PROCEEDINGS

The Breeding and Genetic Resources of Five-Needle Pines Conference

22-26 September, 2008 Yangyang, Republic of Koea

KOREA FOREST RESEARCH INSTITUTE

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Breeding and Genetic Resources of Five-Needle Pines Conference; Ecophysiology, Disease Resistance and Developmental Biology of Five-Needle Pines

by

David Noshad, Eun Woon Noh,
John King and Richard Sniezko

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Breeding and Genetic Resources of Five-Needle Pines : Ecophysiology, Disease Resistance and Developmental Biology

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Breeding and Genetic Resources of Five-Needle Pines : Ecophysiology, Disease Resistance and Developmental Biology

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Abstract

In: Noshad David; Noh Eun Woon; King, John; Sniezko, Richard A. (Eds. 2009) *Breeding and Genetic Resources of Five-Needle Pines.* Proceedings of the Conference 2008, Yangyang, Korea. Korea Forest Research Institute, Seoul 104p. ISBN 978-89-8176-605-4 (93520).

The Proceedings presented here represent the 3rd highly successful conference we have held in recent years for the IUFRO Working Party 2.02.15: The Breeding and Genetic Resources of Five-Needle Pines. This working group highlights work related to quantitative and molecular genetics, resistance to diseases and pests, hybridization, selection and improvement, genecology and conservation genetics in all five-needle pines. Underlying this work is the threat that this species group faces from climate change and disease especially white pine blister rust. This conference was the first time this group has met in East Asia. Participants were grateful to our Korean hosts for their generosity and providing a very well run and informative conference. The other two recent conferences were held at Medford Oregon, USA in 2001 and Valiug, Romania in 2006.

One of the major themes of the meeting, incorporated into these proceedings, is the international efforts and concerns being taken to conserve important gene pools of these ecological and culturally important species. This ranged from: *P. chiapensis* in southern Mexico and Guatemala which plays a key role in ecosystem regeneration in areas managed under slash-and-burn agricultural systems for indigenous peoples; to critically endangered species in Vietnam, to efforts at conservation genetics in Siberia and the North American Rockies.

Another major theme of the conference centered around concern over white pine blister rust caused by the pathogen *Cronartium ribicola.* Efforts through breeding and selection from countries ranging from Canada, Romania. and the USA were discussed as well as the Korean efforts at control in the native *P. koraiensis* forests. A commitment to international cooperation and coordination in understanding and helping control this pathogen was made at the meeting.

There was a commitment to strengthen the international efforts at research in this important species group and encourage ongoing exchange of information and material. The Russian delegation offered to host the next meeting in Tomsk in Siberia and we hope to co-ordinate this with the IUFRO world congress to be held in Seoul, Korea in 2010.

John King
Working group 2.02.15 chair
British Columbia Ministry of Forests, Canada

Key words: five needle pines, white pines, Cronartium ribicloa

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 Ferre (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

Message of the Director General

Five needle pines are important tree species both in economic and ecological terms. In Korea, we have three native and one exotic five needle pines. They have been greatly utilized as sources for timber, nuts and landscape materials. Korean pine has been a good source of timber and edible nuts in Korea. During the past 50 years, we have established Korean pine seed orchards to provide good quality seeds for plantation. We have also performed various research to understand genetic structure of natural stands and to control genetic make-up of the seed orchards. In contrast, a remnant population of Pinus pumila in the peak of the Sorak Mountain represents the Southern most population in Korean peninsula. It warns us how fast the global warming progresses. Eastern white pine (*Pinus strobus*) is a promising species in Korea since it grows fast and straight. Several provenance tests are being done to select best seed sources. However, the health of both the natural stands and the artificial plantations has been greatly threatened by white pine blister rust, pine nematodes and recently by global warming. We need more knowledge on five needle pines in context to their conservation and utilization. Therefore, KFRI, together with the Korean White Pine Society, hosted the IUFRO Working Party 2.02.15 (Breeding and Genetic Resources of Five-needle pines) in September 22, 2008. The scientists in Korea had the opportunity to exchange views and discuss problems and possible solutions with the experts from 12 countries. This issue summarizes the results of the conference.

