assessment period. Selections from the top individuals within these trials have been grafted into seed orchards. Breeding efforts are focused on the development of seedlings with more durable rust resistance for reforestation and restoration.

Key words: blister rust, resistance responses, screening, pines

Introduction

The introduced disease white pine blister rust, caused by the pathogen *Cronartium ribicola* J.C. Fisch. in Raben., has devastated populations of five-needle pines in many parts of western North America. Development of genetic resistance to this pathogen will be a key to restoration of these species. Due to land management changes and limited opportunities for planting seedlings on federal lands, maintaining these species as viable components of the ecosystem will depend even more on the development of durable resistance.

Evaluation of white pine blister rust resistance and/or operational screening has occurred for a number of species in North America, Asia, and Europe (Kim and others 1982; Blada, this proceedings; Stephan, this proceedings; Daoust and Beaulieu, this proceedings; McDonald and others, this proceedings; King and Hunt, this proceedings; Bingham 1983; Zsuffa 1981). In North America, the identification of some genetically resistant individuals (Bingham 1983; Kinloch and others 1970; McDonald and others, this proceedings) led to the initiation of several resistance breeding programs for western white pine (*Pinus monticola* Dougl. ex D. Don.) and sugar pine (*P. lambertiana* Dougl.) (Bingham 1983; King and Hunt, this proceedings; Sniezko 1996; Kinloch and Davis 1996; Samman and Kitzmiller 1996). In Oregon and Washington, Region 6 of the USDA Forest Service, works began on operational screening and breeding of western white pine (WWP) and sugar pine (SP) for resistance to *C. ribicola* in the late 1950s, but screening of whitebark pine (*P. albicaulis* Engelmann) has only recently begun. The screening program for all three species is based at the Dorena Genetic Resource Center (Dorena); the Center staff work with geneticists and land managers throughout Oregon and Washington to develop breeding populations and seed orchards with resistant parent trees. Seedling common-garden studies (Campbell and Sugano 1987; Campbell and Sugano 1989) have provided the foundation for establishing breeding zones in Oregon and Washington for both SP and WWP. Rust resistance of progeny from parent trees in natural stands or plantations has been evaluated for most of these breeding zones; moreover, breeding among resistant progeny has begun for several of the zones.

Since the 1970s blister rust resistance has been evaluated using half-sib progeny of phenotypically selected trees from National Forest lands as well as lands of the USDI Bureau of Land Management (BLM), and other landowners in Oregon and Washington. The phenotypic selections were made on sites varying from low to high incidence of blister rust. In general the selected trees were vigorous and either free of rust cankers or showed fewer cankers than other WWP or SP in the local area. Progeny of approximately of 4,900 WWP and 4500 SP phenotypic selections have now been screened at Dorena. Based upon screening results, selections among the progeny of the selected parents have been made and placed into seed orchards.

The Region 6 program was based on protocols and resistance mechanism studies in WWP by Forest Service re-

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

A.J. Kegley and R.A. Sniezko are with the USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, Oregon, 97424, USA. Phone (541) 767-5716; Fax (541) 767-5709; e-mmail: akegley@fs.fed.us; rsniezko@fs.fed.us.

searchers in Idaho (Bingham 1983; McDonald and others, this proceedings; Sniezko 1996). Recently, screening of seedlings has been modified to incorporate an assessment for a major gene conditioning a hypersensitive response (HR) in needles of sugar pine and western white pine (see Kinloch and others 1999; Kinloch and Dupper 2002 for details on screening for major gene resistance); screening for HR complements but does not replace standard operational screening. Artificial inoculation with blister rust allows seedlings to be categorized into those with complete resistance (no stem infection) and those with partial resistance (stem infection present).

There is little published information on the inherent levels of blister rust resistance in WWP or SP in Oregon and Washington (Sniezko 1996). However, a synthesis of results from the Region 6 program has begun. This paper reports on the levels of resistance for 226 seedling families of WWP and 217 families of SP, representing a large part of their geographic ranges in Oregon and Washington. The data reveal the different types of resistance responses and relative degrees of responses between trials, species, and families, and compares them to existing checklot families.

Some refinements and modifications to Dorena screening summaries are incorporated here for the first time. A summary of these four trials along with analyses of other Region 6 studies will improve the advanced-generation breeding program. Moreover, better comparisons with the resistance screening and breeding programs in British Columbia, California, and Idaho will be possible.

Materials and Methods

Study Design

Four blister rust screening trials were examined, one WWP and one SP, from each of two test years, 1989 and 1994. These trials are denoted as WWP1989, SP1989, WWP1994, and SP1994. The seedling families in these trials are progeny of parent trees that cover much of the range of WWP in Oregon and Washington and of SP in Oregon (fig. 1a,b).

In each of the four trials, seed from 120 families were sown in a randomized complete block design with six blocks. Within each block, 10 seedlings per family were planted in row plots. Each trial contained a maximum of 7,200 seedlings (6 blocks x 1,200 seedlings/block). Seedlings were grown outside in open boxes (0.91 m wide x 1.21 m long x 0.30 m high; 10 boxes per block) for two growing seasons before artificial inoculation with blister rust.

Families

The majority of families included in each of these trials are half-sib seedling progeny ("Wild OP" families) of phenotypic selections from natural forests or plantings (table 1). In the WWP1994 trial, 42 of 110 Wild OP families were from the Quinault Indian Nation in western Washington, 57 were from the Colville National Forest (NF) in eastern Washington, and 11 were from several other National Forests in

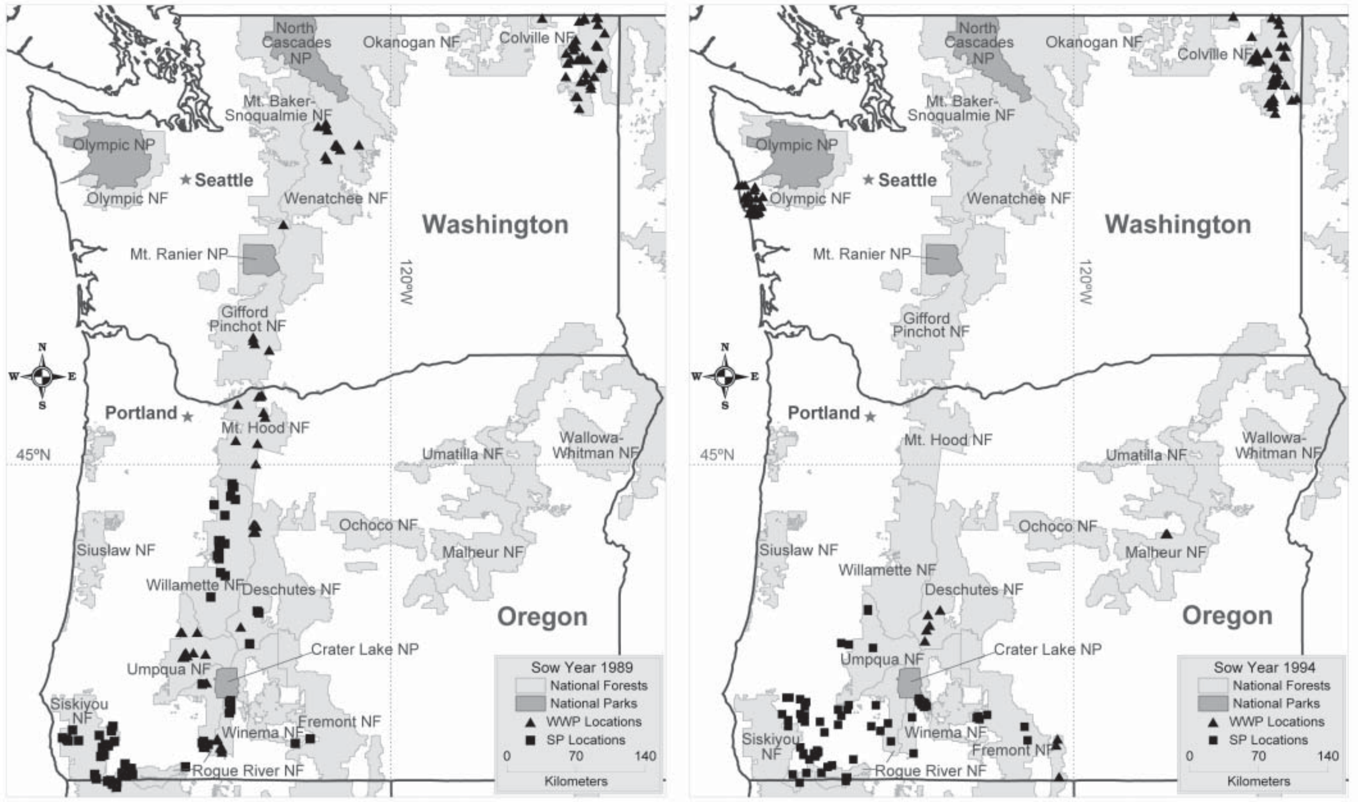


Figure 1—Geographic location of parent trees with half-sib progeny included in the four trials.

Table 1—Geographic location of parent trees of families included in four blister rust screening trials at Dorena Genetic Resource Center.

Species	Breeding Zone	National Forest	Elevation (ft)[c]	Sow Year 1989	Sow Year 1994
western	02180	Fremont	—		3
white	05010	Mt Baker-Snoqualmie	—	1	
pine	06020	Mt Hood	—	20	
	09070	Olympic[b]	—		42
	15040	Umpqua	—	28	
	16140	Wallowa-Whitman	—		3
	17110	Wenatchee	—	13	
	18030	Willamette	—	9	5
	21100	Colville	—	45	57
	Idaho[a]		—		4
	Dorena BR Check[a]	Colville	—		1
	Dorena HR Checks[a]	Umpqua	—	4	4
	Total			120	119
sugar	02176	Fremont	All	5	11
pine	10025	Rogue River	>4000	17	7
	10043	Rogue River	<2500		25
	10044	Rogue River	2500-4000	25	21
	10045	Rogue River	>4000	6	42
	11053	Siskiyou	<2500	3	
	11054	Siskiyou	2500-4000	27	7
	11055	Siskiyou	>4000	2	
	18033	Willamette	<3000	19	
	Dorena HR Checks[a]	Umpqua	—[d]	2	4
	Dorena LR Check	Umpqua	—[d]	1	
	Total			107	117

[a] The checklots are technically not listed by breeding zone but rather by source (Dorena or Idaho) or by resistance type HR (hypersensitive reaction), BR (bark reaction), or LR (low resistance).

[b] Although Breeding Zone 09070 corresponds with Olympic NF, the 42 families from breeding zone 09070 in Sow Year 1994 were from the neighboring Quinault Indian Nation.

[c] Unlike sugar pine, elevation is not a criterion for establishing western white pine breeding zones.

[d] Since these checklots are full-sib crosses between selected parents from different locations, or a mix (LR Check), elevations are not reported for these families.

Region 6. In the WWP1989 trial, 45 of 116 Wild OP families were from the Colville NF, 20 from Mt Hood NF, 28 from the Umpqua NF, and lesser numbers from other NFs (table 1 and fig. 1). The two SP trials sampled progeny of selected trees predominantly from the Rogue River NF and Siskiyou NF in southern Oregon (table 1 and fig.1). Because a few families had insufficient germination (less than 20 seedlings) for inclusion in analyses, the numbers of Wild OP families were as follows: 104 in SP1989; 116 in WWP1989; 110 in WWP1994; and 113 in SP1994.

In addition to the 226 WWP and 217 SP Wild OP families, two to four Dorena full-sib high resistance checklots per trial, one low resistance seedlot (bulk of three half-sib families, used only in SP1989), one half-sib bark reaction checklot (BR checklot) from the Colville NF (only in WWP1994), and four standard WWP full-sib checklot families from the Forest Service rust resistance program in Idaho (only in WWP1994; Aram Eramian, personal communication) were included. The Dorena full-sib high resistance checklots were progeny of crosses among parents that have been confirmed to have a hypersensitive reaction in the needles (HR, see Kinloch and Dupper 2002 for discussion of HR in WWP and SP). Three Dorena full-sib HR checklots were common between the two WWP trials; two full-sib HR checklots were common between the two SP trials.

Inoculation

Details of the inoculation procedure have been previously described by Sniezko (1996) and Samman (1982). Approximately 18 months after sowing, seedlings were moved to a large room (13.7 m x 10.1 m x 3.0 m) where they were inoculated with blister rust (table 2). Temperature within the inoculation chamber was maintained at around 16.7°C (62°F) and relative humidity at 100 percent.

Ribes leaves (the alternate host) infected with *C. ribicola* at the telial stage were collected from various forest sites in Oregon and Washington as well as the Dorena Ribes Garden. These leaves were placed on wire frames above the seedlings, telial side down. *Ribes* leaves were randomly distributed among the six blocks of each trial except in WWP1994; in that trial, leaves from the Dorena Ribes Garden were used on Block 5, and leaves from near Champion Mine on the Umpqua NF were used on Block 6. A virulent pathotype of the rust (with the vcr2 gene) that neutralizes HR in WWP (and the associated Cr2 gene) had been noted in both these locations (Kinloch and others 1999). Blocks 1 through 4 in WWP1994 used *Ribes* leaves from areas without known occurrence of vcr2.

Spore fall was monitored until the desired inoculum density was reached for each box (table 2); the *Ribes* leaves were

Table 2—Inoculation summary statistics for four blister rust screening trials at Dorena Genetic Resource Center.

	WWP1989[a]	**SP1989**	**WWP1994**	**SP1994**
Spore Density[b]				
Target Spore Density	3500	6000	3000	6000
Average Spore Density	3470	5233	3348	7216
Range in Spore Density	3095-3870	3355-6390	2925-3975	6215-8040
% Ribes leaves with vcr2[c]	85%	65%	33.3%	46.2%
Inoculation Temperature	58-63°F	60-62°F	61-64°F	62-63°F
Inoculation Humidity	100%	100%	100%	100%
Spore Germination %	—[d]	86.3%	94.7%	95.6%

[a] Where WWP=western white pine and SP=sugar pine and the year indicates year sown for testing.
[b] The number of basidiospores of *Cronartium ribicola* per square centimeter.
[c] Percentage of total leaves used that originated from areas where vcr2 pathotype virulent to HR in WWP have been confirmed. In WWP1989, SP1989, and SP1994, leaves with vcr2 (Ribes Garden and Champion Creek) were randomly distributed among the blocks. However in WWP1994, potential leaves with vcr2 were placed on blocks 5 and 6 only. Note: vcr2 does not appear to overcome HR in SP.
[d] Basidiospore germination was not assessed in the WWP1989 trial.

then removed. After the target inoculum density was reached, the seedlings were left in the inoculation chamber for approximately 48 hours to ensure spore germination; boxes were then returned to their previous outdoor location. In each of the 2 years, the sugar pine and western white pine trials were inoculated separately.

Assessments of Resistance Traits

Seedlings were assessed for blister rust symptoms six times over a period of 5 years. The first inspection occurred approximately 9 months after inoculation. Seedlings were evaluated for the presence and number of needle lesions (“spots”). The checklots in each trial were used to monitor the presence of needle lesions on all secondary needles until the number of spots reached a maximum. These checklot counts were then used to establish five needle lesion classes specific to each trial. The scale was set up to have approximately 25 percent of the seedlings in each needle lesion class from 1 to 4. Table 3 lists the number of spots in each needle lesion class for each trial.

The second inspection occurred approximately 3 months after the first inspection (1 year after inoculation). Seedlings were assessed for the presence of needle lesions, stem symptoms (cankers and bark reactions), and height. Subsequent inspections of stem symptoms and mortality occurred annually. Bark reaction data was only recorded through the third year after inoculation (inspection 4).

Based upon data collected from the six inspections, presence or absence of resistance responses (table 4) was determined for each seedling. In general, seedlings were characterized by the presence or absence of needle lesions and/or stem symptoms, the type of stem symptom, their survival after 3 and 5 years, and their height after three growing seasons (1 year after inoculation).

Analyses of variance (Proc GLM, SAS 1999) were performed separately for each of the four trials using family block means of each trait for two subsets of data: (1) all families, including checklots and (2) Wild OP families only. The model included Family and Block effects. Although the data for many of the traits were not normally distributed, F-tests are fairly robust against this violation (Cochran and Cox 1967; Zolman 1993).

Table 3—Number of needle lesions (spots) in each class for seedlings in four screening trials.

	# Spots/Needle Lesion Class				
Trial[a]	**0**	**1**	**2**	**3**	**4**
WWP1989	0	1-2	3-5	6-10	11+
SP1989	0	1	2	3-5	6+
WWP1994	0	1-10	11-25	26-48	49+
SP1994	0	1-2	3-5	6-15	16+

[a] Where WWP=western white pine and SP=sugar pine and the year indicates year sown for testing.

Table 4—Traits and derived variables used in assessing seedlings for blister rust resistance.

Category	Trait		Description of Trait
General	Infected		Seedling developed needle lesions and/or stem symptoms
	NOINFECT		Seedling had no needle lesions present at either first or second inspection and had no stem symptoms in subsequent inspections.
	RSURV3		Infected tree is alive 3 years after inoculation
	RSURV5		Infected tree is alive 5 years after inoculation
	TSURV5		Seedling (infected or uninfected) is alive 5 years after inoculation
	HT3		Total height (cm), including any lammas growth, of seedling after three growing seasons
Needle Lesions	NLC	Needle Lesion Class	A categorical classification of number of needle lesions (‘spots’) on all secondary needles on a seedling at first inspection
	SPO	Spots Only	Seedling had needle spots but did not develop stem symptoms through 5 years after inoculation
	SPOT%		Seedling had needle lesions present at either first (SPOT1%) or second inspection (SPOT2%)
Stem Symptoms	SSFREE	Stem symptom free	Seedling was free of stem symptoms (initial orange discoloration of the bark, normal canker or bark reaction) at any of the six inspections following inoculation
	SS	Stem Symptoms	Seedling exhibited normal canker, bark reaction, or was dead of rust
	ESS3	Early Stem Symptoms	Calculated as the ratio of seedlings with stem symptoms (SS) one year after inoculation relative to those with SS three years after inoculation. A lower value indicates families with relatively slower or delayed appearance of stem symptoms[a]
	SSAL3	Stem symptom alive	Seedling with stem symptoms (SS) and alive (AL) 3 years after inoculation[a]
	SSAL5		Seedling with stem symptoms (SS) and alive (AL) 5 years after inoculation[a]
	NCANK	Normal Canker	Seedling exhibits initial orange discoloration of the bark or fusiform swelling with an active orange margin (Kinloch and Davis 1996; Hunt 1997) at any inspection[a]
	BR	Bark Reaction	Seedling exhibits bark reaction, that is, an incompatible interaction with the fungus (Theisen 1988). BR manifests as a sunken necrotic lesion, often at the base of a needle fascicle, on stem tissue. When no fungal activity is observed, the BR is considered ‘complete.’ An ‘incomplete’ or ‘partial’ BR does not completely halt fungal growth (Kinloch and Davis 1996; Franc 1988)[ab]

[a] The denominators for the BR%, NCANK%, ESS3%, and SSAL% calculations included only trees with stem symptoms.

[b] A seedling could be scored as having both a bark reaction and a normal canker due to the presence of (1) multiple stem infections or (2) a transition in status from normal canker to bark reaction or vice-versa in different inspection years.

Results

Survival and Growth

Survival of Wild OP families 5 years after inoculation (TSURV5) was low in all trials, averaging about 2 percent to 14 percent (table 5). TSURV5 was higher in the 1989 trials than in the 1994 trials (table 5). TSURV5 was slightly higher for SP relative to WWP in 1989, but the reverse was true in the 1994 trials. Overall survival of rust-infected Wild OP seedlings (RSURV5) was generally low in all trials, but varied by trial and species (table 5, fig. 2). Like TSURV5, RSURV3 and RSURV5 were higher for the 1989 trials than the 1994 ones (table 5, fig. 2, 3). Fifth-year survival of rust-infected Wild OP seedlings was 9.9 percent and 13.1 percent for WWP and SP, respectively, in the 1989 trials and 3.2 and 1.6 percent in the 1994 trials (table 5, fig. 3). In the 1989 trials the decline in survival began later, between the third and fourth inspections (approximately 2 and 3 years after inoculation), than in the 1994 trials (fig. 2). Despite the very low overall survival, all four trials had at least one family with more than 20 percent survival of infected seedlings (see RSURV5 in table 5 and fig. 3).

RSURV5 of the Idaho full-sib families was moderate, in the 30 to 40 percent range, slightly lower than the Dorena BR checklot (56 percent, table 5). For those seedlings with bark reactions, survival was low but higher than those with normal stem cankers (BRSURV5 and NCSURV5,

Table 5—Trial means and means of the Dorena and Idaho checklots as well as several Wild OP families with relatively high levels of resistance in four blister rust screening trials.

			Survival and Growth[b]										Needle Symptoms[b]					Stem Symptoms[b]			
Trial	Family	Type[a]	infect	no infect	rsurv3	rsurv5	tsurv5	ssal3	ssal5	nc surv5	br surv5	ht	NLC	spo	spot1	spot2	spot	ss	ess3	br	ncank
			Percent									cm		Percent							
WWP 1989	06023-513	Wild OP	97.6	2.4	76.9	52.1	40.0	74.0	36.7	33.9	68.0	31.7	1.6	25.1	88.4	35.3	88.4	72.6	13.9	32.7	61.3
	15045-443	Wild OP	98.3	1.7	83.5	54.8	51.7	74.1	32.4	27.1	66.7	29.5	2.7	30.6	98.3	56.8	98.3	67.8	71.8	20.5	64.1
	15045-642	Wild OP	100.0	0.0	78.0	42.2	40.0	76.3	37.6	26.5	58.3	29.8	2.6	8.9	100.0	48.7	100.0	91.1	41.3	34.0	80.4
	21104-418	Wild OP	100.0	0.0	75.0	35.0	35.0	71.4	28.3	16.1	47.8	37.3	2.2	10.0	100.0	68.3	100.0	90.0	43.2	50.4	78.3
	17114-631	Wild OP	100.0	0.0	86.7	54.8	53.3	84.7	44.6	35.0	71.3	32.0	2.4	22.6	100.0	40.4	100.0	77.4	55.3	27.2	67.2
	15045-861 x 15045-837	HR check	100.0	0.0	73.0	18.9	18.3	68.7	1.9	1.9	0.0	32.8	2.3	17.2	98.3	37.7	100.0	82.8	51.3	4.2	82.8
	15045-861 x 15045-862	HR check	100.0	0.0	88.0	30.6	30.0	83.7	10.0	10.0	0.0	33.5	1.9	22.0	98.3	49.1	98.3	78.0	56.4	2.1	78.0
	15045-862 x 18034-392	HR check	90.0	10.0	97.9	65.5	63.3	95.8	39.8	29.3	61.7	36.5	1.7	39.9	90.0	23.1	90.0	50.1	35.0	25.7	43.3
	15045-896 x 15045-862	HR check	100.0	0.0	91.7	30.7	30.0	87.2	7.0	2.1	0.0	36.1	2.0	25.7	98.3	27.4	98.3	74.3	56.6	2.4	69.3
		Wild OP avg	**99.4**	**0.6**	**66.6**	**9.9**	**9.5**	**65.3**	**6.0**	**4.8**	**10.8**	**30.2**	**2.4**	**4.6**	**98.1**	**59.0**	**98.4**	**94.9**	**65.9**	**12.3**	**93.1**
SP 1989	18033-317	Wild OP	86.3	4.5	68.8	24.5	21.7	77.8	20.8	14.1	33.3	33.2	1.5	6.5	62.5	32.9	64.6	89.0	31.5	8.9	78.3
	18034-112	Wild OP	87.7	11.6	74.6	39.4	36.7	69.8	33.1	6.9	25.0	35.9	1.7	12.2	75.2	31.6	79.5	76.2	40.8	15.4	47.8
	10044-212	Wild OP	84.3	15.7	92.6	42.8	46.7	94.3	23.6	14.6	100.0	39.7	1.5	23.1	68.8	27.7	74.8	61.2	46.7	5.7	53.8
	10044-230	Wild OP	77.4	22.6	83.7	49.8	36.7	72.2	31.7	9.7	83.3	47.1	1.5	18.8	58.8	25.7	71.3	58.6	34.7	23.6	42.1
	11054-877	Wild OP	85.0	15.0	84.8	37.4	45.0	82.1	23.2	16.4	38.9	45.3	1.8	15.4	75.0	33.3	76.7	69.6	45.3	36.9	56.3
	B1054-004 x B1054-034	HR check	78.3	22.8	67.4	60.8	68.3	56.7	44.3	17.3	62.5	40.1	1.7	33.6	66.3	17.7	71.3	43.7	80.0	34.4	27.5
	B1054-034 x 10044-050	HR check	77.4	22.8	50.3	40.4	51.7	24.5	5.8	6.0	0.0	37.3	1.9	30.0	68.5	33.3	74.1	47.1	67.1	27.3	45.5
	Low Resistance Mix	LR Check	94.4	5.6	75.6	10.4	15.0	72.6	3.9	2.1	0.0	41.0	2.0	7.0	76.1	43.0	81.7	87.4	52.7	8.1	80.0
		Wild OP avg	**95.8**	**3.8**	**70.0**	**13.1**	**14.1**	**70.6**	**8.4**	**4.7**	**13.5**	**39.0**	**2.1**	**5.5**	**78.1**	**52.5**	**85.1**	**90.7**	**58.9**	**7.2**	**85.7**
WWP 1994	02187-047	Wild OP	98.1	0.0	10.0	6.7	6.7	11.1	5.6	5.6	—	17.1	2.2	4.2	98.1	46.9	98.1	95.8	50.3	0.0	95.8
	21105-853	Wild OP	100.0	0.0	41.7	20.0	20.0	31.7	8.9	3.9	10.0	45.5	2.2	11.7	98.3	46.7	100.0	88.3	48.8	31.7	83.3
	09070-852	Wild OP	98.3	1.7	30.7	25.2	25.0	9.9	0.0	0.0	0.0	38.0	1.5	25.5	93.1	28.7	93.1	72.8	38.0	7.1	71.2
	09070-892	Wild OP	96.7	3.7	39.6	13.9	16.7	26.6	4.0	0.0	5.6	48.7	1.7	10.9	95.0	61.7	95.0	85.4	32.0	32.8	78.3
	09070-896	Wild OP	100.0	0.0	20.0	13.3	13.3	11.4	5.4	3.7	16.7	46.4	2.2	8.3	100.0	53.3	100.0	91.7	58.5	25.6	88.3
	21104-036	Wild OP	100.0	0.0	34.3	13.7	13.3	32.1	9.6	9.8	17.3	41.1	3.1	5.2	100.0	72.6	100.0	94.8	48.4	38.8	93.1
	21105-052	BR check	100.0	0.0	81.5	55.7	55.0	73.3	35.2	29.1	52.0	46.4	2.3	28.7	96.7	52.4	96.7	71.3	35.7	59.9	62.4
	17 x 293	Idaho	98.3	1.7	54.4	32.2	33.3	47.3	23.1	17.5	41.7	56.2	2.5	11.7	96.7	50.0	98.3	86.7	38.0	48.2	81.7
	221 x 220	Idaho	100.0	0.0	45.0	33.3	33.3	22.4	13.9	2.1	26.7	41.4	3.3	22.8	100.0	53.3	100.0	77.2	43.9	16.5	65.2
	208 x 314	Idaho	96.7	3.5	67.8	40.0	41.7	54.0	23.9	20.4	30.7	45.0	2.0	22.0	95.0	31.7	95.0	74.4	22.4	33.5	69.4
	222 x 225	Idaho	100.0	0.0	38.3	31.7	31.7	24.2	19.0	17.6	30.0	47.2	3.2	17.2	100.0	43.3	100.0	82.8	49.6	26.3	81.1
	15045-861 x 15045-862	HR check	100.0	0.0	40.0	38.3	38.3	2.9	0.0	0.0	0.0	39.8	3.3	43.9	100.0	51.7	100.0	56.1	47.7	2.9	56.1
	15045-862 x 15045-837	HR check	100.0	0.0	53.5	51.9	51.7	5.0	0.0	0.0	0.0	48.3	2.9	51.9	100.0	44.8	100.0	48.1	43.3	15.0	48.1
	15045-862 x 18034-392	HR check	100.0	0.0	51.7	36.7	36.7	26.5	2.1	2.1	0.0	45.8	2.7	35.6	100.0	50.0	100.0	64.4	54.8	20.9	64.4
	15045-896 x 15045-862	HR check	100.0	0.0	40.0	40.0	40.0	0.0	0.0	0.0	0.0	42.4	3.0	40.9	100.0	37.1	100.0	59.1	59.2	2.1	59.1
		Wild OP avg	**99.5**	**0.3**	**6.9**	**3.2**	**3.4**	**4.6**	**0.7**	**0.6**	**1.2**	**42.3**	**2.6**	**2.6**	**98.4**	**76.4**	**98.8**	**97.1**	**70.7**	**7.2**	**96.8**
SP 1994	98-01-018	Wild OP	100.0	0.0	5.0	0.0	0.0	3.7	0.0	0.0	0.0	44.7	2.6	0.0	100.0	75.1	100.0	100.0	79.9	26.1	98.1
	B1053-1380	Wild OP	91.7	8.3	53.6	28.7	33.3	2.4	5.2	2.4	33.3	54.2	1.5	34.7	91.7	43.3	91.7	56.9	62.1	11.3	51.8
	15043-402	Wild OP	100.0	0.0	20.9	13.7	10.0	16.3	6.9	6.9	—	42.9	2.1	7.9	95.5	60.4	95.5	92.1	66.1	0.0	92.1
	B1052-1993	Wild OP	100.0	0.0	5.0	3.3	3.3	5.0	3.3	3.3	0.0	56.7	3.0	0.0	97.9	79.5	100.0	100.0	74.4	4.4	100.0
	B2-2400	Wild OP	98.3	1.7	21.1	10.2	11.7	15.1	3.5	3.5	—	53.4	1.7	6.7	91.7	50.7	96.7	91.7	61.4	0.0	91.7
	B1054-004 x B1054-034	HR check	79.8	20.2	62.6	60.7	63.3	15.7	15.7	10.0	50.0	49.1	1.9	45.3	79.8	24.3	79.8	34.5	69.3	10.7	31.2
	B1054-034 x 10044-050	HR check	94.8	5.4	54.2	50.8	51.7	5.6	5.6	0.0	33.3	51.6	2.3	51.3	91.1	46.3	94.8	43.2	87.8	15.3	40.5
	B1054-005 x B1054-034	HR check	92.6	7.4	67.4	46.4	41.7	23.6	18.1	0.0	100.0	60.9	2.0	43.3	90.7	27.0	90.7	49.3	54.2	18.1	42.8
	B1054-005 x 10044-049	HR check	93.4	0.0	43.3	37.0	26.7	9.7	9.7	5.6	16.7	58.1	1.6	34.8	85.4	38.5	93.4	65.2	65.3	20.8	62.4
		Wild OP avg	**99.5**	**0.2**	**3.3**	**1.6**	**1.6**	**2.0**	**0.5**	**0.4**	**3.4**	**47.6**	**2.9**	**1.2**	**97.8**	**82.9**	**98.6**	**98.6**	**84.5**	**3.5**	**98.4**

[a] Where type refers to the classification of the material: Wild OP=half-sib progeny of phenotypic selections, BR check=half-sib family previously screened and having a high level of bark reaction, Idaho=full-sib checklot from the Idaho blister rust program, HR check=Dorena full-sib checklot with hypersensitive reaction in the needles, LR check=mix of low resistant half-sib material, Wild OP avg=mean of all Wild OP families included in the trial.