I am aware that numerous attempts have already been made to reduce the damage by the pests. However, in many cases, those attempts have not been so successful due to high cost and short-lived effects. We need to understand the resistance as well as adaptation mechanisms. Therefore, I hope to learn much from the research work you present here. By sharing the research results and opinions, we will be better able to cope with our current and future threats to white pine forests. Korea Forest Research Institute has made a clear commitment to collaborate with foreign partners to make forest healthier and more productive. In this context, holding this Conference, “The Breeding and Genetic Resources of Five-Needle Pines” was very appropriate and timely given the various types of biotic and abiotic stresses on pine forests. Through the oral and poster presentations, participants reported many new findings made during the past several years on genetics, physiology, pathology and conservation of five needle pines. We all agree that international research collaboration is needed to minimize the impacts of white pine blister rust. Korea is hosting the XXIII IUFRO World Congress at COEX, Seoul in 2010. I urge you to take your time to attend the congress.

We cordially invite you to participate in the Congress and looking forward to seeing you here next year.

Finally, I would like to express thanks to the editors who worked hard to publish the proceedings.

Wan-Yong Choi

The Director General
Korea Forest Research Institute

'The Breeding and Genetic resources of Five-Needle Pines Conference'
Yangyang, Korea
September 22-26, 2008

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Breeding and Genetic Resources of Five-Needle Pines:
Ecophysiology, Disease Resistance and Developmental Biology
Yangyang, Korea 2008

Korean Successes in Controlling Blister Rust of Korean Pine

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Abstract – Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is a major reforestation species in South Korea. A serious outbreak of blister rust on Korean pine, caused by the pathogen *Cronartium ribicola* J. C. Fischer, was first reported in the northeastern part of South Korea in 1972. By 1979, the disease had spread extensively and had become a serious threat and a major obstacle to the successful reforestation of Korean pine in South Korea. Several management activities were initiated to try to minimize the impact of the rust in pine plantations. An intensive nation-wide blister rust control program, which included eradication of the alternate host (*Pedicularis resupinata*) and removal of all infected Korean pines, was initiated in 1973 by the South Korean government. This ongoing control effort resulted in a drastic decline in blister rust incidence and has successfully prevented further spread of the disease. In addition to the eradication program, a breeding program to develop rust resistant Korean pine was initiated in 1975. The research on resistance concluded that genetic resistance to the rust was present and that it was feasible to develop blister rust resistant clones by rooting individuals of rust resistant trees. However, the work on developing resistant Korean pine was discontinued in 1984 because the incidence of the disease became negligible due to other control methods. Currently, the incidence of the blister rust of Korean pine is negligible, and it is no longer a major immediate threat to Korean pine. However, blister rust cannot be totally eliminated and the underlying susceptibility of Korean pine to this disease means that control efforts will be needed into the foreseeable future unless resistant populations of Korean pine are developed for use in future reforestation.

Introduction

The occurrence of blister rust on Korean pine (*Pinus koraiensis* Sieb. et Zucc.), caused by the pathogen *Cronartium ribicola* J. C. Fischer, has been known in Korea since 1936. However, the incidence and impact from the disease was negligible until 1972 when a serious outbreak of the disease was first reported in the northeastern part of South Korea. The disease spread rapidly to Korean pine plantations throughout the country, killing more than one million trees between 1972 and 1979 (Figure 1). By this time, blister rust had become a serious threat and a major obstacle to the successful reforestation of the Korean pine in Korea. A nation-wide intensive blister rust control program which included eradication of *Pedicularis resupinata* L., the alternative host of *C. ribicola*, and removal of all infected Korean pines, was initiated in 1973 by the South Korean government. This ongoing control effort has resulted in a drastic decline in blister rust incidence and has successfully prevented further spread of the disease. By 1999, the incidence of blister rust had been reduced to only one plantation in the country, and there was no further detection of the

Figure 1. Geographic incidence of blister rust in Korean pine between 1965 and 1975 in South Korea

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rust elsewhere from 1998 to 2002. However, a small sporadic occurrence of the disease has been observed in three plantations since 2003. Currently, the incidence of the blister rust of Korean pine is negligible, and if control measures are continued it is no longer a threat to Korean pine. This paper reviews the history of occurrence of blister rust in South Korea and the government control efforts that have reduced it to near negligible impacts in recent years, and the early work on a program to breed rust resistant Korean pine.

Economic Importance of Korean Pine

Four species of five-needle pine are grown in Korea. They are *Pinus koraiensis* Sieb. et Zucc., *P. parviflora* Sieb. et Zucc., *P. pumila* Regel and *P. strobus* L. The first three species are native to Korea while *P. strobus* is introduced. Of these four species, *P. koraiensis* is by far the most widely distributed throughout the country. *P. koraiensis* has a large natural range that includes Korea, eastern Manchuria and southeastern Siberia, with outlier populations on the Japanese islands of Honshu and Shikoku (Fig. 2, Critchfield and Little, 1966). The remaining three species occur only to a very limited extent in South Korea (Fig. 3). In South Korea, *P. parviflora* is native only to Ulleung Island, and some natural stands of *P. parviflora* can be found on this island. *P. parviflora* is grown only as ornamental tree for horticultural use. One small natural stand of *P. pumila* occurs at high elevation on Mt. Seorak in the northeastern part of South Korea. The introduced *P. strobus* is not widely planted, and there are only a few small planted stands of *P. strobus* in South Korea. *P. strobus* is mainly planted as landscape tree in South Korea.

Figure 2. Geographic distribution of *Pinus koraiensis* (after Critchfield and Little, 1966).

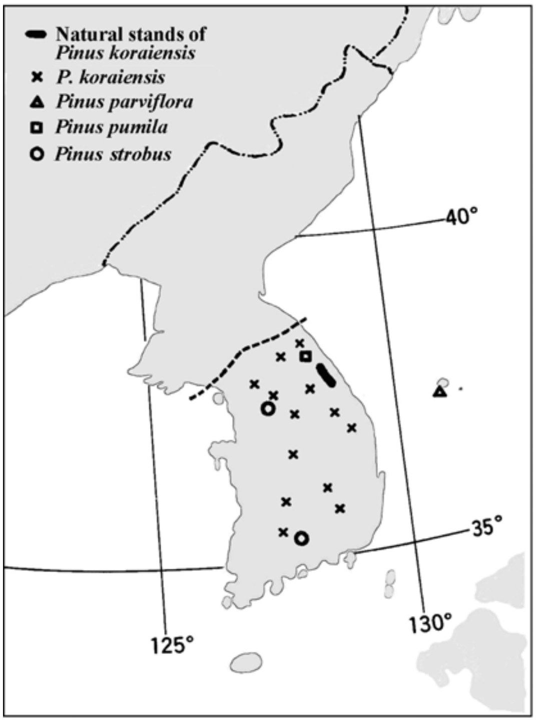

Figure 3. Approximate distribution of five-needle pines in South Korea.

Korean pine is one of the top priority timber species of South Korea, and it has been extensively planted as part of the national reforestation programs in Korea. It is particularly important for the production of quality timbers in South Korea. It also produces edible nuts which are considered a delicacy in Korea and which provide a good source of income for pine growers. These economic advantages together with its good adaptability to cold mountainous conditions are the reasons for its long standing importance as one of the major timber species in Korea. Although some natural stands of Korean pine can still be found on a few high mountains in South Korea, most of the current stands of Korean pine are the result of reforestation efforts. Extensive reforestation of Korean pine began in 1962 after the Korean War. As of 2008, the total area of Korean pine stands in South Korea is approximately 320,000 hectares. Seed orchards provide the majority of seed for reforestation. The area planted to Korean pine is expected to increase further in the future.

Table 1. Incidence of blister rust caused by *Cronartium ribicola* in *P. koraiensis* stands in relation to altitude of the stands, tree diameter (D.B.H.) and age of the trees (compiled from 1975 survey)

Stand Information		Area infected (ha)	% of total area infected	No. of trees infected	% of trees infected
Altitude (m)	Up to 600	60.02	5	4,416	6
	601-1,000	1,018.80	86	49,239	69
	Above 1,000	112.50	9	17,907	25
Tree diameter (D.B.H.) (cm)	Less than 6	695.10	58	59,977	84
	6.1-16	494.22	42	9,085	13
	16.1-20	2.00	-	2,500	3
Age of trees (years)	Less than 10	630.80	53	56,277	78
	11-20	551.52	46	12,679	18
	More than 21	9.00	1	2,606	4
Total		1,191.32	100	71,562	100