[b] Traits are defined in Table 4 except ncsurv5 (survival with normal canker 5 years after inoculation) and brsurv5 (survival with bark reaction 5 years after inoculation).

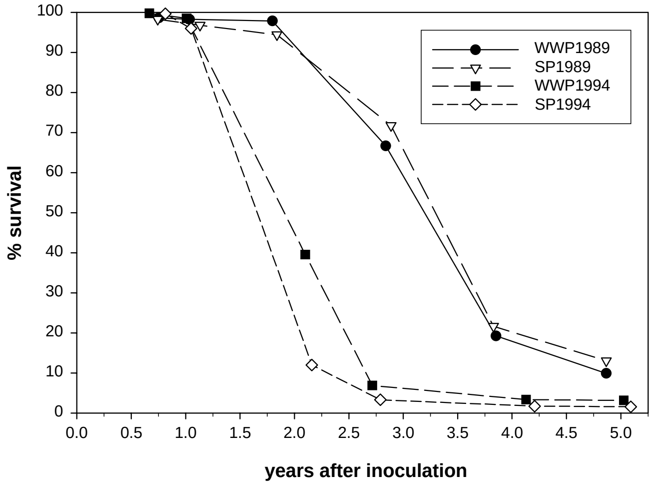


Figure 2—Survival of infected half-sib progeny of western white pine (WWP) and sugar pine (SP) after inoculation with blister rust. Each inflection point represents date of assessment.

respectively, table 5). Survival of the Dorena HR WWP checklots was about 42 percent in the 1994 trial compared to 36 percent in the 1989 trial. Survival of the two HR SP checklots common to both test years was slightly higher, ranging from 40 to 61 percent. RSURV5 of the Low Resistant SP checklot was lower than the average of the Wild OP families (10 percent versus 13 percent) (table 5).

Families varied significantly for height in all four trials (table 6). The checklots were generally intermediate for HT (fig. 4, table 5). Mean height was greater in the 1994 trials than the 1989 trials (table 5, fig. 4).

Rust Infection

Infected seedlings in the Wild OP families (infection percentage) averaged 95.8 to 99.5 percent for the four trials (table 5). Sugar pine seedlings had more variation in the frequency of infection than WWP seedlings. Wild OP family means for SP ranged from 77.4 to 100 percent in the 1989 trial and from 83.3 to 100 percent in the 1994 trial. For WWP

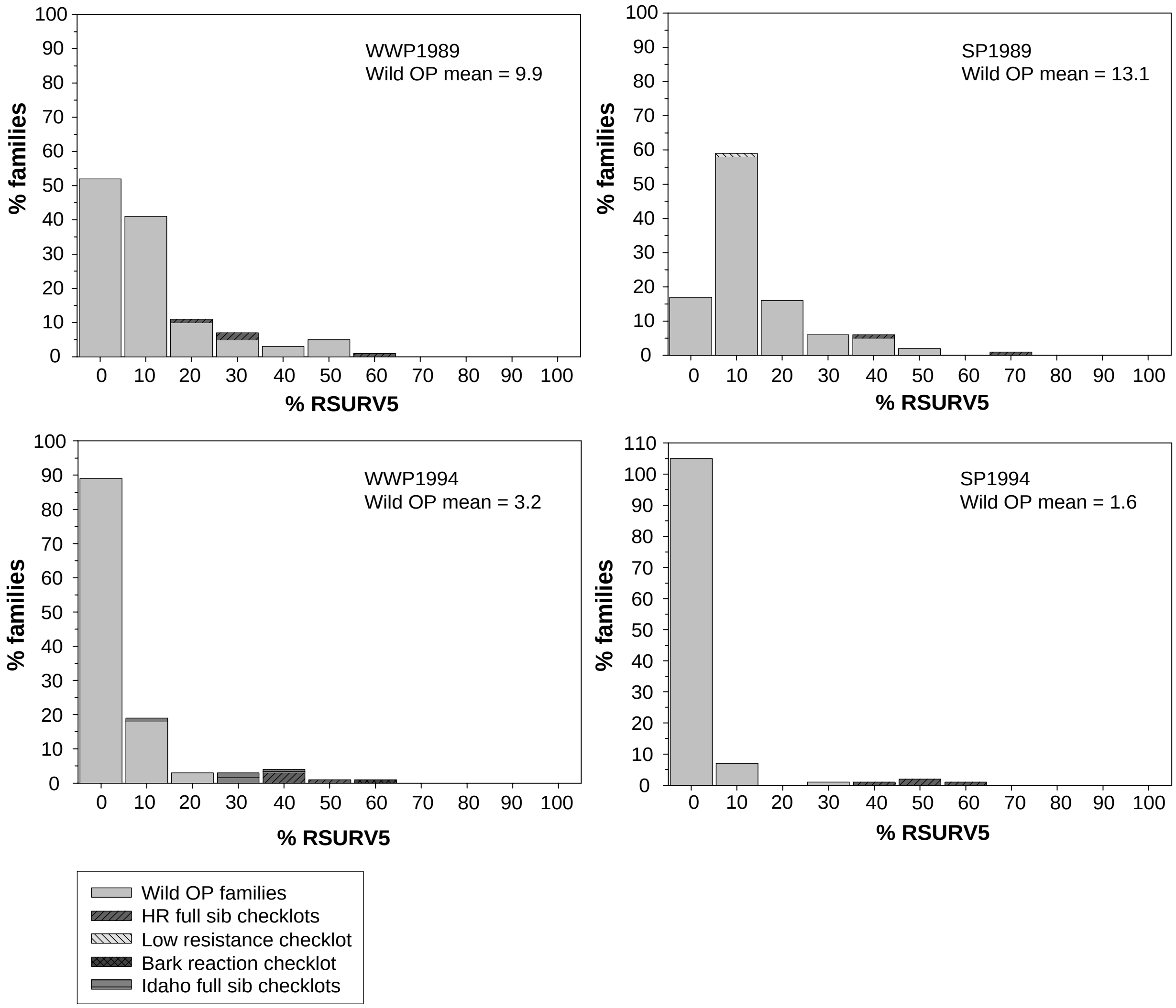


Figure 3—Distribution of family means for survival of infected seedlings 5 years after inoculation (RSURV5) for four blister rust screening trials.

Table 6—P-values associated with F-tests for significant differences among families from analyses of variance for each of four blister rust screening trials at Dorena.

		Wild OP families only				**All families**			
	Trait	**WWP 1989**	**SP 1989**	**WWP 1994**	**SP 1994**	**WWP 1989**	**SP 1989**	**WWP 1994**	**SP 1994**
General	Infect %	**0.2100**[a]	<0.0001	**0.2430**	**0.2635**	0.0113	<0.0001	**0.1656**	<0.0003
	NOINFECT %[b]	**0.2100**	<0.0001	0.0006	0.0183	0.0113	<0.0001	0.0003	<0.0001
	RSURV3 %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	RSURV5 %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	TSURV5 %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	HT	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Needle Lesions	NLC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SPO %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SPOT%	**0.1247**	<0.0001	0.0349	**0.0725**	**0.0836**	<0.0001	0.0152	0.0042
	SPOT1 %	**0.2309**	0.0007	0.0024	0.0197	**0.1929**	0.0009	0.0009	0.0012
	SPOT2 %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Stem Symptoms	SSFREE %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SS %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	ESS3 %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SSAL3 %	<0.0001	<0.0001	<0.0001	0.0108	<0.0001	<0.0001	<0.0001	<0.0001
	SSAL5 %	<0.0001	<0.0001	<0.0001	**0.0747**	<0.0001	<0.0001	<0.0001	<0.0001
	BR %	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	NCANK %	<0.0001	<0.0001	**0.1453**	**0.5214**	<0.0001	0.0045	0.0017	<0.0001

[a] P-values in bold type are not significant at the $\alpha = 0.05$ level.

[b] NOINFECT% is not necessarily equal to 100-infect%. To be classified as NOINFECT, a seedling must survive through the end of the assessment period (6th Inspection) without ever developing needle lesions or stem symptoms.

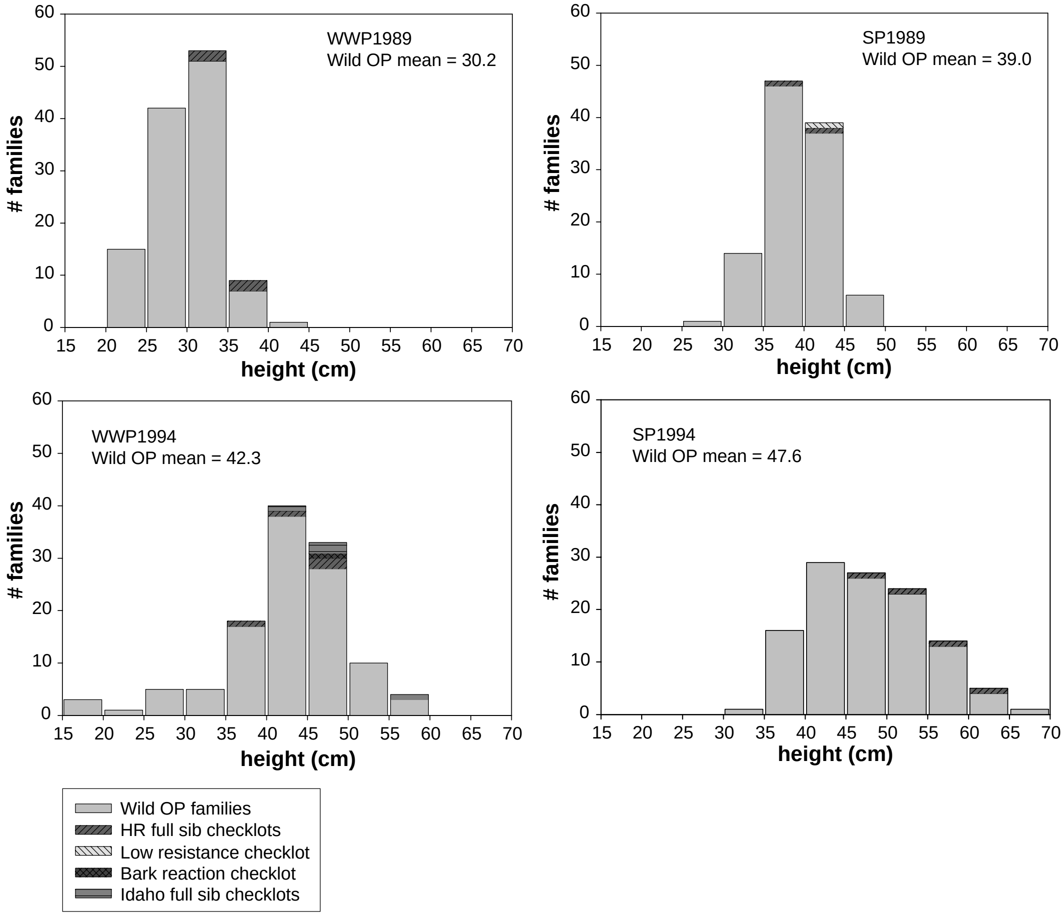


Figure 4—Distribution of family means for height after three growing seasons for the four trials.

family means ranged from 96.7 to 100 percent, including the full-sib families from Dorena and Idaho. Checklots of SP were not consistent in their infection levels between the two trial years. In SP1989, the two resistant Dorena checklots had 77 to 78 percent infection, and the low resistant control had 94 percent infection, whereas the checklots in SP1994 had 80 to 95 percent infection (table 5). Checklots for WWP had 90 to 100 percent infection in the two trials. In three of the four trials, infection percentage was nearly constant over the course of the evaluation, but in SP1989 infection levels increased from first to fourth inspection (fig. 5).

There were very few uninfected seedlings (noninfected seedlings were without any needle lesions and without any stem symptoms). The mean percentage of uninfected seedlings was less than 1 percent for the Wild OP families in the WWP1989 trial and the two 1994 trials; the percentage was about 4 percent for Wild OP families in the SP1989 trial (table 5).

With a few exceptions, families varied significantly ($p<0.0001$) in all traits within each trial, whether or not checklots were included in the analyses (table 6).

Needle Lesions

The percentage of seedlings with needle lesions (spot percent) was very high in all trials, averaging 85 to 99 percent among Wild OP families (table 5). At first inspection, approximately 98 percent of the Wild OP seedlings in WWP1989, WWP1994, and SP1994 had needle lesions, while only 78 percent of SP1989 had spots (spot1, table 5). By the second inspection (approximately 1 year after inoculation), the percentage of trees with needle spots dropped in all four trials; Wild OP run averages for spot2 ranged from 59 to 83 percent (table 5, fig. 6). The shedding of needles was most likely responsible for spot2 values being lower than spot1. The checklots were among the families with the lowest percentage of seedlings with needle lesions at second inspection (table 5, fig. 6).

The number of needle lesions per needle lesion class also varied by trial and species (table 3). In these trials the minimum number of spots required to enter the highest needle lesion class (Class 4) ranged from 6 to as high as 49 (table 3). More needle lesions were present in the 1994 trials relative to the 1989 trials and in western white pine relative to sugar pine (despite the lower inoculum density in the WWP trials). Family mean needle lesion class (NLC) ranged from 1.5 to 4.0, but the distribution of families across classes shifted depending upon the species and the trial (fig. 7). Within a trial, the SP HR checklots generally had fewer than average needle lesions, but the Dorena HR WWP checklots were not consistent between trials (fig. 7, table 5). There was a wide range in NLC among the four Idaho full-sib checklots in WWP1994 (table 5, fig. 7).

The mean percentage of seedlings with needle spots and no stem symptoms (SPO) was low for Wild OP families in all trials, ranging from 1.2 to 5.5 percent (table 5). Some Wild OP families in each trial had more than 10 percent of seedlings with only needle spots with some families showing 30 to 35 percent seedlings with SPO (table 5, fig. 8). All the checklot families, except the Low Resistant checklot in SP1989, showed much higher levels of SPO than the Wild OP trial mean (table 5, fig. 8).

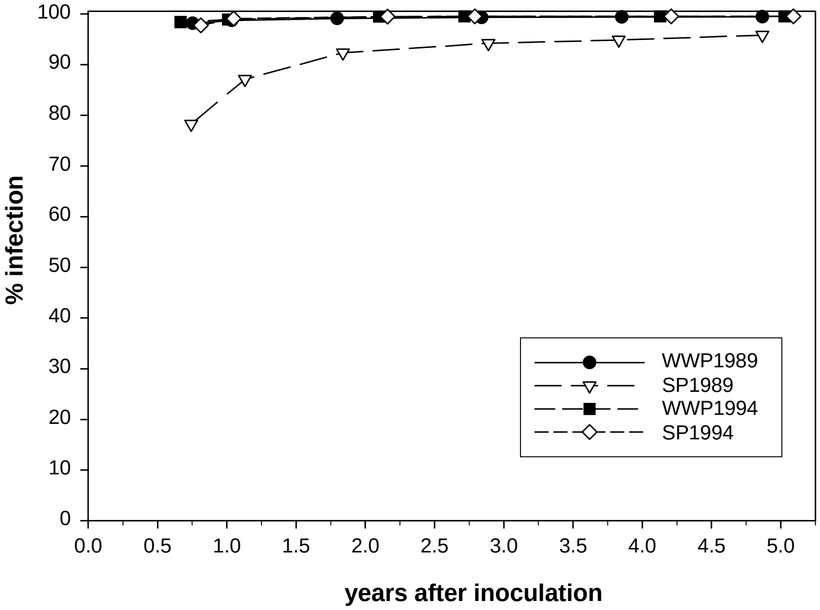


Figure 5—Percent infection of half-sib progeny of phenotypic selections of western white pine (WWP) and sugar pine (SP) in four blister rust screening trials. Each inflection point represents date of assessment.

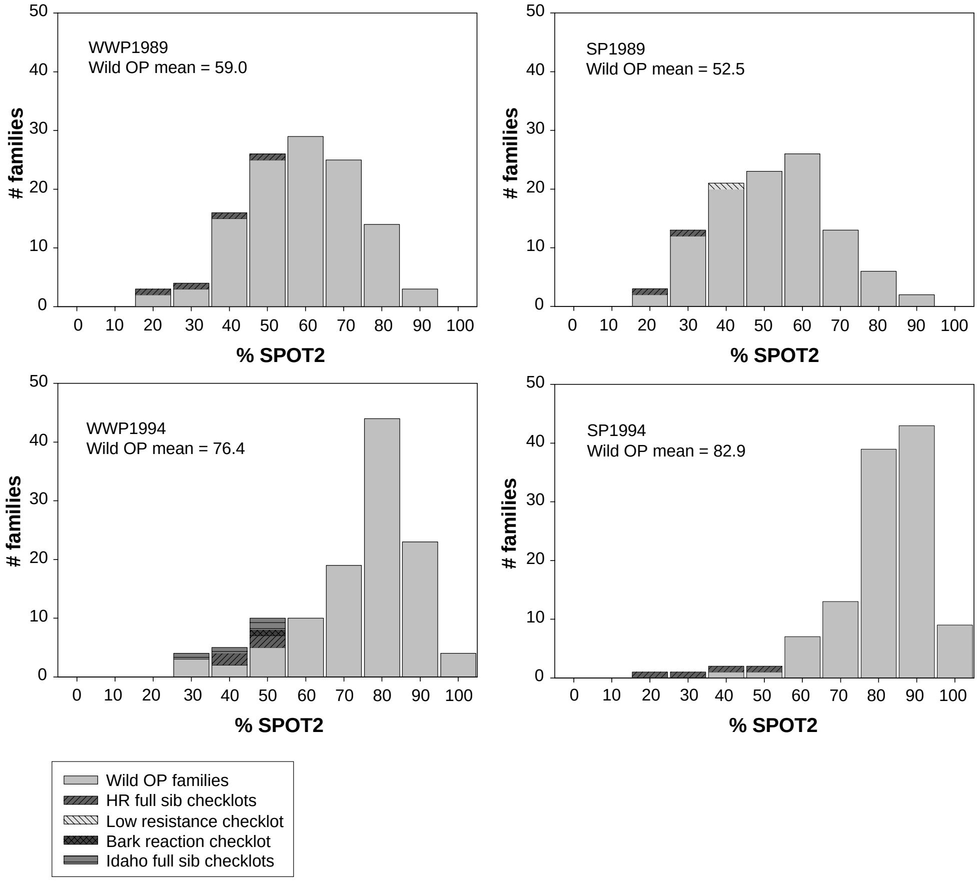


Figure 6—Distribution of family means for the percentage of seedlings with needle spots (SPOT2) 1 year after inoculation (second inspection) for the four trials.

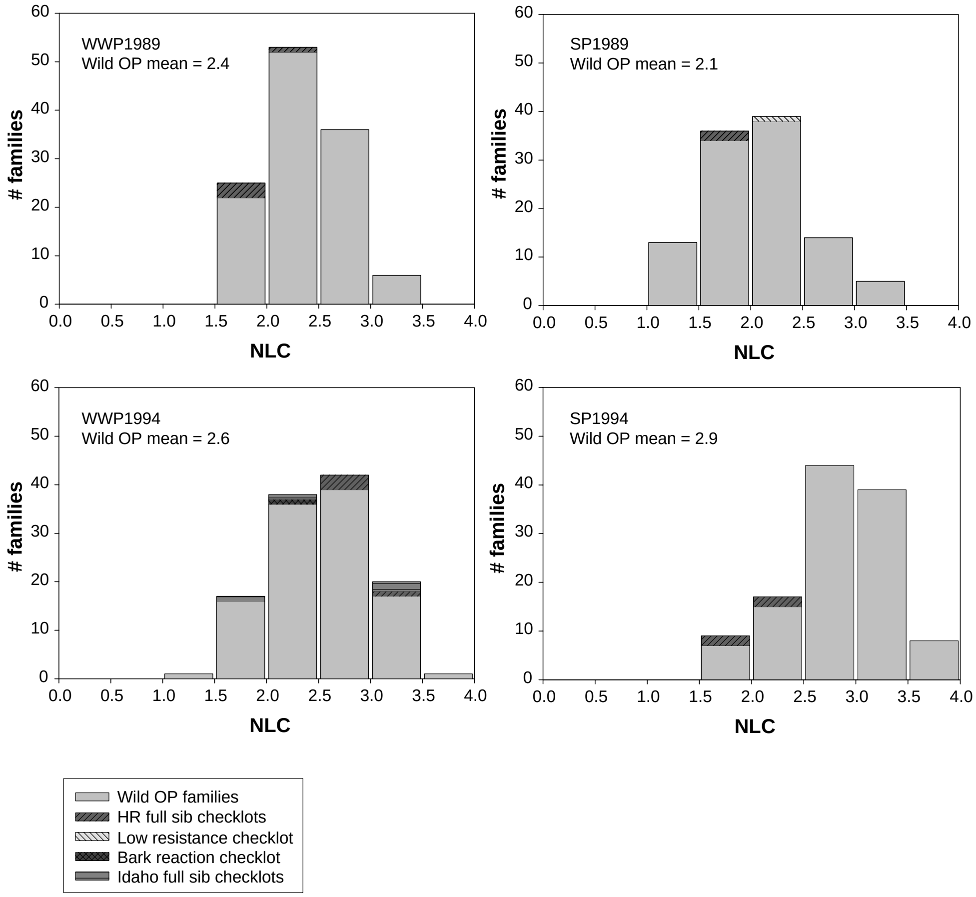


Figure 7—Distribution of family means for needle lesion class (NLC) for the four trials. NLC is a measure of the relative frequency of needle lesions within a test.

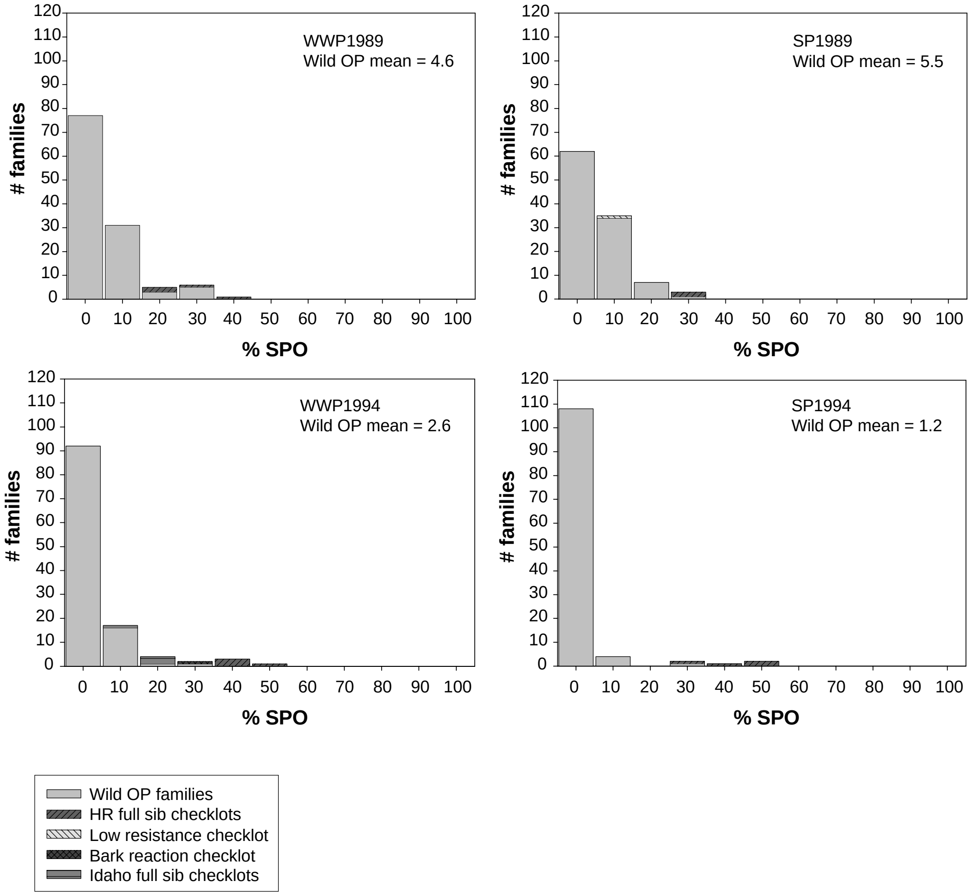


Figure 8—Distribution of family means for percentage of seedlings with needle spots but no stem symptoms (SPO) in the four trials.

Stem Symptoms

More than 90 percent of the seedlings in most Wild OP families developed stem symptoms (SS); the trial average percentages varied from 91 to 99 percent SS (table 5). Even so, there were significant differences among families in all four trials (table 6). The same general pattern of an increase over time in the percentage seedlings with stem symptoms developed in the four trials, although there were some differences in when the SS percent attained a maximum. For example, in SP1989, SS percent continued to increase slightly to the end of the evaluation period (sixth inspection, 5 years after inoculation) whereas SS percent stabilized by the fourth inspection (3 years after inoculation) in the other three trials (fig. 9).

Each of the four trials had at least one Wild OP family with less than 75 percent of the seedlings with stem symptoms (fig. 10 and table 5); SP1989 had nine Wild OP families, WWP1989 had five families, and the two 1994 trials each had one family with less than 75 percent of the seedlings having stem symptoms (table 5, fig. 10). The Dorena WWP and SP checklots with HR (hypersensitive reaction in the foliage) generally had among the lowest SS percent in all trials. The sugar pine checklots with HR averaged 45 percent and 48 percent SS in the two SP trials, respectively; the resistant western white pine checklots averaged 71 percent and 57 percent SS in the WWP trials. In the WWP1994 trial, the Idaho checklots and the Dorena BR checklot also showed low SS percent (71 to 87 percent) relative to the Wild OP families; however, the Dorena HR checklots were even lower with 48 to 64 percent SS in this trial (fig. 10 and table 5). The Dorena Low Resistant checklot in SP1989 had relatively high SS percent (87.4 percent) (table 5, fig. 10).

Normal Cankers—The percentage of Wild OP seedlings with normal cankers in at least one inspection (NCANK) varied from 86 to 98 percent for the four trials. The percentage was higher in 1994 trials relative to 1989, but the ranks between species were not consistent for the 2 years (table 5). The Wild OP sugar pine families with the lowest NCANK had values of 42 and 52 percent, whereas the western white pine families with the lowest NCANK had values of 61 and 71 percent (table 5). Survival of cankered seedlings from Wild OP families 5 years after inoculation (NCSURV5) was very low, ranging from 0.4 to 4.8 percent among the four trials (table 5). Survival of trees with a normal canker was higher for Wild OP families in the 1989 trials than for the

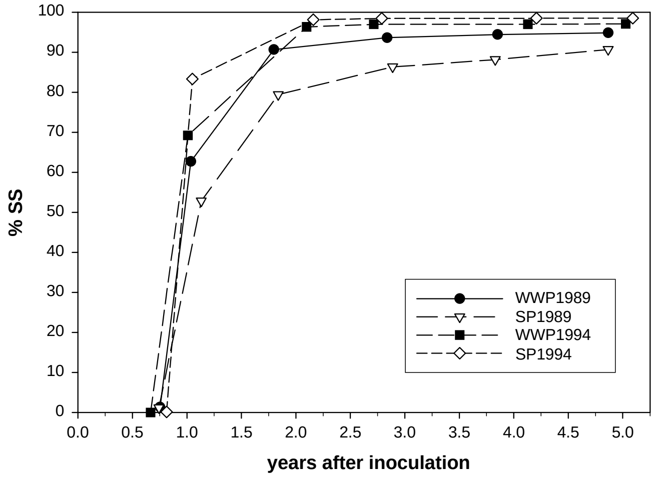


Figure 9—Percentage seedlings with stem symptoms (SS) of half-sib progeny of western white pine (WWP) and sugar pine (SP) in four screening trials. Each inflection point represents date of assessment.

1994 trials. Within a year, survival with a normal canker was slightly higher for WWP than for SP Wild OP families (table 5).

The BR checklot in WWP1994 was among the families with the lowest NCANK percent in that trial (fig. 11, table 5). The Dorena HR checklots had less NCANK percent than the Wild OP average, and the low resistance checklot approached the mean NCANK percent for SP1989 (table 5).

Bark Reaction (BR)—The incidence of trees with bark reactions was low in all four trials, ranging from 3.5 to 12.3 percent (table 5, fig. 12). Significant differences existed among Wild OP families for BR in all four trials (table 6). WWP showed slightly higher amounts of BR than SP (table 5, fig. 12). In all four trials, the majority of seedling families had at least one seedling with bark reaction (unpublished data). In all trials, there was at least one family with more than 20 percent of the seedlings with bark reaction (fig. 12). The Wild OP family with the highest BR (50.4 percent) was from the Colville NF (Family 21104-418) in the WWP1989 trial (table 5). The Dorena SP HR checklots had higher than average BR in both trials whereas the Dorena WWP HR

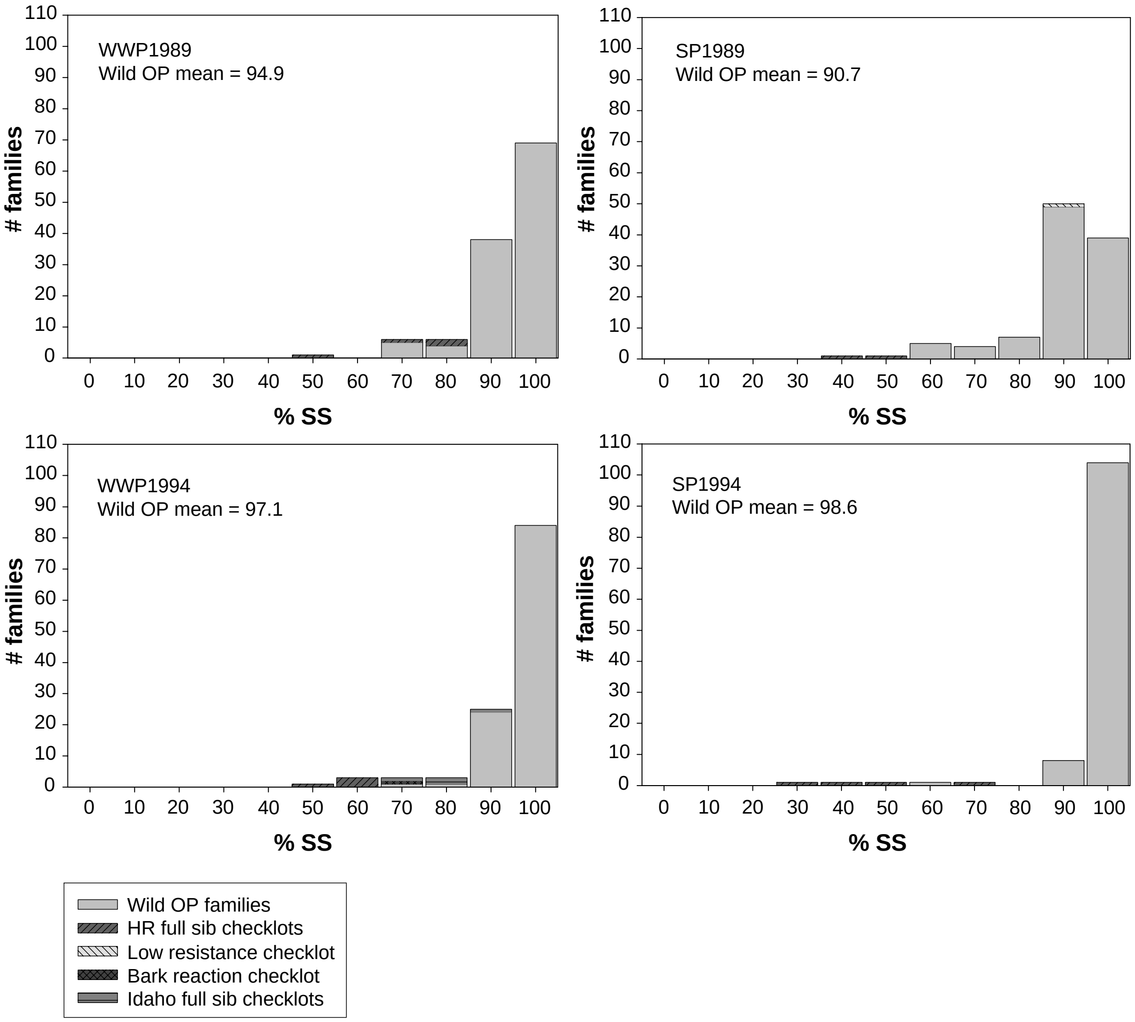


Figure 10—Distribution of family means for seedlings with stem symptoms (SS) in the four trials.

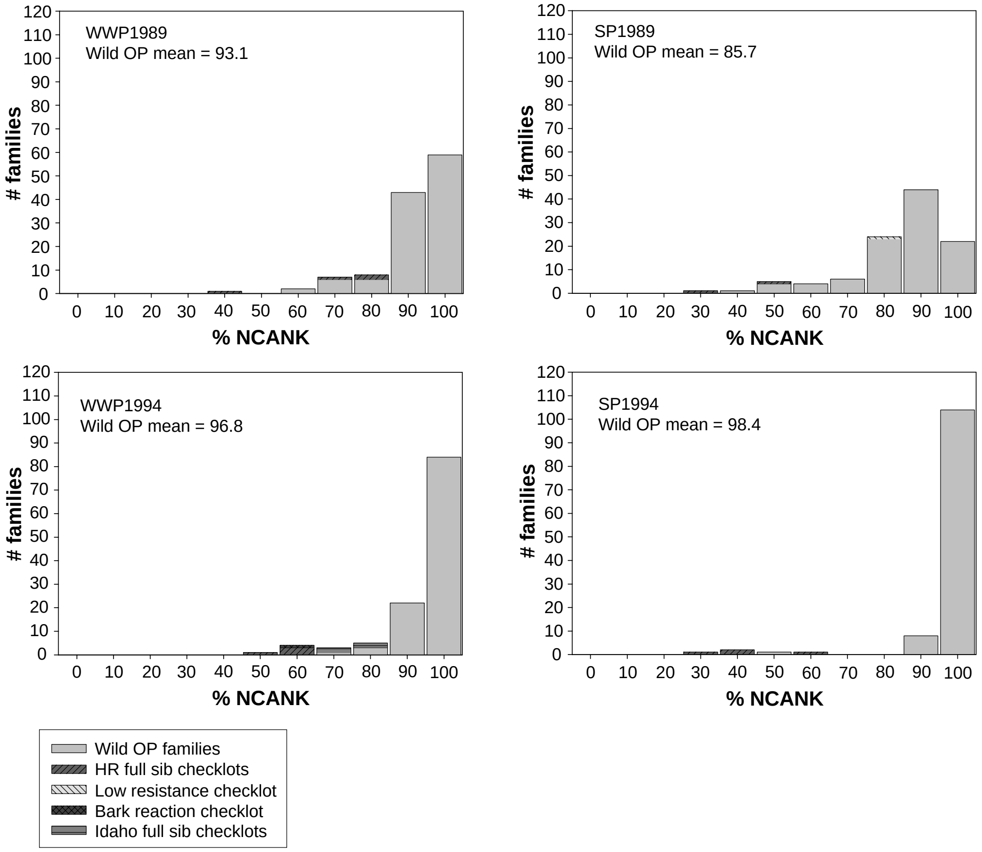


Figure 11—Distribution of family means for the percentage of seedlings with normal cankers (NCANK) in the four trials.

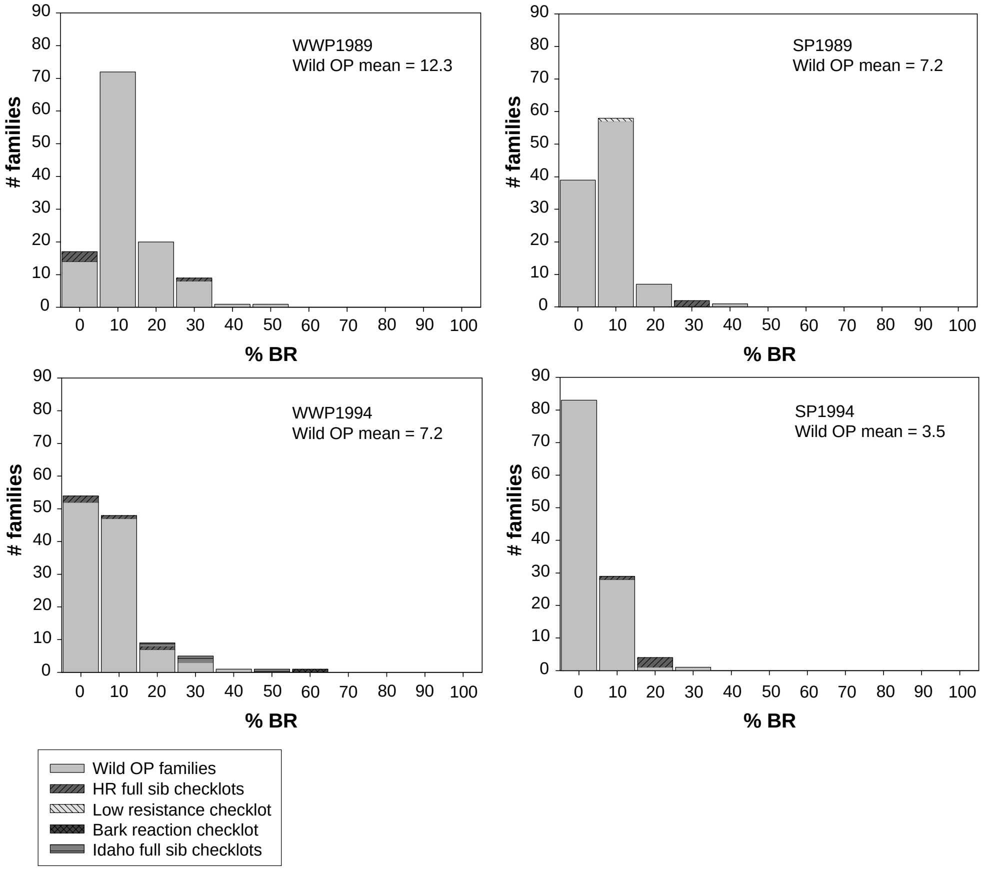


Figure 12—Distribution of family means for the percentage of seedlings with bark reaction (BR) in the four trials.

checklots showed relatively little BR, with a few exceptions (table 5, fig. 12). The Dorena BR checklot, 21105-052 in WWP1994, showed the highest BR percent of any family tested, including the Idaho full-sib checklots (fig. 12, table 5). This family had a high BR percent when first tested as a Wild OP in a 1988 sowing (Sniezko and Kegley 2003). All four of the Idaho checklots in WWP1994 had higher than average BR percent (table 5, fig. 12).

Many of the trees with bark reactions also had normal cankers and died; mean survival 5 years after inoculation of Wild OP seedlings with BR ranged from 1 to 14 percent in the four trials (table 5, fig. 13). Families varied greatly for survival with BR (fig. 13). More of the seedlings with BR in the 1989 trials survived relative to the 1994 trials (table 5), and within a year, slightly more SP survived with BR relative to WWP (table 5).

Early Stem Symptom Percentage (ESS3 Percent)—The average ESS3 for Wild OP families ranged from 58.9 percent (WWP1989) to 84.5 percent (SP1994) (table 5), indicating that over half the seedlings in all four trials showed stem infections approximately 12 months after inoculation. In general the 1989 trials had fewer seedlings with early stem symptoms compared with the 1994 trials. SP1994 showed the highest and least variable family mean ESS3 percent (fig. 14). The trends between species were not consistent in the 2 years (table 5). There were significant differences among Wild OP families in all four trials (table 6). The WWP checklots had families with more delayed onset of stem symptoms (lower ESS3 percent) and included some of the most outstanding families, while the sugar pine checklots were more variable (fig. 14). All four of the Idaho full-sib checklots had among the lowest ESS percent in WWP1994, and the Dorena BR checklot also had relatively few early stem symptoms (table 5, fig. 14).

Survival of Seedlings with Stem Symptoms—Survival of seedlings with stem symptoms (SSAL3) was relatively high (greater than 65 percent) 3 years after inoculation in the 1989 trials but very low in the 1994 trials (table 5). However, by the fifth year after inoculation, many of the seedlings with SS had died; there were few families with high SSAL5 in any trial (table 5). In the 1989 trials SSAL5 of the best families ranged from 21 to 38 percent, but SSAL5 was less than 10 percent in the best Wild OP families in 1994 (table 5). The Dorena HR full sib checklots were mixed in

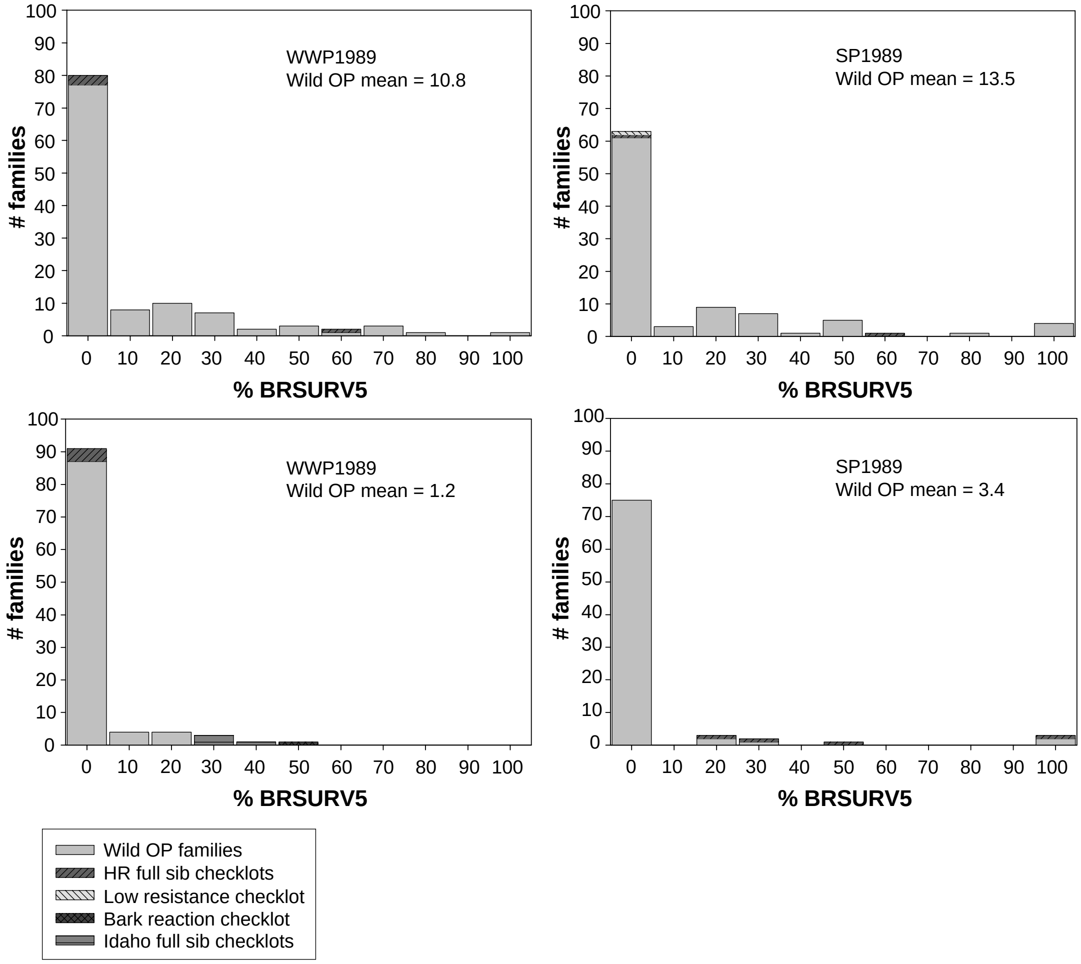


Figure 13—Distribution of family means for percentage of seedlings with bark reaction and surviving 5 years after inoculation (BRSURV5) in the four trials.

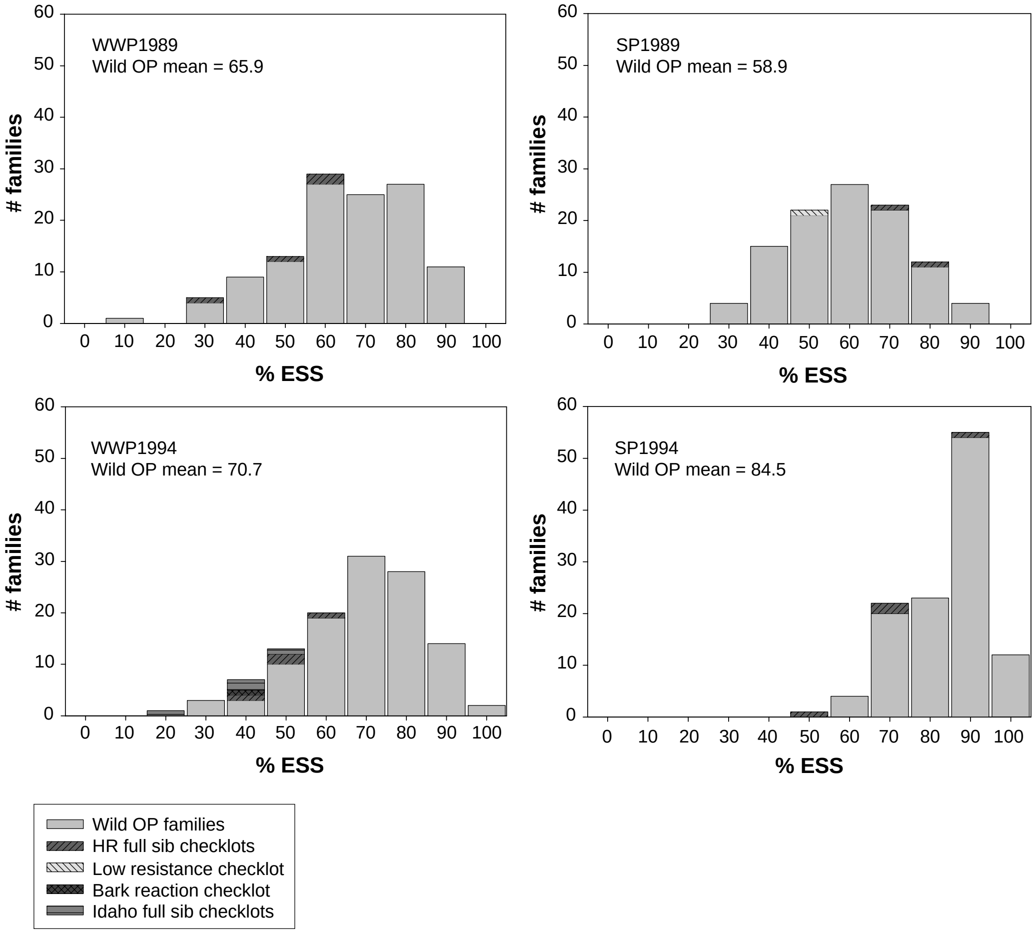


Figure 14—Distribution of family means for the percentage of seedlings with early stem symptoms (ESS3) in the four trials.

performance but generally had low fifth-year survival of seedlings with stem symptoms (SSAL5 percent) (table 5). Two of the full sib HR checklots, SP Family B1054-004 x B1054-034 and WWP Family 15045-862 x 18034-392 were outstanding for SSAL5 in the 1989 trials, and B1054-004 x B1054-034 was also well above the Wild OP mean for SSAL5 in 1994 (table 5). The Dorena BR checklot was well above the Wild OP mean with 35.2 percent SSAL5 in WWP1994 (table 5). Only 3.9 percent of the seedlings in the low resistant sugar pine checklot survived with SS in SP1989 (table 5).

Checklot Performance—More seedlings from Dorena WWP full-sib HR checklots developed stem symptoms (SS percent) in 1989 relative to 1994 (table 5), even though survival of infected seedlings was fairly comparable between the 2 years. In spite of a higher percentage of seedlings with stem symptoms in 1989, those with normal cankers had higher survival than those in 1994 (NCSURV5) (table 5). Mean needle lesion class was higher for the Dorena WWP full sib checklots in 1994 relative to 1989. On average, the Dorena WWP full sib checklots had fewer needle lesions than the Wild OP mean in 1989, while the reverse was true in 1994 (table 5).

For WWP1994, the five Idaho full-sib families generally had higher survival, bark reaction, and survival of seedlings with stem symptoms than the Wild OP families. The Idaho families also had fewer seedlings with stem symptoms and fewer with early stem symptoms than the Wild OP families. The Dorena BR checklot generally had higher levels of resistance responses than the Idaho checklots, and only one of the Idaho families had fewer early stem symptoms (table 5).

Of interest in the WWP1994 trial was whether or not the Idaho full-sib checklots and Wild OP families exhibited a differential reaction when inoculated with a pathotype of rust known to be virulent to HR in WWP. Survival of the infected seedlings from the Dorena and Idaho checklots in WWP1994 was relatively consistent across the first four blocks (in which the pathotype of rust virulent to HR in WWP was excluded), with the Dorena checklots showing higher survival (fig. 15). However, in block 5 and 6 where a pathotype of rust virulent to HR in WWP was present, survival of the four Dorena checkots was dramatically lower, approaching zero in block 6. The Dorena checklot with known bark reaction, however, had higher survival in blocks

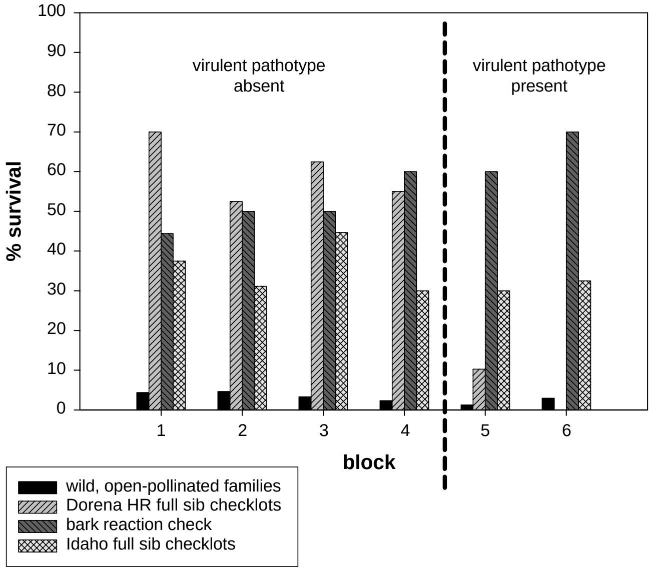


Figure 15—Survival of infected seedlings of several sources of western white pine, by block, in the 1994 trial, with and without a pathotype of rust virulent to HR in western white pine.

5 and 6. There was essentially little or no change in the average survival of the Wild OP families or the Idaho checklots (fig. 15).

SP Checklots—Survival of infected seedlings from the Dorena full-sib checklots was relatively high (37 percent to 68 percent) in both 1989 and 1994 (table 5). The two full-sib checklots common to 1989 and 1994 had similar levels of RSURV5 and SS percent in both trials (table 5). A relatively high proportion (20 percent) of seedlings in the full-sib checklots showed no infection in 1989, and Family B1054-004 x B1054-034 also had a high proportion of uninfected seedlings in 1994 (noinfect, table 5). This family also had a relatively high proportion of seedlings alive with stem symptoms five years after inoculation in both trials (table 5). Bark reaction was higher for the full-sib checklots in 1989 relative to 1994 (table 5, fig. 12). In 1989 mean NLC of the full-sib checklots and the low resistance checklot were close to the run mean of Wild OP families, whereas in 1994, the checklots had fewer than average needle lesions (table 5). In both years, all checklots were below the mean for presence of needle lesions 1 year after inoculation (table 5, fig. 6).

Discussion

Artificial inoculation was successful in infecting more than 95 percent of the 2-year-old seedlings in each trial. This level of infection is probably similar to a high hazard field site but reflects only a single inoculation event. It is unknown whether the use of inoculum in these tests from a wide geographic area increased the infection and mortality levels beyond that of using inoculum from a single geographic source.

SP1989 had 13 percent fewer trees with spots than the other three trials. This is likely attributable to the much lower and more variable inoculum density relative to SP1994 (5,200 spores/cm^2 versus 7,200 spores/cm^2, respectively, table 2). It has been suggested that prevention of needle infection (corresponds to 'no infect' in this paper) is a threshold trait, dependent upon inoculation intensity (Hoff and McDonald 1980a). Results from experiments using different inoculum densities of fusiform rust (*C. quercuum* f. sp. *fusiforme*) on loblolly (*P. taeda* L.) and slash pine (*P. elliottii* Engelmann) indicated that intermediate levels of resistance were more distinguishable at lower inoculum densities (Laird and others 1974). Even when the inoculum density is within the targeted range for WWP or SP, the number of needle lesions per seedling can vary remarkably by trial. Worth noting is that the two SP HR checklots common to these trials had slightly higher percentages of seedlings with stem symptoms in SP1989 even though the mean for the Wild OP families was nearly 8 percent less than in SP1994. Recent data for SP1989 show stem symptoms on trees previously stem symptom free, presumably from natural infections in 1995 and 1997. This had been noted in western white pine; Hunt (1990) reported the presence of stem symptoms on 84 percent of unspotted seedlings in a 1987 inoculation trial. Furthermore, many of those trees without spots had latent canker development (Hunt 1990).

In general, survival provides the ultimate guideline of utility of resistance for immediate use. Five years after inoculation, the mortality in all four trials was high. Only a few families examined here (including checklots) had moderate levels of survival. Both SP and WWP are very susceptible. However, results are dependent on the trial—families, inoculum source, and environmental conditions—and the traits examined. The low survival of progeny from phenotypic selection described here is similar to other reports; Zsuffa (1981) observed very low survival of progeny of resistant eastern white pine (*P. strobus* L.) selections in artificial screening trials (2 percent, family means ranging from 0 to 11.3 percent). Hoff (1984) reported that nearly 90 percent of the cankered western white pine seedlings are dead by the fourth year after inoculation.

One of the major differences between the 1994 and 1989 tests was the lower final survival (total and/or rust-infected survival) in the 1994 tests. The lower survival may have been influenced by the 1994 nursery regime that resulted in the presence of late season lammas growth on both species, and/or a higher number of needle lesions on the trees. Rust infection on lammas growth may circumvent putative resistance mechanisms that prevent stem infection (McDonald and Hoff 1971).

While sugar pine had higher percentages of seedlings without stem symptoms and higher survival relative to western white pine in the 1989 trials, the reverse was true in the 1994 trials. A previous summary of relative blister rust resistance of five-needle pines ranked sugar pine as slightly more susceptible than western white pine (Bingham 1972) as did a field study (Sniezko and others 2000).

The length of the evaluation period after inoculation can have dramatic results on the interpretation of the level of resistance in families (for example, SSAL3 versus SSAL5 for WWP1989 and SP1989). Much of the mortality at Dorena occurs after the third year following inoculations.

The operational program in Idaho terminates formal assessments following the third year after inoculation (Franc 1988). The availability of longer-term data gives the Region 6 program the option to make selections using either the third year or fifth year data.

Trees with normal cankers surviving 5 years after inoculation (a subgroup of SSAL5) are relatively rare for both species. On the surviving trees, some of these cankers are noted to be inactive during the final inspection. Further tracking of the surviving but cankered trees over time would be informative, as large trees with old basal cankers have been observed in the field for both WWP and SP (Hoff 1984; Sniezko and others, this proceedings; Dean Davis, personal comm.) as well as eastern white pine (Hirt 1948).

Bark reaction was present in low frequency in many families and moderate frequency in a few families. Many of the individuals with bark reaction died by the end of the 5-year test period. Survival of seedlings with bark reaction ranged from 1.2 to 13.5 percent in the four trials, but some families had more than 50 percent. Generally trees with complete bark reactions would be expected to live (unless the seedling was girdled or the seedling also had a normal canker). All of the SP resistant checklots had bark reaction levels higher than the Wild OP mean and fewer trees free of stem symptoms than expected. Incompatible or aborted bark reactions have been reported on SP seedlings with HR; these symptoms were associated with infected primary needles (Kinloch and Littlefield 1977; Kinloch and Comstock 1980). It is possible that some of the SP HR seedlings with these atypical symptoms were classified as having stem symptoms.

In examining the Wild OP families for a given trial, there were a few families that approached the levels of survival, bark reaction, or stem-symptom-free of the Dorena or Idaho full-sib checklots. In the 1989 tests, several families had survival levels as high or higher than some of the HR checklots, but in the two 1994 tests, the survival of the Wild OP families was generally much lower than the checklots. In the 1994 tests, SSAL5 levels were very low in the Wild OP families; not many infected trees survived 5 years after inoculation. There may be opportunities for within-family selection for families with a very low incidence for some of the resistant responses.

The Dorena full-sib checklots used in these trials are known to segregate for HR, and they provide linkages between tests. The presence of a pathotype virulent to the HR in WWP (vcr2) notably reduced the expected survival of the Dorena full-sib checklots in WWP1989, as well as in the two blocks of WWP1994 in which the *Ribes* sources with this pathotype were used. In comparison the Idaho full-sib families, the bark reaction checklot, and many Wild OP families in WWP1994 did not show increased levels of stem symptoms when challenged with the virulent pathotype.

Although there was wide variation in family mean needle lesion class (NLC) within each of the four trials, most trees developed stem infections. Individuals with reduced needle lesion frequency (fewer spots) were hypothesized to have fewer stem infections and higher survival (Hoff and McDonald 1980b). However, number of needle lesions in artificial screening has been found to be a poor predictor of cankering in the field (Hunt 2002). In a paired test of high and low spotting individuals within a family planted in the field, the low spotting individuals were as likely to develop stem infections as their highly spotted siblings (Hunt 1990). However, in our trials, there was a trend for the families with higher mean NLC to have a higher percentage early stem symptoms (unpublished data). This has been previously noted with WWP; seedlings with fewer spots generally had fewer cankers 16 to 18 months post-inoculation (Hunt 2002; Meagher and Hunt 1996). Similarly, Hunt (1990) observed that many spot-free WWP seedlings had latent stem symptom development. The implication is that selection for fewer needle lesions may be an indirect selection for delayed stem symptom development.