The Blister Rust Situation in Korea

History

The first documented occurrence of *Cronartium ribicola* in Korea was in 1936 when Takagi (1937) found the aecial stage of the rust on *P. koraiensis* in an 8-year old young plantation in central part of Korean peninsula. He reported that 650 trees of 8-year old Korean pine were infected with the blister rust in a 20 hectare area. The telial stage of the fungus was not observed at that time. The fungus was subsequently confirmed as *Cronartium ribicola* by Hiratsuka (1939). The disease drew no more attention until around 1965 when the disease was reported in several young plantations of *P. koraiensis* in the northeastern part of South Korea. It is assumed that the disease continued to spread slowly over the previous several decades. However, there had been little attention to the disease problems of forest trees during this time because of the chaotic circumstances in Korea, including both World War II and the Korean War. No information regarding the incidence of blister rust of Korean pine in North Korea is available.

Annual survey of blister rust incidence

Annual surveys of the damage caused by blister rust were initiated in 1972 by the Korea Forest Service. These surveys revealed that the disease was most prevalent and severe in stands of *P. koraiensis* that were less than 10 years old and were located at altitudes of between 600 and 1,000 meters (La and Yi, 1976, Table 1). The major reforestation efforts began in 1962 and the high susceptibility of young trees may help explain why blister rust epidemic was noticed in early 1970's in South Korea. The high density of *Pedicularis* in the newly reforested young stands where the trees have not closed canopy is another likely contributing factor to the blister rust epidemic in the 1970's.

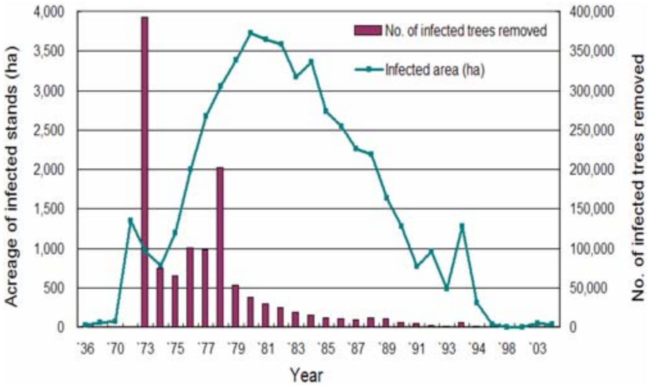

Figure 4. Hectares of Korean pine forests with blister rust infection following eradication of infected pines and alternate host (*Pedicularis*).

The area of Korean pine stands infected with blister rust since its first outbreak in 1972 is given in Figure 4. As shown in Figure 4, the area of infected stands increased rapidly from 1972 until around 1980, then it decreased steadily, becoming negligible by 1997. The decline in the number of infected trees is more drastic than the decline in the acreage of the infected stands. A total of 392,987 infected trees were removed from 954.1 ha of infected stands in South Korea in 1973 (Table 2). In 1997, only a few infected trees were observed and only of 30 ha of infected stands were noted in the country, and no infected stands were observed in 1998. There are two main reasons for this rapid decline of the blister rust in *P. koraiensis* stands. One is the continuing effort of the eradication of *Pedicularis* in and around the infected stands, and the early removal of infected trees. Another reason is that as the Korean pine grew taller and closed canopy, the pines become less vulnerable to infection by blister rust because of fewer low branches, and the density of *Pedicularis* declines dramatically because *Pedicularis* does not grow under the shade of Korean pine.