Several of the resistance responses evaluated here (bark reactions, needle lesion class, latent development of stem symptoms (low ESS3), and stem symptom alive) may be forms of partial resistance. These types of resistances may need several generations of breeding to increase utility for field use. Control crosses among parents with partial resistance traits and testing of their progeny are underway in the Region 6 program. Additionally, field validation of families with complete or partial resistances is in progress (Sniezko and others, this proceedings). Questions remain about whether additional breeding would increase the survival of trees with bark reactions and which of these resistance responses may prove to be more effective in the field as the trees get larger. Many of the families with relatively higher levels of survival expressed more than one resistance response. It is unknown whether these resistances are under the control of genes in tightly linked loci or whether they represent a continuum of response, delaying the expression of disease symptoms or reducing the severity of those symptoms.

At least four mechanisms that lead to complete resistance (lack of stem symptoms) in western white pine have been previously described (Hoff and McDonald 1980a, Hoff and McDonald 1971, McDonald and Hoff 1970, Kinloch and others 1999). There is tentative evidence of a fifth one (unpublished data). One mechanism for complete resistance has been described in sugar pine (Kinloch and others 1970). The hypersensitive reactions in SP and WWP are known to be under the control of separate, single major genes, and premature needle shed and fungicidal reaction in the short shoot are hypothesized to be under the control of separate single recessive genes. However, there may be more than one resistance mechanism or gene underlying a particular phenotypic expression. Differentiating similar phenotypes controlled by different genes will be difficult without virulent strains specific to each, or without the aid of molecular techniques.

The Region 6 program has evaluated blister rust resistance of progeny of thousands of sugar pine and western white pine selections. Results from the trials examined here indicate that progeny of most of these parents are very susceptible. Given the high susceptibility of very young seedlings, natural regeneration may be very unlikely on many sites, exacerbating the decline of these species. Stabilizing and reversing the decline of WWP and SP will be dependent on the development and deployment of resistant material coupled with use of silvicultural tools such as appropriate site selection and pruning. Selections of progeny showing one or more resistance responses have been made and grafts have been established in breeding orchards and seed orchards. Resistant seed is available for some breeding zones for both western white pine and sugar pine. Advance-generation breeding has started for WWP. Major gene resistance in WWP and SP are likely to play an important role for immediate restoration and reforestation efforts, but partial resistance traits will likely be more important in the future development of durable resistance.

Acknowledgments

The Region 6 Area Geneticists and the many tree improvement workers who made all the phenotypic selections and organized the cone collections. Bob Danchok, the late Delbert Albin, and all members of the Dorena rust screening team for the extensive assessments. Jude Danielson for help in organizing the operational elements of the program. The USFS Region 6 Genetics and Forest Health programs for funding and logistical support, and the Bureau of Land Management and Quinault Indian Nation provided seed from their field selections. Joan Dunlap and Scott Kolpak for their reviews of an earlier draft of this paper.

References

Bingham, R.T. 1972. Taxonomy, crossability, and relative blister rust resistance of 5-needled white pines, p. 271-278. In: R.T. Bingham, R.J. Hoff, and G.I. McDonald (eds.) Biology of Rust Resistance in Forest Trees: Proceedings of a NATO-IUFRO Advanced Study Institute. USDA Forest Service Misc. Publ. 1221.

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. USDA Forest Service General Technical Report INT-146. 45 p.

Campbell, R.K. and Sugano, A.I. 1987. Seed zones and breeding zones for sugar pine in southwestern Oregon. USDA Forest Service Research Paper PNW-RP-383. 18 p.

Campbell, R.K. and Sugano, A.I. 1989. Seed zones and breeding zones for white pine in the Cascade Range of Washington and Oregon. USDA Forest Service Research Paper PNW-RP-407.

Cochran, W.G. and Cox, G.M. 1967. Experimental designs, 2nd edition. John Wiley and Sons, Inc. New York. 611 p.

Franc, G.C. 1988. The white pine program in the northern region, p. 21-26. In: R.S. Hunt, ed. Proc. of a western white pine management symposium. Nakusp, BC. Pacific Forestry Centre, Victoria, BC.

Hirt, R.R. 1948. Evidence of resistance to blister rust by eastern white pine growing in the northeast. *J. For.* 46:911-913.

Hoff, R.J. 1984. Resistance to *Cronartium ribicola* in *Pinus monticola*: Higher Survival of Infected Trees. USDA Forest Service Research Note INT-343, 6p.

Hoff, R.J. and McDonald, G.I. 1980a. Improving rust-resistant strains of inland western white pine. USDA Forest Service Research Paper INT-245. 13 p.

Hoff, R.J. and McDonald, G.I. 1980b. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. *Can. J. Bot.* 58:574-577.

Hoff, R.J. and McDonald, G.I. 1971. Resistance to *Cronartium ribicola* in *Pinus monticol*a: short shoot fungicidal reaction. *Can. J. Bot*. 49:1235-1239.

Hunt, R.S. 1990. Blister rust in inoculated and plantation-tested western white pine in British Columbia. *Can. J. For. Res.* 12:279-282.

Hunt, R.S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. *Can. J. Plant Pathol*. 19:352-357.

Hunt, R.S. 2002. Relationship between early family-selection traits and natural blister rust cankering in western white pine. *Can. J. Plant Path.* 24:200-204.

Kim, H.J., Yi, C.K., and La, Y.J. 1982. Present status of Korean pine (*Pinus koraiensis*) blister rust and relative resistance of three species of five-needled pines to *Cronartium ribicola*. Research Report of Forest Research Institute, Seoul, Korea. 29:239-252.

Kinloch, B.B., Jr. and Comstock, M. 1980. Cotyledon test for major gene resistance to white pine blister rust in sugar pine. *Can. J. Bot.* 58: 1912-1914.

Kinloch, B.B. and Davis, D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Res. Publ. 3362.

Kinloch, B.B., Jr. and Dupper, G.E. 2002. Genetic specificity in the white pine-blister rust pathosystem. *Phytopathology* 92:278-280.

Kinloch, B.B., Jr. and Littlefield, J.L. 1977. Whtie pine blister rust: hypersensitive resistance in sugar pine. *Can. J. Bot.* 55:1148-1155.

Kinloch, B.B., Jr., Parks, G.K., and Fowler, C.W. 1970. White pine blister rust: simply inherited resistance in sugar pine. *Can. J. Bot*. 58:1912-1914.

Kinloch, B.B., Sniezko, R.A., Barnes, G.D., and Greathouse, T. E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. *Phytopathology* 89:861-867.

Laird, P.P., Knighten, J.L., and Wolfe, R.L. 1974. Reduced inoculum density enhances sensitivity of resistance detection in fusiform rust resistance testing. USDA Forest Service Southeastern Area, State and Private Forestry, Division of Forest Pest Management Report 74-1-10. 10 p.

McDonald, G.I. and Hoff, R.J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: early shedding of infected needles. USDA Forest Service, Res. Note INT 124. 8 p.

McDonald, G.I. and Hoff, R.J. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: genetic control of needle-spots-only resistance factors. *Can. J. For. Res*. 1:197-202.

Meagher, M.D. and Hunt, R.S. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. *Silv. Gen.* 45(2-3)75-81.

Samman, S.A. 1982. The white pine blister rust program of Region 6, p. 133-183. in Breeding insect and disease resistant forest trees: Proceedings, Servicewide genetics workshop, Eugene, Oregon. USDA Forest Service.

Samman, S. and Kitzmiller, J.H. 1996. The sugar pine program for development of resistance to blister rust in the Pacific Southwest region, p. 162-170. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Davis. Agr. Natural Resources Publ. 3362.

SAS Institute Inc. 1999. SAS OnlineDoc, Version 8. Cary, NC: SAS Institute Inc.

Sniezko, R.S. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Davis. Agr. Natural Resources Publ. 3362.

Sniezko, R.A., Bower, A., and Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance in sugar pine and western white pine. *HortTechnology* 10(3): 519-522.

Sniezko, R.A. and Kegley, A.J. 2003. Blister rust resistance of five-needle pines in Oregon and Washington. Proceedings of Second IUFRO Rust of Forest Trees Working Party Conference. 19-23 August 2002. Yangling, China. Forest Research 16 (Suppl.): 101-112.

Theisen, P.A. 1988. White pine blister rust resistance mechanisms of sugar and western white pines. USDA Forest Service Region 6 Tree Improvement paper 13. 24 p.

Zolman, J.F. 1993. Biostatistics: experimental design and statistical inference. New York, Oxford University Press. 343 p.

Zsuffa, L. 1981. Experiences in breeding *P. strobus* L. for resistance to blister rust. Proc. 18th IUFRO World Cong. Kyoto, Japan. P 181-183.

Confirmation of Dominant Gene Resistance (Cr2) in U.S. White Pine Selections to White Pine Blister Rust Growing in British Columbia

R.S. Hunt
G.D. Jensen
A.K. M. Ekramoddoullah

Abstract—To demonstrate the existence of the dominant Cr2 blister rust resistance gene in Dorena derived stock already producing seed in British Columbia, about 50 seedlings/parent tree were inoculated and examined for hypersensitive needle spots. Seedlings from 33 of 42 canker-free parents produced hypersensitive spots, confirming the presence of Cr2 in the parent trees. To determine if an existing pathotype might have already overcome the Cr2 gene in British Columbia, seedlings from three suspect trees (because they were canker-free for 10 years, but recently became cankered) were inoculated. The Cr2 gene was absent in these three trees. Additionally, seedlings from two seedlots known to possess the Cr2 gene were subjected to two consecutive annual inoculations. This double inoculation was repeated on five different occasions with a composite inoculum. Canker-free seedlings following the first inoculation remained canker-free after a second inoculation, consistent with the uncompromised expression of Cr2.

Key words: Cr2, blister rust, resistance genes

Introduction

As early as the 1960s a single dominant gene for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch.) was suspected in western white pine (*Pinus monticola* D. Don) from the Champion mine region of Oregon. Canker-free trees from this source were incorporated into the USDA Forest Service seed orchard at the Dorena Genetic Resource Center, near Cottage Grover, Oregon. By 1984 this resistance had failed (McDonald and others), which was later attributed to the failure of a single dominant gene, called Cr2 (Kinloch and others). We obtained two seed collections from the Dorena seed orchard - one bulked collection of 32 Champion Mine-derived parents (Champion), and one bulked from six Washington State parents (WA). We out planted the resulting seedlings in plantations across southern British Columbia (BC) in 1986 and 1987 (Hunt 1987). Unaware that these lots could possess the dominant Cr2 gene, we reported that these two lots had greater resistance than other lots in two coastal and two interior plantations (Hunt 1994). Since then, we have tried to (a) confirm that these healthy trees possess the Cr2 gene; (b) determine if a virulent pathotype might exist in BC which overcomes the Cr2 gene; and (c) determine whether the resistant phenotype would be maintained under repeated inoculation.

Materials and Methods

The seven plantations with the Champion and WA seedlots have been monitored for rust incidence annually, or biennially (Hunt 1994). Some of the surviving trees from these two Dorena seedlots have produced seed, particularly in the two most rusted coastal plantations. We collected seed from 42 of these parents and three trees that were canker-free for 10 years but then developed rust. In 2000 50 seeds/parent were stratified and 1-0 seedlings bearing only primary needles were inoculated in a ribes (disease) garden with a composite of six BC rust sources initially collected from throughout the range of western white pine on Vancouver Island. The seedlings were examined for hypersensitive spots, the manifestation of the Cr2 gene (Kinloch and others 1999), in February 2001.

To determine if repeated inoculation would nullify the dominant resistance, or whether it would be maintained, two seedlots known to possess the Cr2 gene respectively were obtained from Dorena (Kinloch and others 1999; registration # 119-15045-845 X OP (our seedlot #1) and #119-15045-845X15045-841 X OP (our seedlot #2)), sown and grown in 1994, 1996, and 1997. Some were first inoculated at age 4-months (only primary needles present) others at 16-months (secondary needles present) in the ribes (disease) garden. Seedling survival was attributed to the Cr2 gene. All survivors were re-inoculated the following year. From the 1994 inoculation, 40 grafted ramets were produced from seven seedlings, and these were inoculated.

Results

The most severely damaged plantation had 49 percent of the Dorena derived trees canker-free compared to only 0.2 percent for the other provenances after 15 years (fig. 1); comparable numbers for the other plantation were 66 and 13

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are with the Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

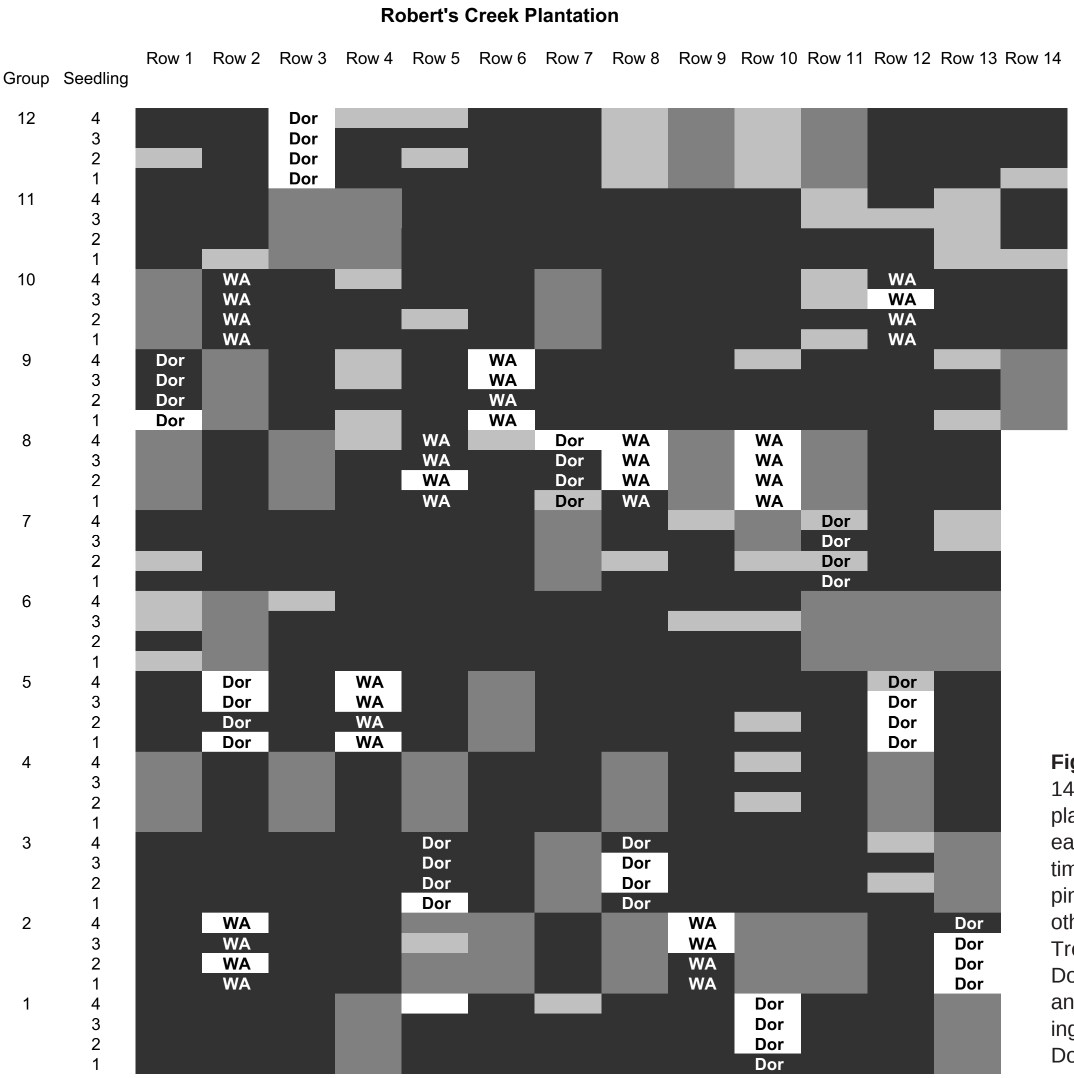


Figure 1—A plantation depicting 14 provenances of western white planted in four-tree row plots, with each provenance replicated 10 times. White denotes canker-free pine, black cankered pine, grey other conifers and missing trees. Trees labelled Dor = a bulked Dorena seed orchard collection, and WA = a collection from Washington State trees growing in the Dorena seed orchard.

percent. Seventeen of 18 canker-free plantation trees from the Champion seedlot produced seedlings with hypersensitive spots (fig. 2) typical for Cr2 (family mean percent Cr2 49; range 21 to 100). None of the seedlings produced by the parent lacking Cr2 possessed Cr2. From the WA seedlot 16 of 24 plantation trees also produced hypersensitive spots (family mean percent Cr2 41; range 21 to 60). The seven WA plantation trees lacking Cr2 processed few seedlings with hypersensitive spots (family mean percent 1; range 0 to 5). The percentage of Cr2 in the offspring of the three recently cankered parents was 0, 0, and 5.

Many of the seedlings from the two registered Dorena seedlots possessing the Cr2 gene died from fusarium root rot making survival rates variable. Nevertheless, the over-all mean survival was greater than 50 percent for each seedlot. None of the canker-free survivors from the first inoculation developed rust cankers from a second inoculation (table 1). In addition, none of the 40 ramets became infected, but in six the rootstock became infected. In these, the rust failed to cross the graft union over 4 years (fig. 3).

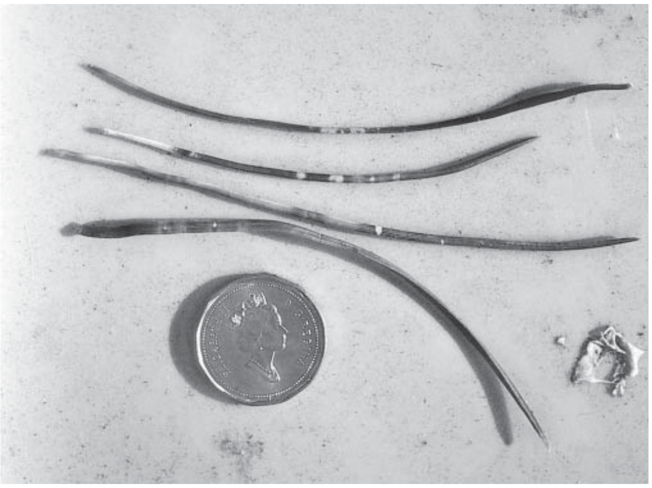


Figure 2—Blister rust infection spots in pine needles. The top two needles show normal spotting; the bottom two have necrotic halos typical of hypersensitive spots in western white pine as a result of the Cr2 gene.

Table 1—Percentage Cr2 gene in two western white pine families based on survival after an initial inoculation with *Cronartium ribicola* and canker-free survivors after a second inoculation. No survivors from the first inoculation became cankered from a second inoculation, but numbers were reduced by fusarium root rot.

Seedlot	Seedlings	Inoculation	Canker-free	Cr2
	(no.)	(year)	(no.)	(%)
1	44[a]	1995&6	10	29
1	24[a]	1996&7	12	54
1	20[b]	1997&8	10	50
1	446[a]	1997&8	214	55
1	498[b]	1998&9	324	74
2	76[a]	1995&6	28	74
2	40[a]	1996&7	28	77
2	37[b]	1997&8	22	59
2	336[a]	1997&8	182	73
2	448[b]	1998&9	305	80

[a] First inoculated at 4 months.
[b] First inoculated at 16 months.

Figure 3—Failure of *Cronartium ribicola* to infect a western white pine scion possessing the Cr2 gene from an infected root stock.

Discussion

No apparent failure of the dominant resistance gene has been observed in seven BC plantations over 15 years. Inoculation of seedlings from 33 healthy parents derived from these plantations produced hypersensitive spots, confirming they possessed the Cr2 gene. Additionally, inoculation of seedlings possessing the Cr2 gene with a composite inoculum resulted in high survivor ratios as would be expected from a heterozygous parent pollinated with orchard pollen. Re-inoculated survivors remained canker-free, confirming the stability of the Cr2 gene. Even scions possessing the Cr2 gene failed to become infected from *C. ribicola* infected rootstock after 4 years. In contrast, seedlings from three recently cankered parents derived from the same Dorena stocks produced normal spots and thus lack the Cr2 gene. We conclude that we have failed to demonstrate the existence of a virulent pathotype of *Cronartium ribicola* to the Cr2 gene in BC and so far the Cr2 gene appears stable in BC even when nonselected stocks are devastated (fig. 1).

Most blister rust cankers in BC occur within 3 m of the ground (Hunt 1991); that is, when the plantations are young (usually less than 15 years old). It appears that the Cr2 gene could be deployed successfully in isolated areas in BC, and if the resistance failed, the trees likely will have out-grown their most susceptible age before a virulent pathotype could have an opportunity to build-up damaging levels of inoculum. However, if a series of age classes were planted in contiguous areas, a new virulent pathotype could be limiting to the younger plantations.

Acknowledgments

We thank Drs. Ray Steinhoff and Richard Sniezko for supplying seedlots from the Dorena Genetic Resource Center.

References

Hunt, R.S. 1987. Is there a biological risk of western white pine provenances to root diseases? In G.A. DeNitto (complier) West. Int. For. Dis. Wk. Conf. 35: 29-34. USDA For. Serv., San Francisco CA.

Hunt, R.S. 1991. Operational control of white pine blister rust by removal of lower branches. For. Chron. 67: 284-287.

Hunt, R.S. 1994. Transferability of western white pine within and to British Coumbia – blister rust resistance. Can. J. Plant Pathol. 16:273-278.

Kinloch, B.B., R.A. Sniezko, G.D. Barnes, and T.E. Greathouse. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. Phytopathology 89: 861-867.

McDonald, G.I., E.M. Hansen, C.A. Osterhaus, and S. Samman. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Disease 68: 800-804.

Age Trends in Genetic Parameters of Blister Rust Resistance and Height Growth in a *Pinus strobus* x *P. peuce* F_1 hybrid population

Ioan Blada
Flaviu Popescu

Abstract—This paper reports information about genetic variation in blister rust resistance (BRR), survival (TS) and total height growth (H) over 20 years in a *Pinus strobus* x *P. peuce* hybrid population. Highly significant ($p<0.001$) differences among hybrid families were found for the three traits. With minor exceptions, female and male x female effects were significant ($p<0.05$) or highly significant ($p<0.01$; $p<0.001$) over the whole testing period for BRR, TS and H. However, male effects were significant ($p<0.05$) only for H. This suggested that the traits were controlled by additive and non-additive genes. Over 20 years, the additive variances ranged between 26 and 39 percent for BRR, between 17 and 62 percent for TS and between 31 and 44 percent for H. Non-additive variances ranged between 37 and 60 percent for BRR, between 34 and 78 percent for TS and between 31 and 54 percent for H. Therefore, both variances were important for the traits involved. Narrow-sense heritability estimates at the family level ranged between 0.290 and 0.430 for BRR, between 0.177 and 0.633 for TS and between 0.382 and 0.531 for H. Heritabilities at the individual level ranged between 0.024 and 0.067 for BRR and between 0.064 and 0.167 for H. Parents of good general combining ability were found for both BRR and H. Strong age-age genetic correlations were found between BRR and TS while correlations between BRR and TS on one hand and H on the other were low. The high-parent heterosis was negative while the mid-parent one was positive, accounting for 21.3 percent for BRR, 35.4 percent for TS and 10.4 percent for H. A variable genetic gain for the three traits could be expected, suggesting that hybrid planting could be profitable.

Key words: *Pinus strobus, P. peuce,* factorial cross, hybrid, additive variance, heritability, combining ability, genetic correlation

Introduction

It is well known that eastern white pine (*Pinus strobus* L.) has wide genetic variability and high productivity in plantations in North America, Europe and the Far East (Kriebel 1983).

The blister rust (*Cronartium ribicola* J.C. Fisch. in Rabenh.) migrated from its Siberian gene center to Europe (Leppik 1967) and by 1900 to North America where it caused losses in *P. strobus, P. monticola* Dougl. and *P. lambertiana* Dougl. (Bingham and Gremmen 1971). The inefficiency of conventional control methods of the pathogen has stimulated interest in genetic improvement of resistance. The eastern white pine improvement program was based on both intraspecific crosses (Riker and others 1943; Heimburger 1972a; Zsuffa 1981) and interspecific ones (Patton 1966; Heimburger 1972; Zsuffa 1979).

Interspecies breeding with white pines was tried in attempts to introduce resistance factors into eastern white pine from related more resistant species, like Balkan pine (*Pinus peuce* Griseb.) and Himalayan pine (*Pinus wallichiana* Jacks.). According to Heimburger (1972a), introduction of resistance genes in eastern white pine may be the only way to form a realistic program with this species. In a previous investigation, an F_1 hybrid population from reciprocal crosses between eastern white pine and Balkan pine supported evidence that extranuclear genes controlling blister-rust resistance and growth traits could be found in eastern white pine (Blada 1992). In addition, more recent investigations (Blada 2000a) suggested that parents with a good general combining ability were found in eastern white pine, not only for growth traits but also for blister-rust resistance.

The *P. strobus* x *P. peuce* F_1 hybrids have demonstrated good blister rust resistance (Patton 1966; Heimburger 1972a; Blada 1989; 2000a) and growth traits (Leandru 1982; Blada 2000a; 2000b).

Introduction to Romania of eastern white pine took place at the end of the 19th century, but it was intensified after 1960 when about 300,000 ha were planted with this species; with its 20.2 m^3/year/ha it proved to be a fast growing species (Radu 1974). At the same time, blackcurrant (*Ribes nigrum L.*), one of the alternate hosts of the blister rust pathogen, was planted on large scale to produce fruits. Very soon, coincident with blackcurrant spreading, heavy attacks of blister rust occurred all over the country (Blada 1987; 1990).

Because the blister rust cannot be controlled by conventional methods (Bingham and others 1953), a genetic improvement

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

The authors are Forest Geneticists, Forest Research Institute, Sos. Stefanesti 128, Post Office 11, Bucharest, Romania. E-mail: ioan_blada@yahoo.com and flaviu popescu@toptech.ro, respectively.

program was launched in Romania in 1977 (Blada 1990). The program included both intra- and inter-specific crossing. The practical objective of the program was to establish hybrid seed orchards consisting of parents with high general combining ability for blister rust resistance (Blada 1982). Because of financial reasons, this objective has been only partially fulfilled. Several progress reports on this subject were previously published or presented in symposia (Blada 1987; 1989; 1990; 1992; 1994; 2000a; 2000b).

The objective of this paper is to present the age trends in genetic parameters for blister rust resistance, tree survival and total height growth over 20 years of testing in a *P. strobus* x *P. peuce* F_1 hybrid population.

Materials and Methods

Parents, Mating Design, and Progenies

The initial material consisted of five eastern white pine female and five Balkan pine male parents selected in non-improved planted populations free of blister rust and of unknown origin, The selections could be considered random samples with respect to any trait, except reproductive fertility. In 1979, a 5 x 5 factorial mating design (Comstock and Robinson, 1952) was completed. The seed samples were stratified according to Kriebel's (1973) recommendations and then sown (spring 1981) in individual polyethylene pots (22x18 cm) in a potting mixture consisting of 70 percent spruce humus and 30 percent sand. The progenies grew in pots throughout the first six years and they were the subjects of the nursery test reported earlier (Blada 1987).

Inoculation and Experimental Design

The 25 full-sib hybrid families and two open pollinated progenies were artificially inoculated three times with blister rust, in late August 1982, 1983 and 1984, when they were two, three and four years old, respectively. During each inoculation period, the potted trees were introduced into a polyethylene tent and arranged in randomized complete block design with 14-seedling row-plots in each of the three blocks. The two open pollinated progenies, representing the means of the two parent species, were included as controls. Inoculum consisted of heavily infected leaves of *Ribes nigrum* L. collected from a single plantation. Other details concerning inoculation and inoculation tents were similar to those described by Bingham (1972).

At age six, the hybrids and controls were planted out at 3x3 m spacing, in the Caransebes-Valisor Forest District, at about 45°27' N latitude and 22°07' E longitude and 310 m altitude. As expected, owing to the heavy controlled artificial inoculation, a variable number of seedlings per family were killed during the nursery stage, so that only about 10 seedlings per plot could be used for planting in the field test. Hence, a randomized complete block design with 10-seedling row-plots in each of the three blocks was used. No thinning was carried out by age 20.

Measurements

Several traits were assessed when the hybrids were 5-, 9-, 11-, 13-, 17- and 20-years-old. However, only three traits for each age were presented in this report. (table 1). The blister rust resistance was scored using an index that took into account both the number and severity of the lesions. Its numerical values assigned were: 1 = dead tree or total susceptibility (all trees killed by rust in previous years were included in this cumulative category); 2 = four or more serious stem lesions; 3 = three severe stem lesions; 4 = three more or less severe stem lesions; 5 = two severe stem lesions; 6 = two more or less severe stem lesions; 7 = one severe stem lesion; 8 = one more or less severe stem lesion; 9 = branch or very light stem lesions; 10 = free of lesions or total resistance. Percentages of the trees surviving were calculated based on blister rust resistance index data, i.e. all trees with a score 2 to 10 were considered tree survivors. All percentages were transformed to the arc sin square root for analysis.

Plot-mean data were subject to randomized block and factorial analysis of variance (Hallauer and Miranda 1981) and within-plot variance was calculated by a separate analysis (Becker 1984) where only six (out of 10) planted trees per plot were taken into account. It should be stressed that a few families had only six surviving trees / block; this is the reason why only six trees per block, taken at random, were included in the analysis.

Statistical Analyses

In order to estimate the genetic components of variance the following statistical model, applied to plot means, was assumed:

$$x_{ijkh} = m + M_i + F_j + (MF)_{ij} + B_k + e_{ijkh} \qquad (1)$$

where: x_{ijkh} = the observation of the *h-th* full-sib family from the cross of the *i-th* male and *j-th* female in the *k-th* block; m = general mean; M_i = the effect of the *i-th* male ($I = 1,2,...I$); F_j = the effect of the *j-th* female ($j= 1,2,...J$); $(MF)_{ij}$ = the effect of the interaction of the *i-th* male and *j-th* female; B_k = the effect of the *k-th* block ($k = 1,2,...K$) and e_{ijkh} = the random error.

Table 1—Traits measured for 25 full-sib hybrid families and two open pollinated families.

Traits	Measured at ages….	Units	Symbols
Blister-rust resistance	5, 9, 11, 13, 17, 20	Scale 1…10	BRR. 5 … BRR. 20
Survival	5, 9, 11, 13, 17, 20	%	S. 5 … S. 20
Total height growth	5, 9, 11, 13, 17, 20	dm	H. 5 … H. 20

Since the parents were assumed to be random samples from a random mating population, and the hybrid families were planted in a complete randomized block design, a random model for statistical analysis (table 2) was used (Comstock and Robinson 1952).

Standard errors (SE) of variance components were computed by the formula given by Anderson and Bancroft (1952).

To estimate effectiveness of selection for early traits, three types of heritabilities were calculated.

The first heritability is the one commonly used for estimating the ratio of genetic (additive and non-additive) to total phenotytic variance which is appropriate for estimating gain from selection among hybrid families when they are vegetatively propagated. This is broad-sense heritability (h^2_1) and was estimated by Grafius and Wiebe's (1959) formula:

$$h^2_1 = \sigma^2_G / \sigma^2_{Ph.1} = (\sigma^2_M + \sigma^2_F + \sigma^2_{MF}) / (\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_e / k) \tag{2}$$

where k = number of blocks.

Mass selection genetic gain (Falconer 1981) was estimated by:

$$\Delta G_1 = i_1 h^2_1 \sigma_{Ph.1} \tag{3}$$

where: i_1 = the selection intensity taken from Becker (1984); $\sigma_{Ph.1}$ is the phenotypic standard deviation of the family mean.

The second heritability is appropriate for estimating gain from selection among half-sib hybrid families when they are sexually propagated. This is narrow-sense heritability (h^2_2) at the family level and was estimated by Grafius and Wiebe's (1959) formula:

$$h^2_2 = \sigma^2_A / \sigma^2_{Ph.1} = (\sigma^2_M + \sigma^2_F) / (\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_e / k) \tag{4}$$

and gain from half-sib family selection was estimated by:

$$\Delta G_2 = i_1 h^2_2 \sigma_{Ph.1} \tag{5}$$

The third heritability is the one commonly used for estimating genetic gain from mass selection among randomly placed seedlings. This is individual tree narrow-sense heritability (h^2_3) and was estimated by:

$$h^2_3 = \sigma^2_A / \sigma^2_{Ph.2} = (\sigma^2_M + \sigma^2_F) / (\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_p + \sigma^2_W) \tag{6}$$

where: σ^2_W = within plot variance; σ^2_p = plot error = $\sigma^2_e - \sigma^2_W/n$; n = seedlings per plot.

The mass selection gain was estimated by:

$$\Delta G_3 = i_1 h^2_3 \sigma_{Ph.2} \tag{7}$$

where σ^2_{Ph2} is the phenotypic standard deviation and it refers to individual tree values.

If the best general combining ability parents (*gca*) are to be selected and intermated, then the genetic gain was calculated as twice the average of *gca*'s or the average of the breeding values of the three selected parents for the next breeding works (table 10).

The heterosis was calculated according to Hallauer and Miranda's (1981) formula:

$$He_1 = [(H_y - HP) / HP] \cdot 100 \tag{8}$$

$$He_2 = [(Hy - MP) / MP] \cdot 100 \tag{9}$$

where: H_y, HP and MP are the hybrid mean, the high-parent mean and the mid-parent mean, respectively. As shown above, two estimates of heterosis were computed: one that compared to the best parent (He_1) and the other that compared to the mean of the parents from open pollinated controls (He_2). According to the broad, modern concept, there exists positive or negative heterosis, luxuriant, adaptive, selective or reproductive heterosis and labile or fixed heterosis (Mac Key 1976). Only positive and negative heterosis was estimated in this experiment.

Genetic coefficient of variation (*GCV*) was calculated by formula:

$$CGV = (\sqrt{\sigma^2_G} / X)\ 100 \tag{10}$$

where: σ^2_G and X are the genetic variance and trait mean, respectively.

General combining ability (*gca*) effects of each parental tree were calculated, using Griffing's (1956) method 4, adapted to a factorial mating design. The statistical model was:

$$X_{ij.} = X_{...} + g_i + g_j + s_{ij} + e_{ijk} \tag{11}$$

where: $X_{ij.}$ is the mean of the *i-th* female tree crossed to the *j-th* male tree over k replications; $X_{...}$ is the general mean; g_i is the gca_i effect associated with the *i-th* female tree; $g_{.j}$ is the gca_j effect associated with *j-th* male tree; s_{ij} is the sca effect associated with the cross between the *i-th* female tree and *j-th* male tree; e_{ijk} is the residual effect.

The computational formulae were as follows:

$$gca_i = x_{i.} - X_{...} \tag{12}$$

$$gca_j = x_{.j} - X_{...} \tag{13}$$

Table 2—Analysis of variance of factorial meting design random effects model, in a random complete block in one environment.

Source of variation	Df	MS	E (MS)
Total	KIJ-1		
Blocks (B)	K-1	MS_B	
Hybrids (Hy)	IJ-1	MS_{Hy}	
-Females (F)	J-1	MS_F	$\sigma^2_W + \sigma^2_p + K\sigma^2_{MF} + KI\sigma^2_F$
-Males (M)	I-1	MS_M	$\sigma^2_W + \sigma^2_p + K\sigma^2_{MF} + KJ\sigma^2_M$
-M x F	(J-1)(I-1)	MS_{MF}	$\sigma^2_W + \sigma^2_p + K\sigma^2_{MF}$
Pooled error	(IJ-1)(K-1)	MS_E	$\sigma^2_W + \sigma^2_p$
Within plot	KIJ(n-1)	MS_W	σ^2_W

where: $x_{i\cdot}$ = the mean of the F_1 resulting from crossing the *i-th* female tree with each of the male parent; $x_{\cdot j}$ = the mean of the F_1 resulting from crossing the *j-th* male parent with each of the female tree;

To examine relationships among traits, genetic correlations coefficients were estimated using Falconer's (1981) formula.

Results and Discussions

Blister Rust Attack Evolution

Table 3 summarizes survival at ages 6 and 20. At the end of the nursery test, survival in the F_1 hybrid population was 74.3 percent, while in open pollinated control populations of *P. strobus* and *P. peuce,* the survival was 22.7 percent and 98.2 percent, respectively (table 3, column 3). The field test showed that, at age 20, survival in hybrids, *P. strobus* and *P. peuce* was 70.7 percent, 15.5 percent and 88.9 percent, respectively (table 3, column 5). Blister rust susceptibility was high in eastern white pine, very low in Balkan pine and low in hybrids. The mortality was due mostly to blister rust and only a few to other causes (see table 3, columns 7 and 8). It should be emphasized that the field test had been laid out in a site free of any *Ribes* species and blister rust, as well. Consequently, no new infections took place, so that the trees were killed only due to the infections that resulted from the control inoculation. Also, it was noticed that during the nursery test, both stem and branch cankers were evident, but later only stem basal cankers occurred. This suggests the absence of the local secondary infections.

Concerning the tree growth, the following phenomenon was observed:

- when the canker was marginal to the stem, the infected tree grew normally up to about 12-15 years of age;
- when the canker spread and reached the half stem diameter, the growth was slow and the tree was dead after a few years;
- when the canker had spread over half the stem diameter, the tree was dead in the same or in the next season of vegetation.

Genetic Variation

The analysis of variance indicated highly significant ($p < 0.001$) differences among hybrid family means for blister rust resistance, survival and total height growth at all ages (table 4, row 2). At age 20, blister rust resistance varied from 6.3 to 9.1, survival between 49.9 percent and 91.3 percent and total height from 88.3 dm to 110.0 dm. Hence, selection at the family level within the hybrid population could be carried out for the three economically important traits.

There was a large genetic variation among parents within each sex (species) for the three traits examined over years. An important finding of this experiment was that the effects of eastern white pine female parents were significant ($p < 0.05$) for blister rust resistance and highly significant ($p < 0.01$; $p < 0.001$) for survival at most ages. Significant differences among eastern white pine female parents for total height growth were found by the end of the testing period, i. e. at ages 17 and 20 (table 4, row 3).

Balkan pine as male parents had significant ($p < 0.05$) effects on height growth through age 17 but had no significant effects on blister rust resistance and survival at any age (table 4, row 4). The results suggested that: (i) an additive genetic control in these three traits occurred; (ii) high *gca* parents could be selected within the eastern white pine parental population. Similar results were found in other experiments with *P. strobus* x *P. peuce* hybrids (Blada 1989).

Male x female interaction effects were highly significant ($p < 0.01$; $p < 0.001$) for the three traits at all ages (table 4, row 5), suggesting non-additive gene action.

The genetic coefficient of variation at the family level (table 5) was, in general, moderate ranging between 8.0 and 12.3 percent for blister rust resistance, between 7.6 and 18.3 percent for survival and between 5.0 and 10.8 percent for total height growth.

Variance Components

Variance component estimates, standard errors and dominance ratios were listed in table 5.

The contribution of the *GCA* variance to the total phenotypic variance ranged over years from 26 to 39 percent for blister rust resistance, 17 to 62 percent for survival and 31 to 44 percent for total height growth. The contribution of *SCA* variance for the same traits ranged from 37 to 60 percent, 34 to 78 percent and 31 to 54 percent, respectively.

The ratio of dominance to additive variance for blister rust resistance was greater than 1 at all ages indicating that dominance variance was of higher importance. However, the additive variance was only slightly lesser than dominance variance, suggesting that both variances could be used in a

Table 3—Tree survival and evolution of blister rust attack.

	Nursery test at age 6		Field test at age 20				
Genotype	Inoculated trees at age 2 No.	Survival No. (%)	Planted trees No. (%)	Survival No.(%)**	Total killed trees No. (%)**	Killed trees by rust No.(%) ***	Killed by other causes No.(%)***
1	2	3	4	5	6	7	8
P. strobus	150	34 (22.7)	34	23 (15.5)	127 (84.5)	124 (97.6)	3 (2.4)
Hybrids	1050	780 (74.3)	780*	742 (70.7)	308 (29.3)	299 (97.1)	9 (2.9)
P. peuce	109	107 (98.2)	107	97 (88.9)	12 (11.1)	4 (33.3)	8 (66.7)

* Some families had more and some other less than 10 trees / block; ** calculated as against inoculated trees (column 2).
*** Calculated as against total killed trees (column 6).

Table 4—Mean squares and F-tests for the *P. strobus* x *P. peuce* F_1 hybrid factorial analyses.

Source of variation	DF	Traits[a] BRR.5	BRR. 9	BRR. 11	BRR. 13	BRR. 17	BRR. 20	S. 5	S. 9	S. 11
Replications	2	0.054	0.035	0.036	0.054	0.171	0.146	2.675	1.418	2.13
Hybrids	24	2.520***	1.503***	2.775***	2.520***	2.537***	2.590***	112.328***	295.800***	332.71***
-Females (F)	(4)	5.527*	2.692	6.702*	5.527*	7.261*	7.512*	184.804	904.224**	1259.58***
-Males (M)	(4)	2.398	2.248	1.121	2.398	1.526	1.671	108.036	229.358	191.20
-M x F	(16)	1.799***	1.020**	2.207***	1.799***	1.609***	1.589***	95.282***	160.304***	136.37***
Error	48	0.142	0.246	0.154	0.142	0.130	0.120	1.953	2.241	5.80
Within plot [b]	540	3.480	6.407	8.348	8.684	14.980	14.950	-	-	-

Source of variation	DF	Traits S. 13	S. 17	S. 20	H. 5	H. 9	H. 11	H. 13	H. 17	H. 20
Replications	2	7.842	3.03	3.26	0.075	0.242	0.314	0.792	5.796	3.647
Hybrids	24	287.044***	312.92***	262.08***	0.491***	2.243***	7.730***	14.793***	50.217***	113.538***
-Females (F)	(4)	832.042**	1145.85**	901.62**	0.123	3.408	6.967	19.628	105.040*	243.275*
-Males (M)	(4)	249.610	95.72	101.45	1.765**	4.410*	19.029*	35.662*	87.649*	149.623
-M x F	(16)	160.153***	158.99***	142.35***	0.265**	1.410***	5.096***	8.366***	27.153***	72.082***
Error	48	4.240	5.27	2.88	0.056	0.263	1.013	0.862	5.215	2.765
Within plot [b]	540	-	-	-	2.484	3.006	10.568	14.124	38.801	75.871

[a] See Table 1 for list of traits.

[b] The within plot variance was calculated by a separate analysis; *p = 5 percent; **p = 1 percent;***p = 0.1 percent.

breeding program. The ratio of dominance to additive variance for both survival and total height growth did not show a clear trend over the testing period. But each of these variances was sufficient for their practical use for the improvement of blister rust resistance, survival and total height growth. The trend in the contribution of the error variance to the phenotypic variance for blister rust resistance and height growth declined significantly with age, that is, from 35 percent at age nine to 12 percent at age 20 and from 29 percent at age 11 to 7 percent at age 20, respectively. In contrast, for survival, the error variance displayed a slight continuous decline, ranging from 5 percent at age five to 3 percent at age 20. This decline supports the expectation that genetic estimates of the three traits become more accurate with age.

The additive variance component associated with female parent (*P. strobus*) effects ranged from 16 to 39 percent for blister rust resistance, from 15 to 59 percent for survival and from 0 to 27 percent for total height growth of the phenotypic variance. The contribution of male parent effects to the same traits ranged from 0 to 12 percent, 0 to 6 percent and 12 to 44 percent, respectively. Thus, it is evident that the magnitude of the variance component associated with female parent effects was far greater than that associated with male parent effects over the whole testing period. These results were consistent with those reported elsewhere (Blada 1989) for another experiment with *P. strobus* x *P. peuce* hybrids.

The female additive variance components were associated with standard errors smaller than the estimates themselves in all but four cases thus making heritability estimates fairly reliable. However, the standard errors of the male variances for blister rust and survival were higher in six cases than the estimates themselves, at most ages, and suggesting non-accurate estimates.

Heritability

The broad-sense (h^2_1) and narrow-sense (h^2_2) heritabilities at the family level, as well as individual-tree narrow-sense (h^2_3) heritabilities calculated over testing period are represented in table 6.

The magnitude of heritability estimates for blister rust resistance, survival and total height growth indicated that these traits may be under moderate to high genetic control, but there is an apparent age dependency. For blister rust resistance, the estimated narrow-sense heritability at the family level was 0.325 at age 5 and increased to 0.430 at age 20. Similarly, for survival the heritability was lowest at age five ($h^2_2 = 0.177$), but increased to 0.516 at age 20. The family narrow-sense heritability for total height growth ranged between 0.382 and 0.531. According to table 6, the trend in the three types of heritability for blister rust resistance and height growth was in general consistent over the 20 years testing period. The change in heritability in long rotation crops such as trees is not surprising since genes involved in height growth control may change with age (Namkoong and others 1988) and these changes may be related to different growth phases (Franklin 1979). Perhaps the accumulative nature of blister-rust resistance and tree survival was responsible for increasing heritability with age and the age-age genetic correlations.

Individual narrow-sense heritabilities differed over years. Their estimates were low ranging from 0.024 to 0.067 for blister rust resistance and low to moderate ranging between 0.064 and 0.167 for height growth.

As expected, the broad-sense heritability estimates were much greater than the narrow-sense estimates. The narrow-sense heritabilities are used in conventional breeding while broad-sense ones are also important as vegetative propagation methods and economical methods of producing specific

Table 5—Variance components (percents in brackets), standard errors (SE), dominance ratios, genetic coefficient of variation (GCV) and trait means (X).

	Traits[a]								
Parameters	**BRR. 5**	**BRR. 9**	**BRR. 11**	**BRR. 13**	**BRR. 17**	**BRR. 20**	**S. 5**	**S. 9**	**S. 11**
σ^2_{GCA-F} ± SE	0.248 (25) ±0.216	0.111 (16) ±0.106	0.300 (26) ±0.263	0.249 (25) ±0.216	0.377 (38) ±0.282	0.395 (39) ±0.291	5.968 (15) ±7.421	49.594 (46) ±34.985	74.881 (59) ±48.576
σ^2_{GCA-M} ± SE	0.040 (4) ±0.100	0.082 (12) ±0.089	–0.072 (0) ±0.065	0.040 (4) ±0.101	–0.006 (0) ±0.069	0.005 (0) ±0.073	0.850 (2) ±4.666	4.604 (4) ±9.520	3.655 (3) ±7.959
Total σ^2_{GCA}	0.288	0.193	0.300	0.289	0.377	0.400	6.818	54.198	78.536
σ^2_{SCA}± SE	0.592 (56) ±0.200	0.258 (37) ±0.114	0.648 (60) ±0.245	0.552 (56) ±0.200	0.493 (49) ±0.179	0.490 (49) ±0.177	31.110 (78) ±10.588	52.688 (48) ±17.812	43.523 (34) ±15.157
Total σ^2_G	0.840	0.451	0.984	0.841	0.870	0.890	37.928	106.886	122.059
σ^2_e± SE	0.142 (15) ±0.028	0.246 (35) ±0.049	0.154 (14) ±0.031	0.142 (15) ±0.028	0.130 (13) ±0.026	0.120 (12) ±0.024	1.953 (5) ±0.390	2.241 (2) ±0.448	5.803 (4) ±1.160
σ^2_{Ph}	0.982	0.697	1.138	0.983	1.000	1.010	39.881	109.127	127.861
σ^2_W	3.480	6.407	8.348	8.684	14.980	14.950	—	—	—
σ^2_p	–0.438	–0.822	–1.237	–1.305	–2.367	–2.372	—	—	—
σ^2_{SCA} : σ^2_{GCA}	1.9 : 1.0	1.3 : 1.0	2.3 : 1.0	1.9 : 1.0	1.3 : 1.0	1.2 : 1.0	4.6 : 1.0	1.0 : 1.0	0.6 : 1.0
σ^2_{GCA-F} : σ^2_{GCA-M}	6.2 : 1.0	1.3 :1.0	1.0 : 0.0	6.2 : 1.0	1.0 : 0.0	1.0 : 0.0	7.0 : 1.0	10.8 : 1.0	20.5 : 1.0
GCV (%)	11.5	8.0	12.1	11.5	12.1	12.3	7.6	14.9	16.7
Mean	8.0	8.4	8.2	7.9	7.7	7.7	81.4 Arc 97.8 %	69.3 Arc 87.4 %	66.0 Arc 83.5 %

	Traits								
Parameters	**S. 13**	**S. 17**	**S. 20**	**H. 5**	**H. 9**	**H. 11**	**H. 13**	**H. 17**	**H. 20**
σ^2_{GCA-F} ± SE	44.793 (42) ±32.222	65.791 (54) ±44.245	50.618 (51) ±34.847	–0.009 (0) ±0.008	0.133 (14) ±0.135	0.125 (4) ±0.291	0.751 (13) ±0.778	5.192 (24) ±4.088	11.413 (27) ±9.500
σ^2_{GCA-M} ± SE	5.964 (6) ±10.245	–4.218 (0) ±5.105	–2.727 (0) ±5.025	0.100 (44) ±0.068	0.200 (20) ±0.173	0.929 (27) ±0.741	1.820 (31) ±1.385	4.023 (18) ±3.427	5.169 (12) ±5.978
Total σ^2_{GCA}	50.757	65.791	50.618	0.100	0.333	1.054	2.571	9.225	16.582
σ^2_{SCA}± SE	51.971 (48) ±17.797	51.237 (42) ±17.669	46.492 (46) ±15.818	0.070 (31) ±0.030	0.382 (39) ±0.158	1.361 (40) ±0.325	2.502 (42) ±0.931	7.313 (34) ±3.037	23.106 (54) ±8.011
Total σ^2_G	102.728	117.028	97.110	0.170	0.715	2.415	5.073	16.538	39.688
σ^2_e± SE	4.240 (4) ±0.848	5.276 (4) ±1.055	2.877 (3) ±0.575	0.056 (25) ±0.111	0.263 (27) ±0.053	1.013 (29) ±0.203	0.862 (14) ±0.172	5.215 (24) ±1.043	2.765 (7) ±0.553
σ^2_{Ph}	106.978	122.304	99.987	0.225	0.978	3.428	5.935	21.753	42.453
σ^2_W	—	—	—	2.484	3.006	10.568	14.124	38.801	75.871
σ^2_p	—	—	—	–0.192	–0.238	–0.748	–1.492	–1.252	–9.880
σ^2_{SCA} : σ^2_{GCA}	1.0 : 1.0	0.8 : 1.0	0.9 : 1.0	0.7 : 1.0	1.1 : 1.0	1.3 : 1.0	1.0 : 1.0	0.8 : 1.0	1.4 : 1.0
σ^2_{GCA-F} : σ^2_{GCA-M}	7.5 : 1.0	1.0 : 0.0	1.0 : 0.0	0.0 : 1.0	0.7 : 1.0	0.1 : 1.0	0.4 : 1.0	1.3 : 1.0	2.2 : 1.0
GCV (%)	15.8	18.3	17.2	10.8	5.9	5.6	5.0	5.5	6.6
Mean	64.1 Arc 80.8 %	59.0 Arc 73.5 %	57.2 Arc 70.7 %	3.8	14.2	27.8	44.7	73.6	96.1

[a] See Table 1 for list of traits.

σ^2_{GCA-F} and σ^2_{GCA-M} = additive variance due female and male parent trees, respectively.

Table 6—Estimates of phenotypic variance ($\sigma^2_{Ph.1}$, $\sigma^2_{Ph.2}$), phenotypic standard deviations ($\sigma_{Ph.1}$, $\sigma_{Ph.2}$), family broad sense and narrow sense heritabilities (H^2_1, h^2_2) and individual narrow sense heritabilities (h^2_3).

	Traits[a]								
Parameters	**BRR. 5**	**BRR. 9**	**BRR. 11**	**BRR. 13**	**BRR. 17**	**BRR. 20**	**S. 5**	**S. 9**	**S. 11**
$\sigma^2_{Ph.1}$	0.888	0.533	1.035	0.888	0.913	0.930	38.579	107.633	123.993
$\sigma^2_{Ph.2}$	4.320	6.858	9.332	9.525	15.850	15.840	—	—	—
$\sigma_{Ph.1}$	0.942	0.730	1.017	0.942	0.955	0.964	6.211	10.375	11.135
$\sigma_{Ph.1}$	2.078	2.619	3.055	3.086	3.981	3.980	—	—	—
$H^2_1=h^2_{bs}$	0.947	0.847	0.951	0.947	0.953	0.957	0.983	0.993	0.984
$h^2_2=h^2_{ns}$	0.325	0.363	0.290	0.325	0.413	0.430	0.177	0.503	0.633
$h^2_3=h^2_W$	0.067	0.028	0.032	0.030	0.024	0.025	—	—	—

	Traits								
Parameters	**S. 13**	**S. 17**	**S. 20**	**H. 5**	**H. 9**	**H. 11**	**H. 13**	**H. 17**	**H. 20**
$\sigma^2_{Ph.1}$	104.141	118.786	98.089	0.188	0.803	2.753	5.359	18.276	40.610
$\sigma^2_{Ph.2}$	—	—	—	2.654	3.721	12.983	19.197	55.339	115.559
$\sigma_{Ph.1}$	10.205	10.899	9.904	0.434	0.896	1.659	2.315	4.275	6.373
$\sigma_{Ph.1}$	—	—	—	1.629	1.929	3.603	4.381	7.439	10.750
$H^2_1=h^2_{bs}$	0.966	0.985	0.990	0.901	0.891	0.877	0.946	0.905	0.977
$h^2_2=h^2_{ns}$	0.487	0.554	0.516	0.531	0.415	0.382	0.480	0.505	0.408
$h^2_3=h^2_W$	—	—	—	0.064	0.089	0.081	0.134	0.167	0.143

[a] See Table 1 for list of traits.
$H^2_1=h^2_{bs} = \sigma^2_G / \sigma^2_{Ph.1}$; $h^2_2=h^2_{ns} = \sigma^2_{GCA} / \sigma^2_{Ph.1}$; $h^2_3=h^2_W = \sigma^2_{GCA} / \sigma^2_{Ph.2}$; $\sigma^2_G = \sigma^2_M + \sigma^2_F + \sigma^2_{MF}$; $\sigma^2_{GCA} = \sigma^2_M + \sigma^2_F$;
$\sigma^2_{Ph.1} = \sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_e/R$; $\sigma^2_{Ph.2} = \sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_p + \sigma^2_W$; σ^2_p = plot error = $\sigma^2_e - \sigma^2_W/n$; $n = 6$; σ^2_e = variance error.

crosses, such as supplemental mass pollination, become available (Zobel and Talbert 1984). However, heritability estimates were high enough to ensure genetic progress in improving blister rust resistance, survival and height growth using *P. strobus* x *P. peuce* F_1 hybrids.

Combining Abilities

The general combining ability (*gca*) effects estimated for 10 parents and 18 traits over years were presented in table 7.

Both positive and negative *gca* effects which differed from the test mean were found for both male and female parents for most traits. The range of estimated *gca* effects among parents suggested that it may be possible to select parents with superior breeding values for blister rust resistance, tree survival and height growth.

At age 20, (fig. 1), the eastern white pine female Parent 7 had the largest positive *gca* effects for both blister rust resistance (*gca* = 0.827 points) and survival (*gca* = 8.070 arc sin) whereas the Parent 1 was the second highest for blister rust resistance but the fourth for survival. At the same age, among Balkan pine parents, the male Parent 20 had the largest positive *gca* effects for both blister rust resistance (*gca* = 0.420 points) and total height growth (*gca* = 5.185 dm). On the other hand, the female Parent 2 and Parent 8 were the worst because of their negative effects for all traits at age 20. With one exception, the Balkan male parents had low effects on both blister rust resistance and height growth. A primary objective of tree breeding involves choosing the best parents for mating, especially when the trait to be improved is quantitatively inherited. Hence the parents 7 and 1 should be selected as good *gca* parents for blister rust resistance and, on the other hand, parents 3 and 20 should be selected as good parents for height growth. Taking into account that blister rust resistance trait has the first priority in improvement, the parents 7, 1, and 20 should be used for blister-rust resistance breeding, as they have the ability of transmitting to their offspring a good level of resistance.

Genetic Correlations

Genetic correlations for traits involved in 5-, 9-, 11-, 13-, 17- and 20-year-old hybrids are presented in table 8.

Within trait age-age genetic correlations ranged from 0.406 to 1.00 for blister rust resistance, 0.106 to 0.963 for survival and 0.010 to 0.926 for total height growth. Correlations between blister rust resistance and survival at the same age, except age five, were moderate to high ranging between 0.425 and 0.897. They appear to have a significant predictive value for early selection purposes. Based on these data, one may expect that selection for high blister rust resistance at age five or survival at age nine should result in high blister rust resistance and a high survival at age 20.

Trait-trait genetic correlations between blister rust resistance or survival, on one hand, and height growth on the other, were low and very low. These results suggested that

Table 7—General combining ability (*gca*) effects of 10 parents for tested traits.

Parents	Traits[a]																	
	BRR. 5	**BRR. 9**	**BRR. 11**	**BRR. 13**	**BRR. 17**	**BRR. 20**	**S. 5**	**S. 9**	**S. 11**	**S. 13**	**S. 17**	**S. 20**	**H. 5**	**H. 9**	**H. 11**	**H. 13**	**H. 17**	**H. 20**
									***gca* - females**									
1	0.338	–0.014	0.660	0.338	0.490	0.480	–3.875	0.678	3.358	0.917	6.148	2.964	–0.017	–0.277	–0.388	–1.392	–1.731	–0.068
2	–0.876	–0.508	–0.860	–0.876	–0.730	–0.726	–1.695	–7.335	–9.228	–6.443	–9.112	–8.216	0.094	0.463	0.112	–0.192	–1.511	–2.928
3	0.138	0.092	0.600	0.138	0.156	0.154	4.072	3.025	3.872	0.370	2.435	5.484	–0.146	–0.444	0.992	2.288	4.529	6.252
7	0.698	0.639	0.093	0.698	0.796	0.827	3.365	11.065	11.545	11.690	9.715	8.070	0.036	–0.310	–0.834	–0.645	0.189	0.812
8	–0.296	–0.208	–0.493	–0.296	–0.710	–0.733	–1.869	–7.435	–9.548	–6.536	–9.188	–8.303	0.032	0.570	0.119	–0.059	–1.477	–4.068
									***gca* - males**									
13	–0.289	–0.561	–0.340	–0.289	–0.137	–0.153	1.178	–2.215	–1.135	–1.656	1.008	–0.636	0.464	–0.250	0.232	0.015	–1.697	–1.528
14	0.031	–0.054	0.060	0.031	0.010	–0.013	1.152	–3.455	–2.082	–3.090	–3.138	–1.870	0.168	–0.290	–1.281	–1.539	–0.131	0.478
15	–0.509	0.019	–0.207	–0.509	–0.450	–0.453	2.125	–1.642	–2.568	–3.996	–1.405	–1.350	0.022	–0.564	–0.728	0.832	–0.411	–1.095
18	0.371	0.072	0.340	0.371	0.196	0.200	–4.642	0.925	–0.428	4.144	3.562	4.564	–0.402	0.330	–0.092	–0.279	–1.864	–3.042
20	0.398	0.526	0.147	0.398	0.383	0.420	0.185	6.385	6.212	4.597	–0.028	–0.710	–0.254	0.776	1.686	2.635	4.103	5.185

[a] See Table 1 for list of traits.