Table 2. Incidence of blister rust in *Pinus koraiensis* in South Korea in 1973

Total acreage of infected stands (ha.)	Total number of trees in the infected stands	Total number of trees infected (removed)	% infected trees
954.1	2,868,518	392, 987	13.7

Table 3. Incidence of blister rust of *Pinus koraiensis* in 2007 at Jungsun[a]

Plantation No.	Planted year	Acreage (ha)	No. of trees planted	No. of trees infected & removed	% infection	Altitude of the plantation (m)
Plantation 1	2002	2.0	6,000	1,572	26	1.200-1,300
Plantation 2	1994	13.2	39,600	12,670	32	1,200-1,300
Plantation 3	2002	0.5	1,500	885	59	1,300-1340

[a] 2-2 seedlings of *P. koraiensis* were planted in the stand

Level of blister rust resistance in *P. koraiensis*

Some information on the inherent susceptibility of Korean pine to the Korean strain of blister rust is evident in Tables 2 and 3. Blister rust infection has been wide-spread in Korea and in 1973 13.7% of all trees were infected (Table 2). In a 2007 survey one area showed 59% infection (Table 3). The very recent high incidence of blister rust in a small area in Jungsun in 2007 indicates that a new epidemic of the disease could take place at any time when continuing effort of eradication of *Pedicularis* and sanitation of infected Korean pine are not properly practiced. The apparent inherent susceptibility of Korean pine to blister rust in Korea strengthens the case for examining a potentially more permanent solution of developing genetic resistance in Korean pine.

Host-fungus relationships

The alternative host of blister rust in Korea is a perennial herb *Pedicularis resupinata* L. (Orobanchaceae) (Yi and La, l974). By contrast, in North America and Europe the primary alternate host of blister rust are species in the genus *Ribes*. *Pedicularis resupinata* is particularly abundant throughout areas blister rust infected *P. koraiensis* stands occur. It is almost exclusively limited to altitudes above 500 meters, which corresponds with those altitudes where the blister rust is most severe on *P. koraiensis* (La and Yi, l976).

Although a number of species of *Ribes* are known to occur in Korea (Table 4), *C. ribicola* infection has never been encountered in *Ribes* species found in and around the rust infected Korean pine stands. Yi and La (1974) demonstrated that native *Ribes fasciculatum* var. *chinense* as well as the non-native *R. nigrum* and *R. hudsonianum* var. *petiole* obtained from United States became heavily infected when they were artificially inoculated with aeciospores of *C. ribicola* collected from diseased Korean pines. This indicates that *C. ribicola* strain occurring in Korea infects both *Pedicularis* and *Ribes* species. On the other hand, Hyun and Koo (1981a, 1981b) reported that *R. nigrum* obtained from West Germany was not infected when it was artificially inoculated with *C. ribicola* from infected Korean pine. Thus, the results of artificial inoculation of *Ribes nigrum* give conflicting results, and further study would be necessary to clarify the infectivity of Korean isolates of *C. ribicola* to *Ribes nigrum*.

Table 4. Species of *Pedicularis* and *Ribes* occurring in Korea (after T. B. Lee, 2003, and Y. N. Lee, 2006)

Pedicularis spp.	*Ribes* spp.
P. resupinata	*R. burejense*
P. resupinata var. *gigantea*	*R. diacantha*
P. resupinata var. *oppositifolia*	*R. distans*
P. resupinata var. *spicata*	*R. fasciculatum* var. *chinense*
P. resupinata var. *umbrosa*	*R. fasciculatum* var. *japonicum*
P. resupinata var. *vaniotii*	*R. grossularia*
P. adunca	*R. horridum*
P. grandiflora	*R. komarovii*
P. hallaisanensis	*R. latifolium*
P. ishidoyana	*R. mandshuricum*
P. lunaris	*R. maximowiczianum*
P. manshurica	*R. maximowiczianum* var. *umbrosum*
P. nigrescens	
P. palustris	*R. nigrum*
P. sceptrum-carolinum	*R. sativum*
P. songdoensis	*R. triste*
P. spicata	*R. ussuriense*
P. verticillata	

Cronartium ribicola on other five-needle pines in korea

Over the last several decades, there has been increasing interest in planting of eastern white pine (*Pinus strobus*) in Korea

Table 5. Blister rust infection levels of seedlings of three species of five-needle pines exposed to natural infection

Pine species	No. trees tested[a]	No. trees infected[b]	Percent Infection
Pinus koraiensis	182	40	22
P. strobus	217	85	39
P. parviflora	181	0	0

[a] 4~5 years old seedlings planted in 3 locations of high blister rust incidence