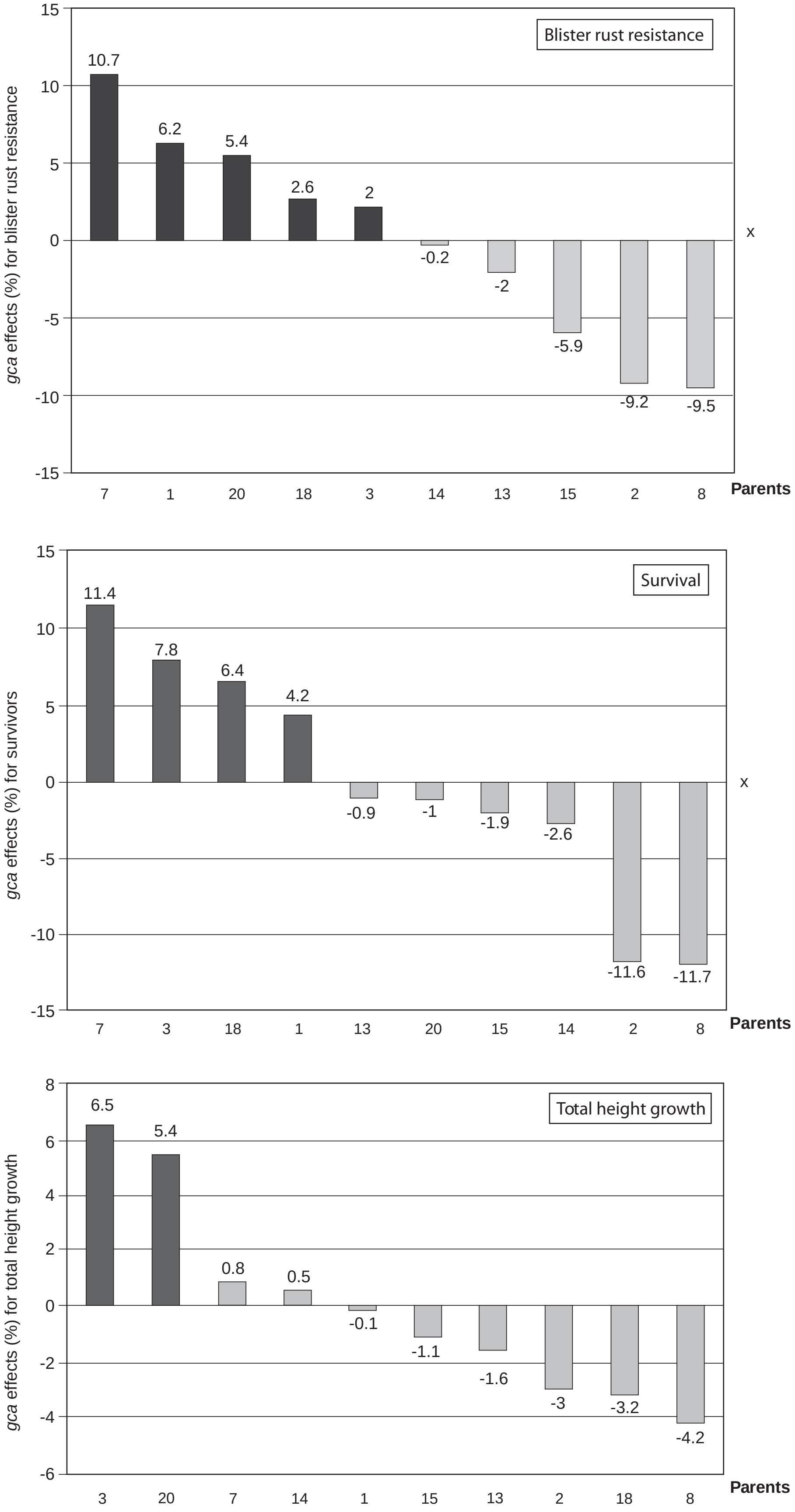


Figure 1—General combining ability (*gca*) effects for blister-rust resistance, survival and total height growth at age 20.

Table 8—Genetic correlations among traits.

Traits[a]	**BRR. 9**	**BRR. 11**	**BRR. 13**	**BRR. 17**	**BRR. 20**	**S. 5**	**S. 9**	**S. 11**	**S. 13**	**S. 17**	**S. 20**	**H. 5**	**H. 9**	**H. 11**	**H. 13**	**H. 17**	**H. 20**
BRR. 5	0.809	0.676	1.000	0.926	0.918	0.281	0.816	0.803	0.874	0.751	0.754	–0.405	–0.178	0.019	0.078	0.227	0.232
BRR. 9	—	0.406	0.809	0.759	0.759	0.312	0.868	0.774	0.783	0.568	0.661	–0.561	–0.187	–0.108	0.031	0.236	0.148
BRR. 11		—	0.676	0.644	0.626	0.106	0.474	0.506	0.425	0.559	0.487	–0.397	–0.285	0.037	0.135	0.328	0.352
BRR. 13			—	0.926	0.918	0.281	0.816	0.803	0.874	0.751	0.754	–0.405	–0.178	0.019	0.078	0.227	0.232
BRR. 17				—	0.998	0.342	0.869	0.871	0.891	0.790	0.778	–0.306	–0.265	0.053	0.098	0.303	0.344
BRR. 20					—	0.348	0.877	0.880	0.897	0.792	0.775	–0.316	–0.253	0.070	0.114	0.325	0.361
S. 5						—	0.398	0.414	0.379	0.324	0.390	0.229	–0.383	0.110	0.232	0.395	0.363
S. 9							—	0.963	0.906	0.762	0.817	–0.378	–0.278	0.061	0.174	0.351	0.309
S. 11								—	0.917	0.841	0.851	–0.247	–0.290	0.087	0.175	0.365	0.408
S. 13									—	0.845	0.839	–0.323	–0.208	0.054	0.086	0.210	0.237
S. 17										—	0.921	–0.232	–0.452	–0.038	–0.032	0.148	0.304
S. 20											—	–0.287	–0.430	–0.045	–0.001	0.143	0.231
H. 5												—	0.043	0.073	0.010	0.093	0.093
H. 9													—	0.567	0.454	0.065	0.016
H. 11														—	0.926	0.660	0.564
H. 13															—	0.829	0.702
H. 17																—	0.876

[a] See Table 1 for list of traits.

the two categories of traits were inherited independently, and hence, tandem selection cannot be applied.

Contradictory age-age within trait correlation estimates was found in total height growth. Thus, correlations involving height growth at ages five and nine were very low with two exceptions. In contrast, correlations involving ages between 11 and 20 were high and very high, ranging between 0.564 and 0.926. Consequently, early selection at age 11 could result in height growth improvement at age 20, and presumably at rotation age, too.

Heterosis

Parent and hybrid performances and the two types of heterosis exhibited at age 20 are illustrated in figure 2.

It should be pointed out that the eastern white pine is the best parent species for growth whereas the Balkan pine is the best parent species for blister rust resistance.

At age 20, the estimates of the high-parent heterosis were negative for the three traits, that is, the hybrid performances were lower than those of the best parent for each trait. For example, for height growth, the hybrid mean was 9.8 percent lower than the mean of the white pine but greater than Balkan pine. Similarly, the mean blister rust resistance and tree survival were 16.3 and 20.4 percent, respectively, lower than the mean of Balkan pine bulk lot.

Mid-parent heterosis was positive for the three involved traits. The heterosis estimates accounted for 21.3 percent for blister rust resistance, 35.4 percent for survival and 10.4 percent for height growth.

The hybrids inherited a high blister rust resistance and were fast growing. Thus, the eastern white pine measured an average of 3.5 points in blister rust resistance and 15.5 percent in tree survival while the hybrid measured 7.7 points and 70.7 percent, respectively; that is, 120 percent and 356 percent more. Also, the hybrid mean exceeded the mean of the Balkan pine in height growth. The Balkan pine measured 67.6 dm in height while the hybrid measured 96.1 dm, or 42 percent more. Therefore, the hybrids inherited high blister rust resistance and faster growth from their parents.

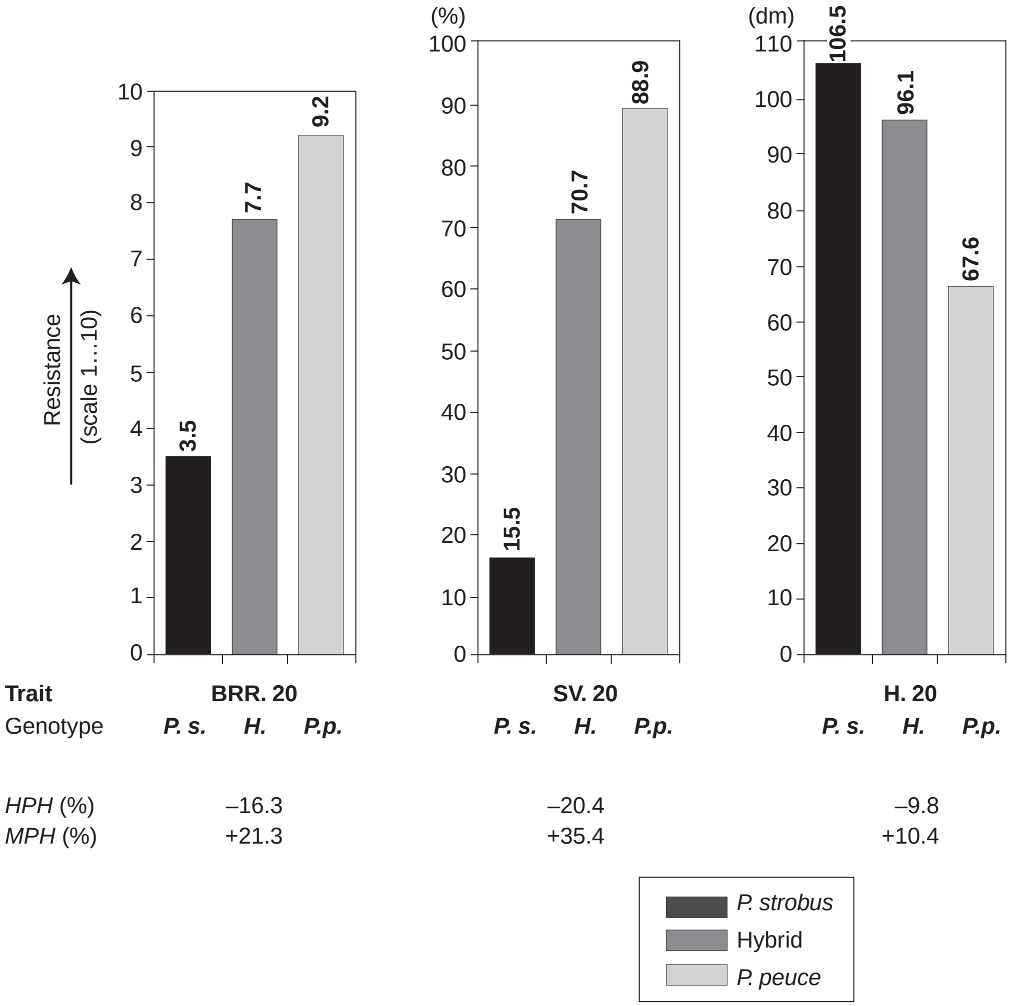


Figure 2—*Pinus strobus, P. peuce* and *P. strobus* x *P. peuce* F1 hybrid performance at age 20, high-parent heterosis (HPH) and mid-parent heterosis (MPH).

Selection and Genetic Gain

Based on these results, selection could be made at both the family and individual level. The genetic gain that could be achieved in all traits and all ages are presented in table 9.

If the best 5, 8, 11, or 14 out of 25 hybrid families were selected at age 20, a genetic gain of 7.2,, 5.8, 4.7,and 3.7 percent in blister rust resistance, 12, 9.7, 7.7 and 6.1 percent in survival and 3.6, 2.9, 2.3 , and 1.8 percent in height growth could be expected.

Selection at individual level could make an additional gain. So, if at age 20 the best 5, 10, 15, and 20 percent individuals within the best hybrid families were selected, a genetic again of 2.7, 2.3, 2.0, and 1.8 percent in blister rust resistance and 3.3, 2.8, 2.5, and 2.2 percent in total height growth could be achieved.

Genetic gain was calculated as twice the average of the *gca*'s, at age 20. The average breeding value of the best parents was presented in table 9.

The best three parents for blister rust resistance were female trees 7 and 1 and male tree 20. Their average breeding value was 1.151 points, which represent a genetic gain of 15 percent in the overall mean (7.7 points), for blister rust resistance (table 10, column 4). Similarly, for total height growth, the best three parents were 3, 7, and 20, and their average breeding value was 8.165 dm, which represent a genetic gain of 8.5 percent in the overall mean (96.1 dm) for height growth

The estimated genetic gains indicated that a program aimed at improving blister rust resistance and height growth through interspecific hybridisation could be successfully achieved.

Table 9—Expected genetic gain (ΔG) according to the intensity of selection and hybrid age.

	ΔG (%) selecting the best 5, 8, 11 or 14 hybrid families of 25 tested				**ΔG (%) selecting the best 5, 10, 15 or 20% individuals within the best hybrid families**			
Traits[a]	**5**	**8**	**11**	**14**	**5**	**10**	**15**	**20**
BRR. 5	5.1	4.1	3.3	2.6	3.6	3.0	2.7	2.4
BRR. 9	4.2	3.4	2.7	2.2	1.8	1.5	1.4	1.2
BRR. 11	4.8	3.9	3.1	2.5	2.5	2.1	1.8	1.7
BRR. 13	5.2	4.2	3.4	2.6	2.4	2.1	1.8	1.6
BRR. 17	6.9	5.5	4.4	3.5	2.6	2.2	1.9	1.7
BRR. 20	7.2	5.8	4.7	3.7	2.7	2.3	2.0	1.8
S. 5	1.8	1.5	1.2	0.9	—	—	—	
S. 9	10.1	8.1	6.5	5.1	—	—	—	—
S. 11	14.4	11.5	9.3	7.3	—	—	—	—
S. 13	10.4	8.4	6.7	5.3	—	—	—	—
S. 17	13.8	11.1	8.9	7.0	—	—	—	
S. 20	12.0	9.7	7.7	6.1	—	—	—	—
H. 5	8.2	6.6	5.3	4.1	5.7	4.8	4.3	3.8
H. 9	3.5	2.8	2.3	1.8	2.5	2.1	1.8	1.7
H. 11	3.1	2.5	2.0	1.6	2.2	1.8	1.6	1.5
H. 13	3.3	2.7	2.2	1.7	2.7	2.3	2.0	1.8
H. 17	3.9	3.2	2.5	2.0	3.5	3.0	2.6	2.4
H. 20	3.6	2.9	2.3	1.8	3.3	2.8	2.5	2.2

[a] See Table 1 for list of traits.

Table 10—General combining ability (*gca*) estimates, breeding values (BV) and genetic gains (ΔG) if selected the best three parents for blister-rust resistance, tree survivors and total height growth.

Blister-rust resistance				**Survival**				**Total height growth**			
Select parents	***gca***	**BV**	**ΔG**[a]	**Select parents**	***gca***	**BV**	**ΔG**[a]	**Select parents**	***gca***	**BV**	**ΔG**[a]
	- - - Points	*- - -*	*%*		*- - - - - - - -%*	*- - - - - - -*			*- - - - - dm*	*- - - - -*	*%*
7	0.827	1.654	21.5	7	11.4	22.4	31.7	3	6.252	12.50	13.0
1	0.480	0.960	12.5	3	7.8	15.6	22.1	20	5.185	10.37	10.8
20	0.420	0.840	10.9	18	6.4	12.8	18.1	7	0.812	1.624	1.7
Mean	0.577	1.151	15.0		8.5	17.1	24.0		4.083	8.165	8.5

[a] Calculated against test mean, that is, 7.7 for blister-rust resistance, 70.7 percent for survival and 96.1 dm for total height growth.

Conclusions

At age 20, the *P. strobus* x *P. peuce* F_1 hybrids exhibited a mid-parent heterosis in blister rust resistance, survival and total height growth.

Highly significant genetic variation over 20 years was detected in the hybrid population to warrant improvement for the three traits involved, using additive as well as non-additive genetic variances.

Additive variance for height growth was found within both *P. strobus* and *P. peuce* parent populations, whereas the additive variance associated with blister rust resistance and survival was found within female parent (*P. strobus*) population, only. Non-additive variance was also consistently involved in the three tested traits.

The magnitude of variation in *gca* effects suggested that in both initial populations it is possible to detect parents with high breeding values for the traits under study. An important finding was that good *gca* parents were found within eastern white pine, not only for growth but for blister rust resistance, as well.

Narrow sense heritability estimates at the hybrid family level suggested that the tested traits were under moderate to high genetic control over all ages.

The within trait age-age genetic correlations suggested that selection for blister rust resistance and height growth at an early age should result in high improvement of the respective traits, at age 20.

Because of the weak trait-trait correlations, at all ages, between growth and blister rust resistance, no tandem selection can be applied.

The estimated genetic gains indicated that planting *P. strobus* x *P. peuce* F_1 hybrids in operational planting programs seems to be promising

Acknowledgements

The authors wish to acknowledge the substantial technical assistance of S. Tanasie, A. Dragila and C. Dinu. Also, we express our gratitude to Professor Scott Schlarbaum from the University of Tennessee and to Dr. Richard Sniezko from the Dorena Genetic Resource Center, Oregon, who reviewed an early draft of this paper and made useful suggestions. Also, the authors acknowledge the thorough comments of the two, unknown for us, reviewers.

References

Anderson, R. L. and Bancroft, T. A. 1952. Statistical theory in research. McGraw-Hill Book Co., New-York.

Becker, W. A. 1984. Manual of procedures in quantitative genetics. Fourth Edition. Acad. Enterp. Pullman, Washington, 190 p.

Bingham, R. T., Squilaace, E. A. and Duffield, J. E. 1953. Breeding blister rust resistant western white pine. J. For. 51:161-163.

Bingham, R. T. 1972. Artificial inoculation of a large number of *Pinus monticola* seedlings with *Cronartium ribicola*. In: Bingham, R. T. and others, eds. Biology of rust resistance in forest trees; Proceedings of a NATO-IUFRO Advanced Study Institute, 1969 August 17-24; USDA Forest Service, Misc. Publ. 1221: Washington: 357-372.

Bingham, R. T. and Gremmen , J. 1971. A proposed international program for testing white pine blister rust resistantance. Eur. J. Path. 1: 93-100.

Blada, I. 1982. Relative blister-rust resistance of native and introduced white pines in Romania. In: Heybroek H. and others, eds. Resistance to diseases and pests in forest trees: Proceedings; PUDOC: Wageningen, The Netherlands, 415-416.

Blada, I. 1987. Genetic resistance to *Cronartium ribicola* and height growth in some fine needle pines and some interspecific hybrids. Ph D Thesis, ASAS: Bucharest 146 p.

Blada, I. 1989. Juvenile blister-rust resistance and height growth of *Pinus strobus* x *P.peuce* F_1 hybrids. Silvae Genetica 38 (2): 45-49.

Blada, I. 1990. Blister-rust in Romania. Eur. J. Path. 20: 55-58.

Blada, I. 1992. Nuclear and extranuclear genetic effects in F1 reciprocal hybrids between *Pinus strobus* and *P.peuce*. *Silvae Genetica* 41 (1): 34-38.

Blada, I. 1994. Interspecific hybridisation of Swiss Stone Pine (*Pinus cembra L.*). Silvae Genetica 43 (1): 14-20.

Blada, I. 2000a. Genetic variation in blister-rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17 (Experiment 1). Forest Genetics (7): 109-120.

Blada, I. 2000b. Genetic variation in blister-rust resistance and growth traits in *Pinus strobus* x *P. peuce* hybrid at age 17 (Experiment 2). Silvae Genetica 49 (2): 71-78.

Comstock, R. E. and Robinson, H. F. 1952. Estimation of average dominance of genes. In: Gowen, J. W. (Ed.) Heterosis, Iowa State Coll. Press, Ames. 522 p.

Falconer, D. S. 1981. Introduction to quantitative genetics. Longman and Co., New York: 340 p.

Franklin, E. C. 1979. Model relating levels of development of four North American

Grafius, J. E. and Wiebe, G. A. 1959. Expected genetic gain in yield in small grain. A geometrical interpretation. Agron. Jour. 51: 560-562.

Griffing, G. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Austral. J. Biol. Sci. 9: 463-493.

Hallauer, A. R. and Miranda, F. O. 1981. Quantitative genetics in maize breeding. Iowa State University, Ames, 468 p.

Heimburger, C. 1972. Relative blister rust resistance of native and introduced white pines in eastern North America. In Bingham, R. T. and others (eds.), Biology of rust resistance in forest trees; Proceedings of a NATO-IUFRO Advanced Study Institute, 1969 August 17-24; USDA Forest Service, Misc. Publ. 1221: Washington.

Kriebel, H. B. 1973. Methods for germinating seed of five needle pines. IUFRO Instructions, 2 p.

Kriebel, H. B. 1983.Breeding white pine : a world wide perspective. For. Ec. And Manag. 6: 263-279.

Leandru, L. 1982: Hibrizi interspecifici la pin. ICAS Bucuresti, Seria II, 78 p.

Leppik, E. E. 1967. Some viewpoints on the phylogeny of rust fungy. VI. Biogenic radiation Mycologia 59: 568-579.

Mac Key, J. 1976. Genetic and evolutionary principles of heterosis. *Eucarpia* 7: 17-33.

Namkoong, G.; Kang, H. C. and Brouard, J. S. 1988. Tree breeding principles and strategies. Springer-Verlag, New York: 180 pp.

Patton, R. T. 1966. Interspecific hybridization in breeding for white pine blister rust resistance. In: Gerhold, H. D. and others (eds.), Breeding pest resistant trees. Perg. Press, Oxford.

Radu, S. 1974. Cultura si valorificarea pinului strob. Editura Ceres, Bucuresti. 304 p.

Riker, A. J., Kouba, T. F., Brener, W. H. and Byam, L. E. 1943. White pines selections tested for resistance to blister rust. J. For. 41: 753-760.

Zsuffa, L. 1979. The genetic improvement of eastern white pine in Ontario. In: Scarrat, J. B. (ed.), Prrocc. Tree improv. symp. COJFRC Publ. 0-P-7: 153-160.

Zsuffa, L. 1981. Experiences in breeding *P. strobus* for resistance to blister rust. In: Proc. 17-th IUFRO World Congress, Kyoto, 181-183.

Zobel, B. and Talbert, J. 1984. Applied forest tree improvement. John Wiley and Sons, New York, 505 p.

Field Resistance to *Cronartium ribicola* in Full-Sib Families of *Pinus monticola* in Oregon

Richard A. Sniezko
Bohun B. Kinloch Jr.
Andrew D. Bower
Robert S. Danchok
Joseph M. Linn
Angelia J. Kegley

Abstract—Two field sites were established between 1968 and 1974 using canker-free western white pine seedlings from full-sib families previously inoculated with white pine blister rust (*Cronartium ribicola*) at Dorena Genetic Resource Center. Many individuals planted on these sites had been identified as the resistant segregants for a major gene for resistance (Cr2). However, a strain of rust with specific virulence (vcr2) to this gene has been found at high frequency at and near these sites. In 1997, 27 and 92 families had surviving individuals at Blodgett Creek (BC) and Grass Creek (GC), respectively. Most of the trees on both sites were infected (99.1 percent at BC; 92.5 percent at GC). Despite heavy incidence of infection, there was striking variation in its intensity. Individual trees ranged from 0 to more than 200 cankers, and families also varied dramatically. Many of the trees at both sites continue to grow well, despite heavy infection. Wide variation in infection frequency and survival among and within families on these sites demonstrates that even the earliest selections from the program possess mechanisms of resistance other than Cr2.

Key words: white pine blister rust, field resistance, western white pine, virulence

Introduction

Since its introduction to western North America near Vancouver, B.C. in 1910 (Mielke 1943), white pine blister rust (*Cronartium ribicola* J. C. Fisch.) has caused widespread damage and mortality to western white pine (*Pinus monticola* Dougl. ex D. Donn) and other five-needle pines. In the mid-1950s, the USDA Forest Service began an operational breeding program for blister rust resistance in Oregon and Washington (USDA Forest Service Region 6) to produce seedlings of western white pine for reforestation. The development of resistant populations of western white pine through breeding was seen as the best avenue for re-establishing this species. The early program included tree selection in the field, controlled pollination of selections, and artificial inoculation of the progeny.

Many of the early field selections for which progeny tests showed the most dramatic genetic resistance came from candidate trees in two areas in Oregon. The area with the highest frequency of canker-free trees was a natural second-growth stand dating from approximately 1920 in the Champion Mine area on the Cottage Grove Ranger District of the Umpqua National Forest in the Western Cascade Range. The second source of highly resistant parent trees was a plantation established with seedlings of unknown origin between 1916 and 1935 in the Bear Pass area on the Sweet Home Ranger District of the neighboring Willamette National Forest.

Survival of the parent trees at Champion Mine and Bear Pass after repeated natural epidemics was due primarily to a single dominant gene (Cr2) for resistance (Kinloch and others 1999). However, a new strain of rust appeared in the Champion Mine area around 1970 (McDonald and others 1984). Trees formerly free of infection became heavily infected. By 1994, all resistant parent trees in the Champion Mine area were dead from rust. Many of the resistant trees in the Bear Pass area still show no infection even though a low frequency of the virulent strain (vcr2) has recently been detected in this area (unpublished data).

Few long-term plantings (25 years or more) have tracked occurrence of blister rust in individual resistant families of western white pine. The plantings at Blodgett Creek (BC) and Grass Creek (GC) were primarily established with canker-free survivors of full-sib families after artificial inoculations (geographic origins are indicated in fig. 1). Soon after planting BC and GC, budgetary constraints and personnel departures resulted in their virtual abandonment. In 1996 and 1997 BC and GC were remonumented and assessed for survival, growth, and incidence of blister rust. This paper reports on the status of these two plantings following 23 to 29 years of exposure to blister rust.

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Richard A. Sniezko, Robert S. Danchok, Joseph M. Linn, and Angelia J. Kegley are with the USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, Oregon, 97424, U.S.A., Phone (541) 767-5700, Fax (541) 767-5709, Email: rsniezko@fs.fed.us. Bohun B. Kinloch Jr. is with the USDA Forest Service, Pacific Southwest Research Station, Box 245, Berkeley, California, 94701, U.S.A. Email: bkinloch@fs.fed.us. Andrew D. Bower is with the University of British Columbia, Forest Sciences Department, Vancouver, B.C. V6T 1Z4 CANADA Email: adbower@interchange.ubc.ca.

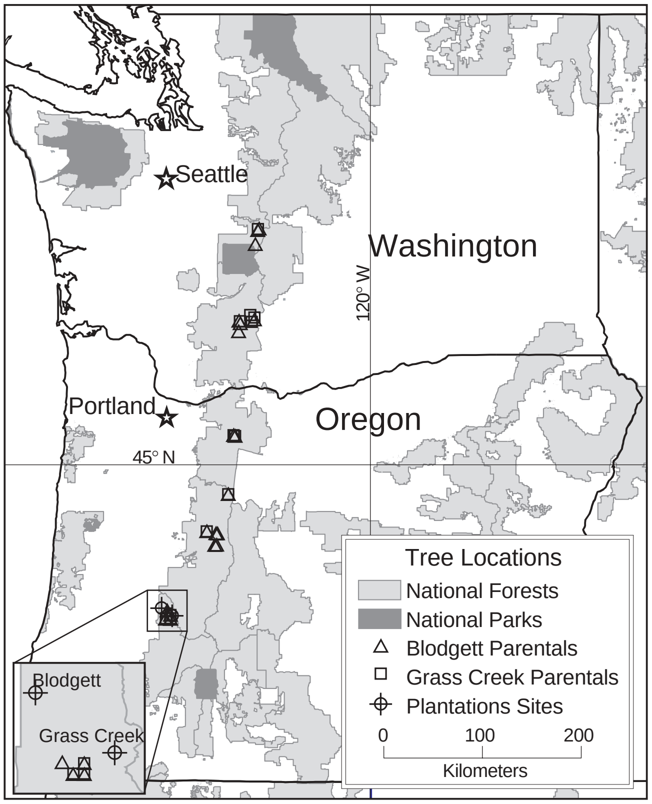


Figure 1—Geographic distribution of western white pine parent trees represented at experimental plantings at Blodgett Creek and Grass Creek. Insert represents plantations in Champion Mine area.

Material and Methods

The BC and GC plantings were established between 1968 and 1974 with family row plots or blocks consisting primarily of healthy, canker-free seedlings from families that had been artificially inoculated and assessed for blister rust infection in the Region 6 program (table 1). The seedling families had been inoculated two to three times and assessed for stem symptoms. The seedlings planted at BC were from two different sowings: 1968 and 1969. Seedlings from both sowings were artificially inoculated two times with *C. ribicola* by suspending *Ribes* sp. leaves bearing mature telia over them in a moist enclosure at ambient temperatures. Seedlings planted at GC from sowings in 1959, 1962, 1963, and 1964 were artificially inoculated three times, and the seedlings at GC from the 1965 and 1966 sowings were inoculated twice. Seedlings were held for several years after the last inoculation, and age at planting varied. The number of trees planted per family varied depending upon results from the artificial inoculation. A few noninoculated families were also included.

The Blodgett Creek site is on a low elevation, relatively flat bench area and appears to have a higher exposure to rust than the Grass Creek site, which is on a steep, high elevation, south-facing slope. Both sites are within 10 miles of the Champion Mine area, where many of the parents originated, and where a strain of rust virulent to Cr2 was first identified (McDonald and others 1984, Kinloch and others 1999).

A total of 62 families (1,726 seedlings total) from 65 parents with one to 90 trees per family were planted at BC in late 1973 and early 1974. A total of 120 families (3,751 seedlings total) from 82 parents with one to 180 trees per family were represented in three plantings (1968, 1970, and 1972) at GC (table 1). The majority of families at both sites were full-sibs from controlled crosses made among 120 ortets in natural stands. The parents represented at BC came from four National Forests: Umpqua, Willamette, Mt. Hood, and Mt. Baker-Snoqualmie (fig. 1); parents at GC were predominantly from the Umpqua, Willamette, Mt. Hood, and Mt. Baker-Snoqualmie, with a few selections from BLM lands and an unknown source from Idaho.

Data on survival after first growing season were available for all plantings at the BC site and for the first (1968) planting at GC (table 2). After extensive remonumentation of each site in 1996, every identifiable tree (living and dead) was assessed for survival, diameter, and frequency and location (bole versus branch) of rust infection. When possible, the presence of cankers was determined for dead trees. Frequency of infections was assessed using a scale of 0 to 6 that was geometric, rather than arithmetic, in which each

Table 1—Establishment and background information for two plantings of western white pine in Oregon.

	Blodgett Creek	Grass Creek
Latitude	43.678° N	43.601° N
Longitude	122.718° W	122.580° W
Elevation	2250 ft (690 m)	3800 ft (1160 m)
Aspect	Southwest	South
Topography	Mostly flat with some areas sloped 5-35%	Slopes 5-45% with several flat bench areas
Distance from Champion Mine (km)	~8 miles (12.8 km) NW	~3 miles (~4.8 km) ENE
Year(s) Established	1973, 1974	1968, 1970, 1972
Number of Families Planted	62	120
Total Number of Trees Planted	1726	3751
Families Surviving in 1997	27	92
Trees Remaining in 1997	404	1579

Table 2—Summary of mean survival, growth and rust status of two plantings of western white pine in Oregon.

	Blodgett Creek	**Grass Creek**
First year survival	41.2%	n/a
Total survival in 1997	332 trees (19.2%)	974 trees (26.0%)
Survival in 1997 as % of first year survival	46.7%	41.1% (1968 planting) n/a (1970 and 1972 plantings)
Identifiable trees in 1997	404 total 313 healthy (77.5%) 19 sick or dying (4.7%) 33 dead <5 years (8.2%) 39 dead >5 years (9.7%)	1579 total 755 healthy (47.8%) 219 sick or dying (13.9%) 190 dead <5 years (12.0%) 395 dead >5 years (25.0%) 20 dead, rust status unknown (1.3%)
Mean diameter	19.9 cm	16.3 cm (overall) 17.9 cm (1968 planting) 18.3 cm (1970 planting) 15.2 cm (1972 planting)
Mean canker class per tree[a]	4.33	3.60 (overall) 4.70 (1968 planting) 3.09 (1970 planting) 3.30 (1972 planting)
Range of family mean canker class	1.67-5.60	0-6
Canker-free trees	3 (0.4%)	73 (6.3%)

[a] Frequency of infection was assessed for each tree using a scale of 0 to 6. Trees in class 0 had 0 cankers; class 1, 1-3; class 2, 4-9; class 3, 10-21; class 4, 22-50; class 5, 51-100; class 6, >100.

succeeding class interval was approximately double that of the preceding class interval. Trees in canker class (CCL) 0 had no cankers; class 1 was (arbitrarily) set at 1 to 3 cankers; class 2, 4 to 9; class 3, 10 to 21; class 4, 22 to 50; class 5, 51 to 100; class 6, greater than 100. Because of the long intervals between establishment and assessment, lack of a replicated design, and nonscalar measurement of cankers/tree, no formal statistical analyses were possible.

Results

The 1997 survey of the two sites indicated that 99.1 and 92.5 percent of the living trees have blister rust infections at BC and at GC, respectively. The mean CCL per living tree was 4.33 at BC (approximately 62 cankers) and 3.60 at GC (approximately 42 cankers) (table 2). Family mean CCL ranged from 1.67 to 5.60 at BC and from 0 to 6 at GC (fig. 2), but very few families were in CCL 2 or lower (less than 10 cankers). The number of canker-free trees was greater at GC (73) than at BC (3) (table 2).

Mean diameter (at 1.3 m) of surviving trees was 19.9 cm and 16.3 cm at BC and GC, respectively (table 2). Overall survival (including mortality within the first year following planting) was 19.2 percent at BC and 26 percent at GC. Survival varied by family (fig. 3). Some of the families with the highest survival (greater than 50 percent) differed dramatically in numbers of cankers per tree; this is also true for trees within families (fig. 4a – d for examples). When first-year mortality is excluded, survival was 46.7 percent at BC and 41.1 percent for the 1968 planting at GC (table 2).

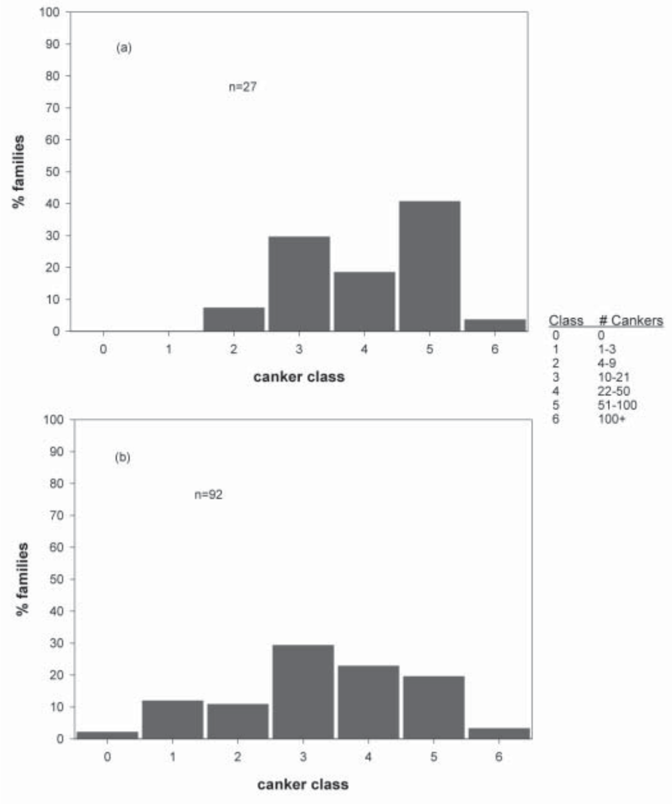


Figure 2—Distribution of family mean canker class (based on numbers/tree of individual trees) of 1997 survivors at (a) Blodgett and (b) Grass Creek.

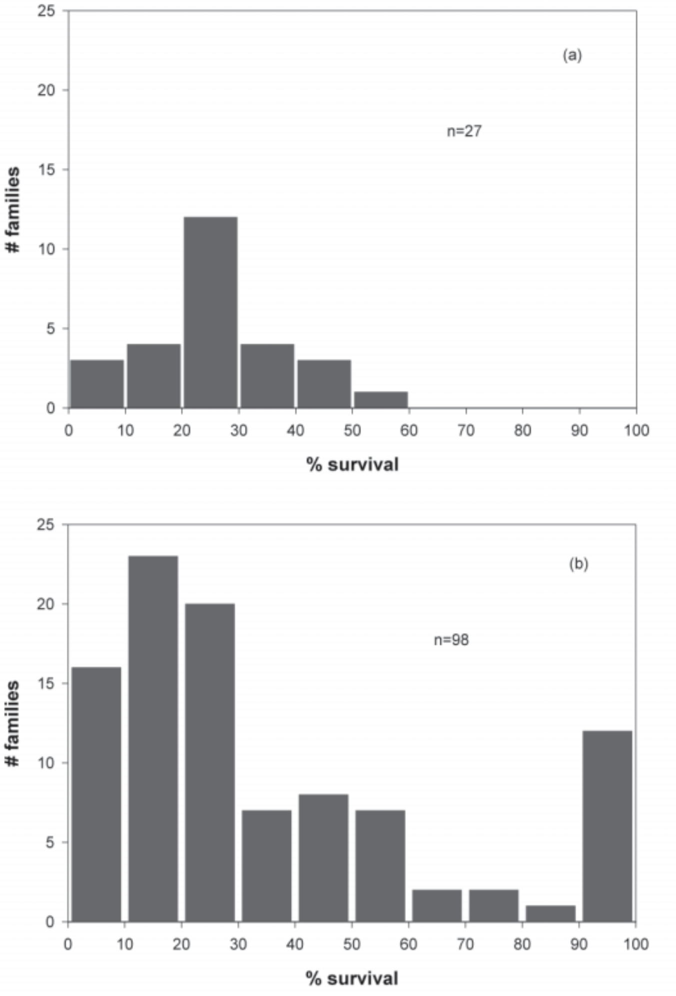


Figure 3—Distribution of family means for percent survival from establishment at (a) Blodgett and (b) Grass Creek

Recent mortality was greater at GC (38.3 percent) than at BC (17.9 percent) (table 2).

Atropellis canker (caused by *Atropellis* sp.) was present on trees at both sites (14 trees at BC and 189 trees at GC); some families at GC had more than 50 percent of living trees with *Atropellis*. Care was taken to distinguish this disease from white pine blister rust.

Blodgett Creek

In 1997, 332 of the 404 identifiable trees (82 percent) were still alive, with 27 of the original 62 families still represented (with one to 31 surviving trees per family). Survival from time of planting for these 27 families was low to moderate (fig. 3a) but higher if first-year mortality was excluded. Excluding first-year mortality, survival varied from 18.8 to 85.7 percent, with an overall mean of 46.7 percent. Survival in the past 10 to 15 years was generally high for all families. Of the 72 dead trees, 33 appeared to have died within the previous 5 years (table 2).

The majority of living trees had more than 22 cankers (CCL greater than 3), and more than 20 percent of the trees had over 100 cankers (fig. 5a). On a family mean basis, the number of cankers per tree varied from five to more than 100 (fig. 2). Only three trees were canker-free on this site. Of the living trees, 73 percent had both bole cankers and branch cankers, and no trees had only bole cankers. A small percentage (5.1) of trees had only branch cankers that were relatively new (less than 5 years old), while most trees (82.5 percent) had both recent and old branch cankers. Only six trees had all cankers dead or inactive, and these were also the six trees with the fewest cankers. The two families largest in diameter had among the fewest cankers (fig. 6a).

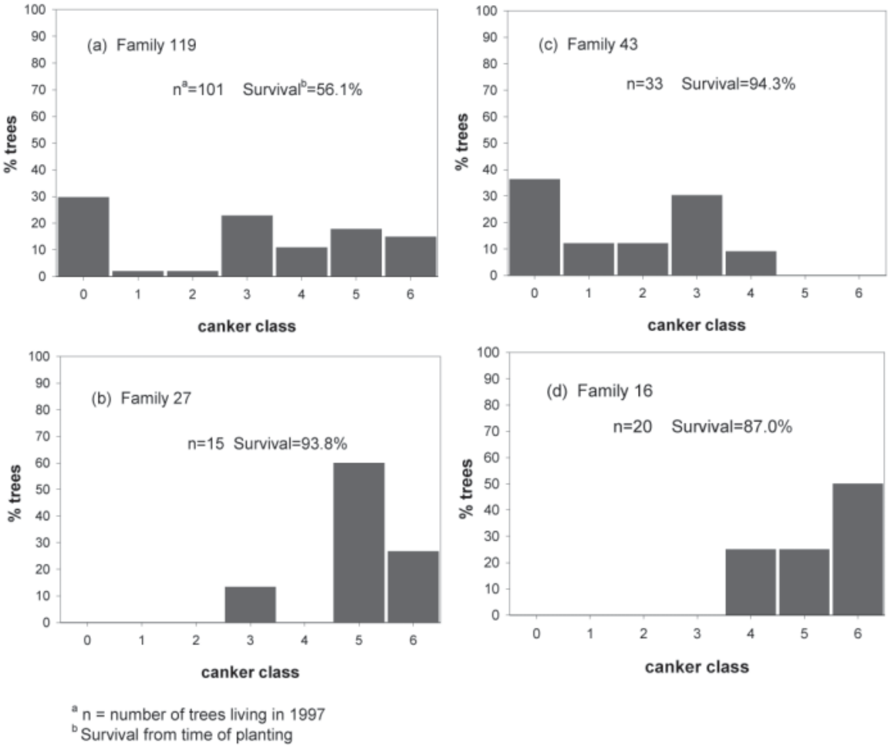
Grass Creek

In 1997, 974 of 1579 remonumented trees were still alive, and 98 of the 120 families were still represented by identifiable living and dead trees (with one to 101 trees per family still alive). Survival from time of planting was only 26.0 percent, but some families had 100 percent survival (fig. 3b); survival in the past 10 to 15 years varied widely by family and somewhat by planting year but was high for many families. Nearly one-third (190 of the 605 trees) of the mortality recorded in 1997 appears to have occurred within the previous 5 years (table 2).

Trees in the 1968 planting averaged more than twice the number of cankers as the 1970 and 1972 plantings (table 2 and fig. 5b-d). Over 50 percent of trees in the 1968 planting had more than 50 cankers (CCL 5 and 6), while less than 15 percent of the trees in any of the three plantings were canker-free (fig. 5b-5d). Most families averaged CCL 3 or higher (minimum of 10 cankers/tree) (fig. 2b). Of the living trees, 63 percent had both bole and branch cankers, while only two trees had bole cankers only. Few trees (64) had only branch cankers that were relatively recent; most had old and recent branch cankers (82.2 percent). All cankers appeared to be dead or inactive on 15 trees. Overall, there was no strong relationship between diameter and number of cankers (fig. 6b).

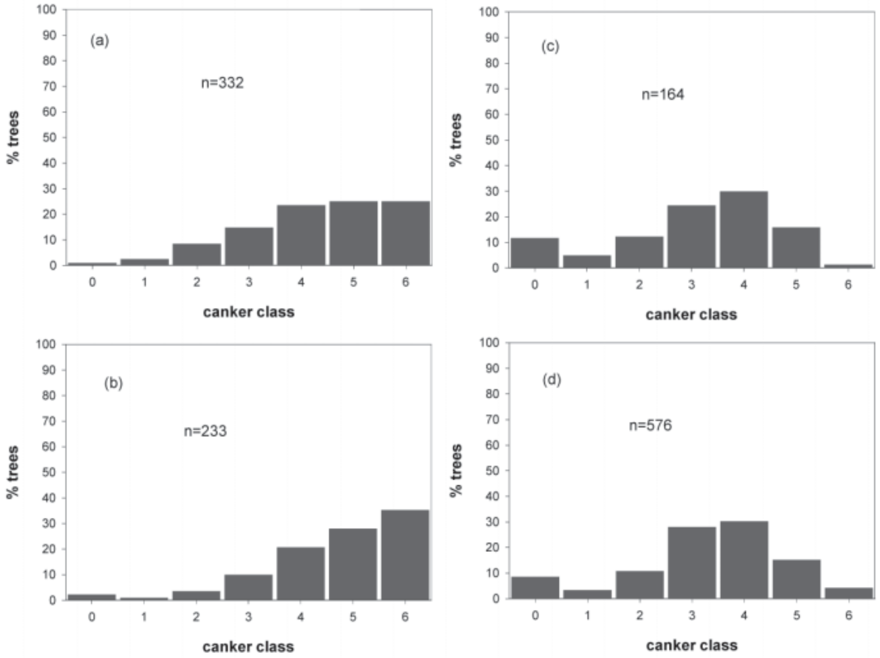
A total of 73 trees, coming from 21 different families, at this site were canker-free, with most of these being in the 1970 and 1972 plantings (fig. 5b-d). These families had from one to 30 trees canker-free, and where more than 15 trees had been planted, the family mean percentage of canker-free trees was generally low (less than 10 percent). Family 103 (18034-374 x 18034-391), which originally had only three trees planted, still had 100 percent survival, and no cankers were apparent in 1997. Of six trees planted in Family 117 (06020-501 x 06020-511), the two survivors were canker-free. Family 43 (18034-395 x 18035-386) had 33 of the original 35 (94.3 percent) trees planted surviving in 1997, and 12 of these (36.3 percent) were canker-free (fig. 4c). Follow-up visits (after 1997) detected a canker in one of the trees in Family 103, and branch cankers are also present on all of the formerly canker-free trees in Family 43.

One parent, 15040-836, was involved in six of the eight crosses with highest survival; overall it was used in 18 crosses at GC. GC Family 16 (15045-835 x 15045-836) had high survival despite the presence of many cankers (fig. 4d). Another interesting parent is 06020-511 from Mt. Hood National Forest. At BC, 06020-511 was the female parent in



Class	# Cankers
0	0
1	1-3
2	4-9
3	10-21
4	22-50
5	51-100
6	100+

Figure 4—Canker class distribution (1997 assessment) of living trees for two relatively resistant (a, c) and two susceptible (b, d) families at Grass Creek.



Class	# Cankers
0	0
1	1-3
2	4-9
3	10-21
4	22-50
5	51-100
6	100+

Figure 5—Canker class distribution of trees alive in 1997 at (a) Blodgett plantings established in 1973-1974 and Grass Creek plantings established in (b) 1968, (c) 1970 and (d) 1972.

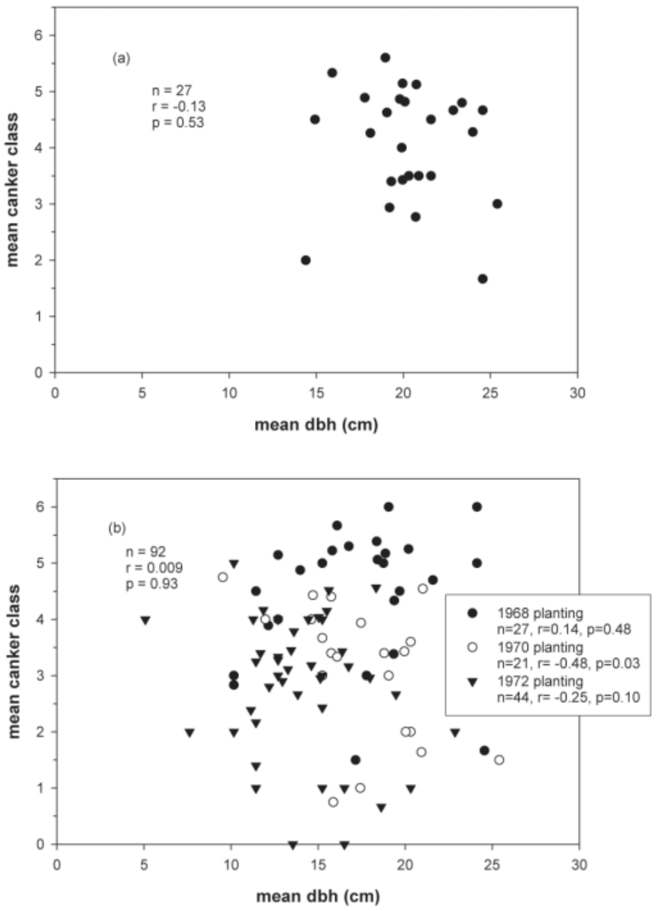


Figure 6—Family mean canker class per living tree versus diameter (dbh) at (a) Blodgett and (b) Grass Creek.

two crosses and the male parent in two crosses. At GC, 06020-511 was represented in three families, twice as the male parent and once as an apparent self (Family 119). GC Family 119 had 180 seedlings planted; in the 1997 inventory, 101 of these trees were still alive, of which 30 (29.7 percent) were canker-free (fig. 4a). Many other trees in this family appeared vigorous despite having large, old bole cankers. Although only one of the two families at BC with 06020-511 as the female parent had surviving trees in 1997, this family had the lowest mean canker class (1.67) and the second largest mean DBH (24.6 cm) (fig. 6a).

Discussion

The high level of rust infection at these sites was somewhat unexpected since most of the seedlings were canker-free after heavy artificial inoculation. However, recent investigations make it apparent that the main cause of the high level of infection is the presence of a virulent strain of rust (Kinloch and others 1999, Kinloch and Dupper 2002). Many of the parents represented in the planting are now known to carry a specific gene for resistance (Cr2) that is neutralized by a corresponding gene for virulence (vcr2) in the pathogen in a gene-for-gene relationship (Kinloch and others 1999, Kinloch and Dupper 2002). High inoculum loads and intense selection pressure caused a sudden and dramatic increase in vcr2 in these plantations.

The families planted on these sites were some of the first selections made in the Region 6 program (Kinloch and others 1999). Despite a relatively narrow genetic base among these early families, wide variation in infection frequency and survival of trees on these sites indicates that non-Cr2 resistance is also present. This became apparent only after Cr2 was neutralized by vcr2, thereby unmasking independent, unrelated mechanisms. These mechanisms are forms of partial resistance that reduce infection rate or allow the tree to survive after infection. Similar results have been reported for progenies of sugar pine (*P. lambertiana* Dougl.) with a different major gene for resistance to blister rust (Cr1). After many years exposure to a strain of rust (vcr1) with specific virulence to Cr1, most of the sugar pine trees were killed, but a significant number exhibited mechanisms of partial resistance unrelated to Cr1 that enabled them to survive and in many cases heal (Kinloch and Davis 1996). One of these mechanisms included infection frequency differences of a similar magnitude to those observed at BC and GC.

Wave years of infection occurred frequently at both sites after plantation establishment. Many trees have dozens or more cankers but are still showing vigorous growth. Some individuals have large bole cankers that have been present for over two decades (an indication of tolerance), for example, family '119' at GC. One reason many of these trees are still alive is that most of the cankers at these two sites are branch cankers more than 0.5 m from the main stem and so are unlikely to reach the bole. These two plantings will continue to be monitored to see if the cumulative blister rust impacts over time lead to mortality directly (bole girdling) or indirectly (crown thinning or predisposition to other agents such as bark beetles), and whether the resistance is durable. From a silvicultural point of view, it is encouraging to see trees that still thrive after nearly 30 years of intense white pine blister rust exposure.

Due to the gap in data collection between trial establishment and the 1997 assessment as well as the substantial first-year postplanting mortality, it is not possible to clearly delineate all of the resistance mechanisms that might be present. Although the physiological basis and inheritance of additional mechanisms is unknown, at least several phenotypes have been documented: canker-free, low infection frequency (fewer than average cankers present), tolerance (vigorous tree with large bole cankers), and bark reaction (healed or inactive cankers present). The gap in data limits some of the specific information that could have been garnered for each family, such as for small, ephemeral bark reactions.

These two sites represent some of the earliest field plantings of the first resistant trees produced by the Region 6 western white pine blister rust resistance program. Although only canker-free seedlings were deployed, survival of these trees over nearly 30 years of exposure to a virulent strain of rust can be attributed to partial resistance mechanisms. These mechanisms may provide the foundation for establishing durable resistance in future generations of western white pine. Collections of wind-pollinated lots from several trees at BC and GC have been made, and these seedlots have been included in recent rust-screening trials.

Since these two sites were established, progeny of thousands of parent trees have been screened for a more diverse set of putative resistance mechanisms (Sniezko 1996), and replicated field validation tests have been established (Sniezko and others this proceedings, Sniezko and others 2000). Modifications to the operational screening program continue as new information on resistance mechanisms becomes available. Resistant seed from the breeding program will be used to help restore and maintain western white pine as a valuable component of the forest ecosystems in Oregon and Washington.

Acknowledgments

The authors acknowledge Gerry Barnes (Dorena Center Manager from 1966-1977) whose first-hand knowledge of the history of early screening and establishment of these stands was invaluable, and Paul Zambino for his review of an earlier version of this paper. We thank those involved in establishing these plantings, including Cliff Woody, Ted Degerstrom, Ron Myers, Mack St. Clair, Gero Mitchelen, Roland Fergason, Ernie Degerstrom, and Marge Janisch as well as the USDA Forest Service's forest health protection group for funding key portions of this work.

References

Kinloch, Bohun B., Jr. and Davis, Dean. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Res. Publ. 3362.

Kinloch, Bohun B. and Dupper, Gayle E. 2002. Genetic specificity in the white pine-blister rust pathosystem. Phytopathology 92:278-280.

Kinloch, B.B., Jr., Sniezko, R.A., Barnes, G.D., and Greathouse, T.E. 1999. A major gene for resistance to white pine blister rust in western white pine from the Western Cascade Range. Phytopathology 89:861-867.

McDonald, G.I., Hansen, E.M., Osterhaus, C.A., and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicol*a from the Cascade Mountains of Oregon. Plant Disease 68: 800-804.

Mielke, J.L. 1943. White pine blister rust in North America. Yale University School of Forestry. Bulletin 52. 155 pp.

Sniezko, R.S. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Natural Res. Publ. 3362.

Sniezko, R.A., Bower, A., Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance in sugar pine and western white pine. HortTechnology 10(3): 519-522.

Influence of Seedling Physiology on Expression of Blister Rust Resistance in Needles of Western White Pine

Kwan-Soo Woo
Geral I. McDonald
Lauren Fins

Abstract—Growth conditions for nursery-grown western white pine seedlings have been shown to affect levels of blister rust infection (from *Cronartium ribicola).* In an experiment initially designed to test the influence of environmental conditions at two nurseries in northern Idaho on the blister rust pathosystem, western white pine seedlings of a single resistant seedlot were unintentionally held in cold storage for 6 months longer at one nursery than at the other. Inoculation of these long-stored seedlings with blister rust spores occurred at 1 month after growth resumed under nursery conditions, versus 7 months for those with shorter storage. Infection percent was nearly double and infection efficiency (infections per unit area of stomata) was 70 times greater on the seedlings with only 1 month of growth than on the seedlings with the more mature foliage. Since the seedlings had originated from the same genetic source, the overwhelming difference suggests that phenology and/or nursery regimes can strongly influence infectability of seedling needles in western white pine using artificial inoculations. If phenology is the key factor, it may help explain why infection levels have been relatively high on northern Idaho resistant selections when grown at milder locations. Furthermore, if resistance genes can be selectively activated by manipulating phenology, molecular tools that examine gene expression might be employed to enhance our understanding of environmental regulation of genes for blister rust resistance.

Key words: *Cronartium ribicola*, blister rust pathosystem, infection efficiency, phenology.

Introduction

Western white pine's (*Pinus monticola* Dougl.) susceptibility to the blister rust fungus (*Cronartium ribicola* J.C. Fisch. in Rabenh.) has been shown to vary with tree age, type, and age of needles, and age of the shoot (Lachmund 1933; Pierson and Buchanan 1938; Bingham 1972; Hunt 1991). But the environment in which seedlings are reared may also influence their susceptibility to infection. This possibility became apparent when, at three years postinoculation with *C. ribicola* spores, 47.6 percent of the seedlings grown in one nursery in northern Idaho were dead, compared to only 29.8 percent mortality of the seedlings grown in a second Idaho nursery. The result was the reverse of expectations because the seedlings grown in the second nursery were from a previously untested seed orchard, which was later determined to have low rust resistance compared to those grown in the first nursery. It appeared that, in addition to genetic variation, factors associated with nursery location and/or growing regimes had influenced “susceptibility,” or perhaps “infectability” of the seedlings to *C. ribicola* (Eramian and Foushee, personal communication).

If rust resistance levels do vary as a function of growth environment, then estimates of resistance levels of the same genetic stock may vary widely depending on the nursery regime in which the stock is grown, or between stocks placed in field environments and their nursery-grown counterparts. Such variation in estimated resistance would imply a need to refine nursery rearing and testing protocols such that they produce comparable test results that are reliable predictors of long-term resistance levels under a variety of field conditions.

The original objective of the study reported here was to address this issue and test the hypothesis that blister rust infection levels in western white pine seedlings are indeed influenced by differences in nursery growing environment. We began by growing seedlings of the same genetic stock in two nurseries in northern Idaho, with the intent of following their routine protocols and exposing the seedlings to blister rust spores at the end of their second growing season after germination. However, an unexpected physiological difference was induced between the stocks when the seedlings at one of the nurseries were accidentally kept in cold storage six months longer than their counterparts at the second nursery. As environmental conditions during inoculation and/or disease development can influence the expression of blister rust-resistance genes in white pines, we

---

In: Sniezko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Kwan-Soo Woo is with the Tree Breeding Division, Forest Research Institute, Korea Forest Service // Suwon, Omokchundong 44-3, Kyonggido, 441-350, South Korea. Telephone: 82-(0)31-290-1106; Fax: 82-(0)31-292-4458, e-mail: woo9431@yahoo.co.kr. Geral I. McDonald is with the USDA Forest Service, Rocky Mountain Research Station, 1221 South Main Street, Moscow, ID 83843, U.S.A. Telephone: (208) 883-2343; Fax: (208) 883-2318, e-mail: gimcdonald@fs.fed.us. Lauren Fins is with the Department of Forest Resources, University of Idaho, Moscow, ID 83844-1133, U.S.A. Telephone: (208) 885-7920; Fax: (208) 885-6226, e-mail: lfins@uidaho.edu.

recognized the possibility that the physiological differences induced by the accidental extension of cold storage might also have important and potentially profound effects on expression of blister rust resistance in the seedlings (Yokata 1983; Bower 1987; Hunt and Meagher 1989; McDonald and others, this volume). As a measure of the relative infectability and early expression of resistance in the two stocks by *C. ribicola*, we compared their levels of needle infection at 5 months postinoculation. Subsequent mortality from rust was not evaluated.

Materials and Methods

Plant Material and Artificial Inoculation

Western white pine seedlings from a single genetic source (blister rust-resistant F_2 from the R.T. Bingham White Pine Seed Orchard in Moscow, ID, as described in Hoff and others 1973) were grown for two growing seasons at two nurseries in northern Idaho: Potlatch Corporation's Nursery in Lewiston, and the University of Idaho Forest Research Nursery in Moscow, hereafter referred to as the Lewiston and Moscow nurseries. The seedlings from both nurseries were inoculated with spores of *C. ribicola* at the USDA Forest Service Nursery in Coeur d'Alene, ID, in September 1999 using the routine procedures described in Mahalovich and Eramian (1995). The rust inoculum was collected from an established *Ribes* garden that had been inoculated with aeciospores collected from blister rust cankers in locations in Idaho, western Montana, and eastern Washington. Spore cast was monitored on slides that were placed at regular intervals among the seedlings.

At the time they were inoculated, the seedlings from the Moscow Nursery were about 36 cm tall and had calipers of 0.6 to 0.8 cm. Seedlings from the Lewiston Nursery, which had inadvertently been left in cold storage from December 1998 to early August 1999, were physiologically immature and substantially smaller, measuring about 22 cm in height, with a caliper of 0.3 to 0.5 cm, and had short (but expanding) secondary needles. We entered 200 seedlings into this study (25 per nursery in each of four replications). Nursery groups were randomized within replications, with one plot per nursery per replication.

In the months following inoculation, the seedlings were irrigated but no nutrients were supplied. At 5 months postinoculation, the seedlings were inspected for needle spots as a measure of successful penetration by basidiospore germ tubes. Overall seedling survival was 98.5 percent (197/200.) Needle spots were counted on all needles. Infection efficiency (IE) was calculated as:

[1] IE = spots per seedling / infective target area per seedling / spore cast

Infection efficiency was determined for each seedling and averaged for each nursery (McDonald and others 1981, 1991). The number used for spore cast in this equation was determined the by spore count on a monitoring slide that was closest to the seedlings during the inoculation process. Infective target area (ITA) (cm^2/seedling) was estimated as:

[2] ITA = SR x N x L (cm) x 0.01 (cm)

where SR= average number of stomatal rows on two adaxial sides per needle, N= number of needles per seedling, and L= average length of stomatal row (estimated by needle length) (McDonald and others 1981). Average number of stomatal rows and needle length were based on samples of six needles per seedling (one from each of six fascicles, two each from the top, middle, and bottom of each seedling). Lesion frequency was calculated as the number of needle spots/infective target area. Subsequent mortality as a function of blister rust infection was not evaluated.

Statistical Analyses

Data were analyzed using the SAS-PROC GLM (general linear model) statistical package and Type III Sums of Squares (SAS Institute Inc. 1989). Data were transformed by arcsine when the exploratory PROC UNIVARIATE test showed they were not normally distributed.

Results

Seedlings from the Moscow Nursery averaged 3.25 spots per seedling (range: 0 to 50) whereas seedlings from the Lewiston Nursery averaged 87.83 (range: 0 to 710) (table 1). Infection efficiency was significantly higher on seedlings

Table 1—Infection efficiency of *Cronartium ribicola* on western white pine seedlings from two nurseries in northern Idaho[a].

Nurseries	Percent infection	Number of spots/seedling	Infective target area (cm^2/seedling)	Spore concentration (spores/cm^2)	Germination (%)	Lesion freq.	Infection efficiency
Lewiston	95	87.83 (11.04)	94.15 (4.30)	5521.20 (232.04)	44.77 (1.42)	1.050 (0.138)	$2.1x10^{-4}$ ($2.9x10^{-5}$)
Moscow	51	3.25 (0.73)	322.36 (11.31)	4465.75 (116.58)	61.39 (1.08)	0.011 (0.002)	$3x10^{-6}$ ($6x10^{-7}$)

[a] Values are means with SE given in parentheses. P-values (shown below) are based on comparisons between samples of seedlings from the Lewiston versus the Moscow nurseries when P<0.05. Needle spots were counted on all needles per seedling (P=0.0009); Infective target area was estimated by average number of stomatal rows on two adaxial sides per needle x number of needles per seedling x average length of stomatal row (estimated by needle length) x 0.01cm (P=0.0037); Spore concentration = total number of spores in ten fields per slide x 60.2 (P=0.6574); Germination % (P=0.003); Lesion frequency = # spots/infective target area (P=0.0025); Infection efficiency = spots per seedling/infective target area per seedling/spore cast (P=0.0105).

from the Lewiston Nursery (mean=$2.1x10^{-4}$) compared to those grown in the Moscow Nursery (mean=$3x10^{-6}$) (P=0.0105). Also statistically significant were differences in the number of spots per seedling (P=0.0009), infective target area (P=0.0037), spore germination percentage (P=0.003), and lesion frequency (P=0.0025). Only five of 98 seedlings from the Lewiston Nursery had no spots, compared to 49 of 99 for the Moscow seedlings. No statistical difference was found between nurseries in spore concentration (P=0.6574).

Compared to the seedlings grown in the Moscow Nursery, seedlings grown in the Lewiston Nursery had a relatively small infective target area (94 versus 322 cm^2/seedling) and, although spore germination percentage was lower on nearby monitoring slides (45 vs 61 percent), the Lewiston Nursery seedlings developed more rust spots per seedling than did those from the Moscow Nursery.

The mean spore concentration (spores/cm^2) for the two stocks at the end of the inoculation period was 4,994 spores/cm^2, ranging from 2,047 to 11,920 spores/cm^2. Mean spore germination was 53 percent (range 25 to 82 percent).

Discussion

Compared to the Moscow Nursery seedlings, the Lewiston Nursery seedlings had 27 times the number of spots and 70 times the infection efficiency (table 1). As the seedlings from the two nurseries had originated from the same genetic source (open pollinated seed from the same seed orchard), and the seedlings were inoculated at the same time and under the same inoculation conditions, observed differences in infection were not likely to be a function of genetic differences or differences in inoculation conditions. The most likely explanation of the observed differences in infection is either nursery cultural practices and/or a difference in developmental and physiological state of the seedlings at the time of inoculation.

As previous attempts to infect white pine seedlings grown in the Lewiston Nursery had resulted in relatively low infection percentages (Foushee, personal communication), the standard nursery practice used at the Lewiston Nursery is not a likely explanation for the observed, relatively high infection levels in this study. However, the Lewiston Nursery seedlings were kept in cold storage for six months longer than the seedlings from the Moscow Nursery and were removed from cold storage only one month prior to inoculation with rust. The seedlings had small needles (that were probably still expanding) and succulent tissues when they were inoculated. These observations suggest that some needle resistance mechanisms may not be fully operational in needles that have not reached their full development within a current growing season.

In addition, the very low frequency of needle spotting on the seedlings from the Moscow Nursery (compared to target levels of greater than 90 percent for routine rust screenings) may indicate a nursery regime that, at least temporarily, protects seedlings from infection. If so, rust screening that includes only artificial inoculations of their seedlings will not reflect actual long-term rust resistance levels under field conditions.

Data on needle spots were not recorded by needle type or location on the seedlings. However, it was clear that most of the observed rust spots appeared on current-year needles that were at the tops of the western white pine seedlings from both nurseries. Although it is possible that this reflects a purely spatial phenomenon, with uppermost younger needles having greater spore deposition than those lower on the stem, our results with *P. monticola* are consistent with previous findings for *P. monticola* and *P. strobus* that current needles are more susceptible to blister rust than older needles (Snell 1936; Van Arsdel 1968; Hunt 1991). Alternatively, physio-mechanical attributes may explain the differences, since stomata in older needles are less active than those of the current-year needles (Hirt 1938).

Bingham (1973) reported that seedling height was significantly related to the frequency of needle spots on nonresistant western white pine seedlings, with taller seedlings more highly cankered than shorter ones. However, this relationship did not hold true for three types of resistant stocks, one of which consisted of a bulk lot of F_2 seedlings similar to those used in our study. In either case, our result differs from Bingham's in that the relatively small seedlings from Lewiston Nursery had, by a large margin, more needle spots than the seedlings grown in the Moscow Nursery.

The average spore germination for our study was 53 percent, considerably lower than the overall average of 73 percent for the 1999 routine inoculations at the Coeur d'Alene Nursery (Eramian, personal communication; data on file at the USDA Forest Service Coeur d'Alene Nursery). Also, spore germination percentages on the slides near the Lewiston seedlings were consistently lower than those near the Moscow seedlings (45 percent versus 61 percent respectively). The reasons for the overall lower spore germination and the differences between samples near the two stock types are not apparent but may be related to variation in microsite associated with seedling size and foliage density.

Target spore deposition at the Coeur d'Alene Nursery is 6,000 to 7,000 spores per square centimeter and 95 percent or higher infection percent (Eramian, personal communication). Thus the mean spore deposition of 5,258 spores per square centimeter was lower than desired, but the 95 percent infection of the Lewiston Nursery seedlings indicates the deposition of spores and the environmental conditions in the inoculation tent were sufficient to achieve a high level of infection in at least one of the groups. The reason for the low infection percentage for the Moscow nursery seedlings is not known.

We found no relationship between needle spot development and either spore concentration or percent spore germination on slide traps (table 1), suggesting that at relatively high spore concentrations, seedling physiology may have more influence on infection efficiency than either spore concentration or percent germination.

An investigation of actual physiological differences between the groups was beyond the scope of this study. However, in other studies, a western white pine protein, *Pin m* III, was found to increase both during winter months and in tissue infected with blister rust (Ekramoddoullah and others 1995; Ekramoddoullah and others 1998). The normal winter increase in the protein was suppressed in rust-infected trees with the slow canker growth resistance mechanism, further suggesting a relationship between *Pin m* III and rust resistance. If the abundance of *Pin m* III is related to rust resistance, and if it is relatively easy to manipulate by subjecting trees to differing lengths of cold storage, the protein may be a useful indicator of desirable or undesirable genotypes for selection in tree breeding programs. This hypothesis is easily tested by subjecting groups of resistant and susceptible seedlings to long versus short cold storage treatments. If there is a relationship only the resistant stocks exposed to normal cold storage period are predicted to show low levels of the protein.

Our results are also consistent with the infection of Japanese stone pine shoots by *Endocronartium sahoanum* (Kaneko and Harada 1995) after cold storage synchronization. Increased susceptibility in these related situations argues for the existence of a physiological cause associated with cold-storage treatment and/or immature tissue.

In our study, seedlings established under different nursery environments displayed different infection levels, but the likely causal factors were confounded and could not be isolated to explain the differences. Physiological and developmental conditions, growing regimes and nursery environment may all have influenced infectability of the study seedlings.

Needle infection was higher on current-year needles than on older needles (magnitude not quantified in this study) but older needles may have been sheltered from inoculum by the newer foliage, or they may have been less physiologically active. The highest infection levels occurred on physiologically immature needles. It appears that some needle resistance mechanisms may not be fully developed in actively growing, nonmature, succulent needles. If true, movement of stock to other climatic conditions, or differences in annual weather patterns under field conditions may influence the effectiveness of some resistance mechanisms, and may, at least in part, account for suspected “wave year” phenomena. However, it is also possible that, in this study, the effect of needle maturation state was confounded by physiological changes associated with the prolonged period of cold storage and/or the nursery growing regime. The “needle immaturity” hypothesis could be tested by inoculating second year seedlings of the same genetic stock at monthly intervals from June through September, or by inoculating only in September using groups of seedlings (of the same genetic stock) that had been subjected to different periods of cold storage.

Our results suggest that expression of genes related to rust resistance in northern Idaho white pine seedlings is sensitive to physiological state as related to phenology and/or to growing regimes that alter needle infectability in the nursery. If phenology is a critical factor, new molecular tools, such as cDNA PCR detection assays and micro arrays may facilitate experiments designed to explore environmental regulation of resistance genes and their association with phenologic traits. If variation in growing regimes is found to critically affect the infectability of seedling needles, then either protocols for evaluating resistance must be fine-tuned to address this variation, or growth regimes and testing protocols should be standardized for test seedlings across nurseries.

Any program that relies on artificial inoculation for evaluating seedlings for rust resistance must also include long-term field tests under a variety of conditions to fully assess resistance and validate the predictions of early screenings. If studies determine that rust infection under field conditions generally tends to be substantially higher than is indicated by current rust screening procedures for tree improvement programs, it may be useful to alter testing protocols to better mimic field conditions, such as including multiple exposures to rust spores, and exposing seedlings to spores earlier, potentially beginning when teliospores are first produced on the *Ribes* leaves. Further study is needed to test these hypotheses and to refine rust screening procedures (and perhaps nursery rearing procedures) that, together, will provide accurate long-term predictions of rust resistance.

Acknowledgments

The authors thank Paul Zambino and Ned Klopfenstein for their thoughtful reviews of the manuscript, Aram Eramian for providing critical baseline information on routine rust screenings at the Coeur d’Alene Nursery, and the Inland Empire Tree Improvement Cooperative for its support of the original study on which the manuscript is based.

References

Bingham, R.T. 1972. Artificial inoculation of large number of *Pinus monticola* seedlings with *Cronartium ribicola*. *In* Biology of rust resistance in forest trees: proceedings of a NATO-IUFRO advance study institute. R.T. Bingham, R.J. Hoff, and G.I. McDonald, eds. USDA Misc. Publ. 1221, p. 357-372.

Bingham, R.T., Hoff, R.J., and McDonald, G.I. 1973. Breeding blister rust resistant western white pine. VI. First results from field testing of resistant planting stock. USDA Forest Service Research Note INT-179. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah 84401. 12 p.

Bower, R.C. 1987. Early comparison of an Idaho and a coastal source of western white pine on Vancouver Island. West. J. App. For. 2:20-21.

Ekramoddoullah, A.K.M., Davidson, J.J., and Taylor, D.W. 1998. A protein associated with frost hardiness of western white pine is up-regulated by infection in the white pine blister rust pathosystem. Can. J. For. Res. 28:412-417.

Ekramoddoullah, A.K.M., Taylor, D.W., and Hawkins, B.J. 1995. Characterization of a fall protein of sugar pine and detection of its homologue associated with frost hardiness of western white pine needles. Can. J. For. Res. 25:1137-1147.

Eramian, A.D. 1997, 2003. Personal communication. USDA Forest service, Coeur d’Alene Nursery, Coeur d’Alene, Idaho.

Foushee, D. 1997. Personal communication. USDA Forest service, Coeur d’Alene Nursery, Coeur d’Alene, Idaho.

Hirt, R.R. 1938. Relation of stomata to infection of *Pinus strobus* by *Cronartium ribicola*. Phytopathology 28:180-190.

Hoff, R.J., McDonald, G.I., and Bingham, R.T. 1973. Resistance to *Cronartium ribicola* in *Pinus monticola*: structure and gain of resistance in the second generation. USDA For. Serv. Res. Note INT-178, Intermountain Forest and Range Experiment Station, Ogden, Utah. 8 p.

Hunt, R.S. 1991. The effect of age on the susceptibility to blister rust of western white pine seedlings. *In* 3rd International IUFRO Rust of Pine Working Party Conference. Ed. By Hiratsuka, Y., J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Laishley. Sep. 18-22, 1989. Banff, Alberta, Canada, pp. 287-290.

Hunt, R.S., and Meagher, M.D. 1989. Incidence of blister rust on “resistant” white pine (*Pinus monticola and P. strobus*) in coastal British Columbia plantations. Can. J. Plant Pathol. 11 (4):419-423.

Kaneko, S., and Harada, Y. 1995. Life cycle of *Endocronartium sahoanum* and its nuclear condition in axenic culture. Pp 95-100. In: Proceedings of the 4th IUFRO “Rust of Pines” Working Party Conference ed by Kaneko, S., K. Katsuya, M. Kakishima, and Y. Ono. Tsukubo, Japan.

Lachmund, H.G. 1933. Resistance of the current season's shoots of *Pinus monticola* to infection by *Cronartium ribicola*. Phytopathology 23:917-922.

Mahalovich, M.F., and Eramian, A. 1995. Breeding and seed orchard plan for the development of blister rust resistant white pine for the Northern Rockies. Draft tree improvement plan. USDA Forest Service Northern Region. 59 p.

McDonald, G.I., Hoff, R.J., and Samman, S. 1991. Epidemiologic function of blister rust resistance: A system for integrated management. *In* 3rd International IUFRO Rust of Pine Working Party Conference. Ed. by Hiratsuka, Y., J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Laishley. Sep. 18-22, 1989. Banff, Alberta, Canada, pp. 235-255.

McDonald, G.I., Hoff, R.J., and Wykoff, W.R. 1981. Computer simulation of white pine blister rust epidemics. I. Model formulation. USDA For. Serv. Res. Pap. INT-258, Intermountain Forest and Range Experiment Station, Ogden, Utah. 136 p.

McDonald, G.I., Zambino, P. and Sniezko, R. In press. Breeding rust-resistant pines in the western United States: Lessons from the past and a look to the future. This Volume.

Pierson, R.K. and Buchanan, T.S. 1938. Susceptibility of needles of different ages on *Pinus monticola* seedlings to *Cronartium ribicola* infection. Phytopathology. 29:833-839.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6.4th ed. SAS Institute Inc., Cary, N.C.

Snell, W.H. 1936. The relation of the age of needles of *Pinus strobus* to infection by *Cronartium ribicola*. Phytopathology 26:1074-1080.

Van Arsdel, E.P. 1968. Stem and needle inoculations of eastern white pine with the blister rust fungus. Phytopathology 58:512-514.

Yokota, S-I. 1983. Resistance of improved *Pinus monticola* and some other white pines to the blister rust fungus *Cronartium ribicola*, of Hokkaido, Japan. European Journal of Forest Pathology 13:389-402.

Part V: Conference Attendees

Conference attendees at the BLM's Sprague Seed Orchard

Photo courtesy of T. Tuttle

Conference Attendees

Chang-Young Ahn
Korea Forest Research Institute
Omokchun-dong 44-3
Suwon 441-350 Republic of Korea

Alexander H. Alexandrov
Forest Research Institute
132 St. Kliment Ohridski Blvd.
1756 Sofia, Bulgaria
forestin@bulnet.bg

Nabil Atalla
2310 Winslow Park Dr.
Medford, OR 97504

Bruce Barr
Sierra Pacific Industries
P.O. Box 39
Stirling City, CA 95978 USA
Phone: (530)873-9192
Fax: (530)873-2463
bbarr@spi-ind.com

Jerome Beatty
USDA Forest Service
Forest Health Protection
1601 North Kent Street
RPC, 7th Floor (FHP)
Arlington, VA 22209
Phone: (703)605-5332
Fax: (703)605-5353
jbeatty@fs.fed.us

Jordan Bennett
University of Victoria
P.O. Box 3020
Victoria, BC V8W 3N5 Canada
Phone: (250)721-7113
Fax: (250)721-6611
muchachoverde@hotmail.com

Ioan Blada
Forest Research Institute
SOS Stefaresti, NR 128
Bucharest 11 Romania
ioan_blada@yahoo.com

Andrew Bower
University of British Columbia
Forest Sciences Department
Vancouver, B.C. V6T 1Z4 Canada
adbower@interchange.ubc.ca

Patti Brown
Canadian Forest Products LTD
4858 Skylark Rd
Sechelt, BC V0N 3A2 Canada
Phone: (604)885-5905
Fax: (604)885-7614
pabrown@mail.canfor.ca

Todd Burnes
University of Minnesota
495 Borlaug Hall
St. Paul, MN 55108 USA
Phone: (612)625-6231
Fax: (612)625-9728
burne002@tc.umn.edu

Deems Burton
USDA Forest Service
P.O. Box 377
Happy Camp, CA 96039 USA
Phone: (530)493-2243
Fax: (530)493-1796
dburton@fs.fed.us

Mo-Mei Chen
University of California, Berkeley
1001 Valley Life Sciences Bldg
Berkeley, CA 94720 USA
Phone: (510)643-0633
Fax: (510)643-5390
mmchen@nature.berkeley.edu

Wan-Yong Choi
Korea Forest Research Institute
Omokchun-dong 44-3
Suwon 441-350 Republic of Korea
wychoi@foa.go.kr

Russell Cox
Tennessee Division of Forestry
2416 Fletcher Luck Lane
Knoxville, TN 37916 USA
Phone: (865)594-6432
Fax: (865)594-5430
racox@state.tn.us

Tim Crowder
Timberwest
1450 Mt Newton Cross
Saanichton, BC V8M 1S1 Canada
Phone: (250)652-4211
crowdert@island.net

Dean Davis
USDA Forest Service
P.O. Box 377
Happy Camp, CA 96039 USA
Phone: (530)493-2243
Fax: (530)493-1796
dadavis@fs.fed.us

Gaëtan Daoust
Natural Resources Canada
Canadian Forest Service
Laurentian Forestry Centre
1055 du P.E.P.S.
P.O. Box 3800
Sainte-Foy, Québec Canada G1V 4C7
Phone: (418) 648-5830
Fax: (418) 648-5849
gdaoust@cfl.forestry.ca

Abul K. Ekramoddoullah
Canadian Forest Service
506 West Burnside Road
Victoria, B.C. V8Z 1M5
Phone: (250)363-0692
Fax: (250)363-0775
aekramoddoul@pfc.forestry.ca

Danilo Fernando
SUNY Environ. Science and Forestry
1 Forestry Drive
Syracuse, NY 13210 USA
Phone: (315)470-5746
Fax: (315)470-6934
fernando@esf.edu

Lauren Fins
University of Idaho
Department of Forest Resources
Moscow, ID 83843 USA
Phone: (208)885-7920
Fax: (208)885-6226
lfins@uidaho.edu

John Hawkins
Sierra Pacific Industries
P.O. Box 39
Stirling City, CA 95978 USA
Phone: (530)873-9192
Fax: (530)2463
jhawkins@spi-ind.com

Liang Hsin
USDI Bureau of Land Management
P.O. Box 2695
Portland, OR 97208 USA
Phone: (503)808-6079
Fax: (503)808-6021
lhsin@or.blm.gov

Richard Hunt
Pacific Forestry Centre
506 West Burnside Road
Victoria, BC V8Z 1M5 Canada
Phone: (250)363-0640
rhunt@pfc.forestry.ca

Anatoly Iroshnikov
Research Institute of Forest
Genetics and Breeding
105 Lomonosov Str
394087 Voronezh Russia
Phone: (0732)53-95-07
Fax: +(0732)53-94-36
ilgis@lesgen.vrn.ru
mail@lesgen.vrn.ru

Angelia Kegley
Dorena Genetic Resource Center
USDA Forest Service
34963 Shoreview Rd
Cottage Grove, OR 97424 USA
Phone: (541)767-5703
Fax: (541)767-5709
akegley@fs.fed.us

Shams Khan
145/J3 Street #8 Phase 2
Hayatabad, Peshawar Pakistan
shamspfi@yahoo.com

John King
Ministry of Forests
P.O. Box 9519 STN Prov Govt
Victoria BX V8W 9C2 Canada
john.king@gems7.gov.bc.ca

Bohun Kinloch
Institute of Forest Genetics
P.O. Box 245
Berkeley, CA 94701 USA
bkinloch@fs.fed.us

Jay Kitzmiller
USDA Forest Service
2741 Cramer Lane
Chico, CA 95928 USA
jkitzmiller@fs.fed.us

Harvey Koester
USDI Bureau of Land Management
3040 Biddle Rd
Medford, OR 97504 USA
Phone: (541)618-2401
Fax: (541)618-2400
hkoester@or.blm.gov

Jodie Krakowski
Forest Sciences Dept.
University of British Columbia
3041-2424 Main Mall
Vancouver, British Columbia V6T 1Z4
jodiek@interchange.ubc.ca

Howard B. Kriebel
Ohio State University
625 Medford Leas
Medford, NJ 08055 USA
Tel: 609-654-3625
Fax: 609-654-3625
kriebel@medleas.com

Joe Linn
Umpqua National Forest
2900 NW Stewart Parkway
Roseburg, OR 97470
Phone: (541) 957-3204
Fax: (541) 957-3298
jlinn @fs.fed.us

Glenn Lunak
Sierra Pacific Industries
P.O. Box 39
Stirling City, CA 95978 USA
Phone: (530)873-9192
Fax: (530)873-2463
glunak@spi-ind.com

Gordon Lyford
USDI Bureau of Land Management
Sprague Seed Orchard
Phone: (541)476-4432
Gordon_Lyford@or.blm.gov

Mary Frances Mahalovich
USDA Forest Service
1221 S Main Street
Moscow, ID 83843 USA
Phone: (208)883-2350
Fax: (208)883-2318
mmahalovich@fs.fed.us

Sheila Martinson
USDA Forest Service
P.O. Box 3623
Portland, OR 97208 USA
smartinson@fs.fed.us

Geral McDonald
USDA Forest Service
1221 South Main Street
Moscow, ID 83843 USA
Phone: (208)883-2343
Fax: (208)883-2318
gimcdonald@fs.fed.us

Mike Meagher
MDM FORGENE
666 Jones Terrace
Victoria, BC Canada
Phone: (250)727-7675
Fax: (250)727-7609
mmeagher@pfc.forestry.ca

Robert Ohrn
USDI Bureau of Land Management
1717 Fabry Rd SE
Salem, OR 97306 USA
Phone: (503)589-6883
Fax: (503)375-5715
bob_ohrn@blm.gov

David Oline
Southern Oregon University
1250 Siskiyou Blvd
Ashland, OR 97520 USA
Phone: (541)552-6799
OlineD@sou.edu

Dmitri Politov
Vavilov Institute of General Genetics
Russian Academy of Sciences
3 Gubkin Str GSP-1
Moscow 119991 Russia
Phone: (095) 135-5067
Fax: (095) 132-8962
dvp@vigg.ru

Christine Redmond
WA Department of Natural Resources
P.O. Box 47017
Olympia, WA 98504 USA
Phone: (360)407-7576
Fax: (360)459-6872
christine.redmond@wadnr.go

Marc Rust
IETIC
UI College of Natural Resources
Moscow, ID 83844 USA
Phone: (208)885-7109
Fax: (208)885-6226
mrust@uidaho.edu

Safiya Samman
USDA Forest Service
Forest Health Protection
1601 North Kent Street
RPC, 7th Floor (FHP)
Arlington, VA 22209
Phone: (703)605-5341
Fax: (703)605-5353
ssamman@fs.fed.us

Scott Schlarbaum
University of Tennessee
Dept. of Forestry, Wildlife & Fisheries
Knoxville, TN 37901 USA
Phone: (865)974-7993
Fax: (865)974-4733
tenntip@utk.edu

Anna Schoettle
USDA Forest Service
240 W Prospect Rd
Fort Collins, OR 80526 USA
Phone: (970)498-1333
Fax: (970)498-1212
aschoettle@fs.fed.us

Richard Sniezko
USDA Forest Service
Dorena Genetic Resource Center
34963 Shoreview Rd
Cottage Grove, OR 97424 USA
Phone: (541)767-5716
Fax: (541)767-5709
rsniezko@fs.fed.us

Richard Stephan
Institute for Forest Genetics
Siekerlandstrasse 2
D-22927 Grosshansdorf Germany
Tel/Fax: +49 - (0)4102 - 63555
r-c.stephan@t-online.de

Paul Stover
USDA Forest Service
2375 Fruitridge Rd
Camino, CA 95709 USA
Phone: (530)642-5030
Fax: (530)642-5099
pstover@fs.fed.us

Terry Tuttle
USDI Bureau of Land Management
1884 Bristol Drive
Medford, OR 97504 USA
Phone: (541)779-3396
Fax: (541)846-9119
terry_tuttle@blm.gov

Nick Vagle
USDA Forest Service
P.O. Box 440
Grants Pass, OR 97528 USA
Phone: (541)471-6810
Fax: (541)471-6514
nvagle@fs.fed.us

Huo-Ran Wang
Division of Exotic Forestry
Research Institute of Forestry
Chinese Academy of Forestry
Beijing 100091 China
Phone: (086)010 62889683
Fax: (086)010 62884927; 62872015
wanghr@rif.forestry.ac.cn

Kwan-Soo Woo
Tree Breeding Division
Forest Research Institute
Korea Forest Service // Suwon,
Omokchundong 44-3,
Kyonggido, 441-350, South Korea
Telephone: 82-(0)31-290-1106
Fax: 82-(0)31-292-4458,
woo9431@yahoo.co.kr

Publisher's note: Papers in this report were reviewed by the compilers and other external reviewers. Rocky Mountain Research Station Publishing Services reviewed papers for format and style. Authors are responsible for content.

You may order additional copies of this publication by sending your mailing information in label form through one of the following media. Please specify the publication title and series number.

Telephone (970) 498-1392
FAX (970) 498-1396
E-mail rschneider@fs.fed.us
Web site http://www.fs.fed.us/rm
Mailing Address Publications Distribution
Rocky Mountain Research Station
240 West Prospect Road
Fort Collins, CO 80526

RMRS
ROCKY MOUNTAIN RESEARCH STATION

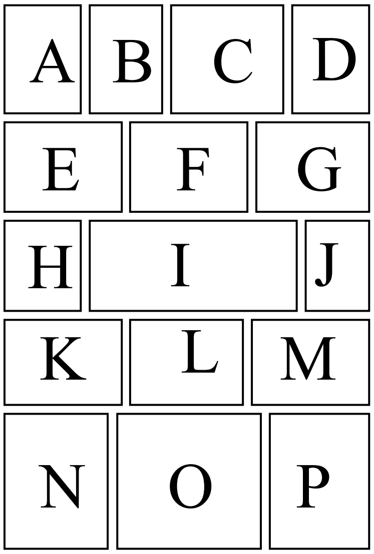
The Rocky Mountain Research Station develops scientific information and technology to improve management, protection, and use of the forests and rangelands. Research is designed to meet the needs of National Forest managers, Federal and State agencies, public and private organizations, academic institutions, industry, and individuals.

Studies accelerate solutions to problems involving ecosystems, range, forests, water, recreation, fire, resource inventory, land reclamation, community sustainability, forest engineering technology, multiple use economics, wildlife and fish habitat, and forest insects and diseases. Studies are conducted cooperatively, and applications may be found worldwide.

Research Locations

Flagstaff, Arizona
Fort Collins, Colorado*
Boise, Idaho
Moscow, Idaho
Bozeman, Montana
Missoula, Montana
Lincoln, Nebraska
Reno, Nevada
Albuquerque, New Mexico
Rapid City, South Dakota
Logan, Utah
Ogden, Utah
Provo, Utah
Laramie, Wyoming

*Station Headquarters, Natural Resources Research Center,
2150 Centre Avenue, Building A, Fort Collins, CO 80526



From upper left corner of back cover:

A. *Pinus sibirica* (D. Politov)
B. *P. strobus* (G. Daoust)
C. *P. sibirica* (D. Politov)
D. *P. cembra* (B.R. Stephan)
E. *P. koraiensis* (A. Boyarinov)
F. *P. wallichiana* x *P. strobus* (G. Daoust)
G. *P. armandii* var. *amamiana* (T. Sugaya)
H. *P. balfouriana* (D. Oline)
I. *P. cembra* (B.R. Stephan)
J. *P. koraiensis* (A. Boyarinov)
K. *P. albicaulis* (R. Sniezko)
L. *P. monticola* (R. Sniezko)
M. *P. parviflora* (B.R. Stephan)
N. *P. lambertiana* (R. Sniezko)
O. *P. pumila* (V. Potenko)
P. *P. peuce* (B.R. Stephan)

Back Cover Design: Ryan Berdeen