[b] Total number of trees that developed blister cankers from 1978 to 1985

because of its fast growth and good timber quality. However, the susceptibility of *P. strobus* to the strain of *C. ribicola* occurring in Korea was not known. Therefore, the susceptibility of *P. strobus* to blister rust was evaluated along with two other five-needle pines occurring in Korea (Kim *et al.*, 1982, La and Yi, 1985). Four to five-year old seedlings were planted in 1978 in three locations and exposed to natural infection by blister rust. Blister rust cankers started to appear on both *P. koraiensis* and *P. strobus* seedlings three years after planting. Blister-like aecia did not develop on any of the 181 *P. parviflora* seedlings over seven years after planting, suggesting that *P. parviflora* is highly resistant to Korean strain of *C. ribicola* (Table 5). Further testing, including artificial inoculation trials would be useful to confirm the apparent high level of resistance of *P. parviflora* to blister rust in Korea. In these small field trials, the incidence of blister rust on *P. strobus* (39%) was almost twice that on *P. koraiensis* (22%) (Table 5). The much higher susceptibility of *P. strobus* relative to that of *P. koraiensis* in these short-term field evaluation agrees with testing done in North America and Europe (Bingham 1972, Hoff *et al.*, 1980, Stephan 1984). The high level of blister rust incidence in *P. strobus* strongly suggests that potential threat of disease on *P. strobus* should be carefully considered before large scale planting of *P. strobus* in Korea is undertaken.

The incidence of blister rust on *P. pumila* under natural condition in Korea is not known. Artificial inoculation of *P. pumila* with basidiospores of *C. ribicola* also has not been carried out in Korea to determine its susceptibility to the strain of blister rust in Korea.

Breeding of blister rust resistant korean pine

The severe outbreak of the Korean pine blister rust during early 1970's stimulated great interest in potentially breeding Korean pine for resistance to the strain of blister rust in South Korea. Breeding efforts to develop blister rust resistant clones of Korean pine were initiated in 1975 by Hyun and Koo of the Institute of Forest Genetics in Suwon, South Korea (1981a, 1981b).

Initially, the focus was on the rootability of cuttings of *Pinus koraiensis* because high rootability is essential for mass propagation of blister rust resistant clones. Rootability of cuttings of Korean pine exceeded 70% when cuttings were from young trees (less than six years old). The youngest plantations were searched for rust-free selections, but there was little rust in those plantations. Therefore, slightly older (8 to 14-year old) heavily rust infected plantations were searched and 1,142 rust-free candidate trees were selected from four locations in the northeastern part of South Korea.

For resistance testing, rooted cuttings from these selected resistant candidate trees, and unselected ordinary 1-0 and 2-0 seedlings of *P. koraiensis* were artificially inoculated using naturally infected *Pedicularis* leaves bearing abundant telial columns as inoculum. For each inoculation, naturally infected *Pedicularis* leaves were collected from heavily infected Korean pine stands 200 km away from the research facility in Suwon. This artificial inoculation testing was carried out for three consecutive years, 1976 to 1978. In each year, 1-0 and 2-0 seedlings of *P. koraiensis* of different seed sources and rooted cuttings of resistant candidate trees were subjected to inoculation for resistance testing. However, the artificial inoculation was not successful, as no visible symptoms such as needle spots or stem cankers developed on any of the seedlings and rooted cuttings. It is suspected that *Pedicularis*, being an subalpine perennial herb, might have wilted during the transportation from it's natural habitat to inoculation chamber in Suwon, resulting in the considerable loss of germinability of teliospores (Koo *et al.*, 1983).

Collecting large quantities of infected *Pedicularis* leaves from the Korean pine stands and transporting them to the moist chamber in the research facility in Suwon during the same day is a rather difficult job. Therefore, an attempt was made to propagate *Pedicularis* in the nursery in Suwon. But, it was soon evident that *Pedicularis*, being subalpine plant, does not grow well in the nursery at low altitude.

Due to these difficulties, the rooted cuttings were planted and exposed to natural infection in a specially designated protection area where rust is severe on both Korean pine and *Pedicularis.* In addition, large numbers of *Pedicularis* were planted in this protected area to increase the inoculum density. The screening of rooted cuttings for blister rust resistance under natural conditions was successful, and characteristic needle spots and stem cankers appeared on 30% of the three year old seedlings, while none of the rooted cuttings from resistant trees showed any symptoms two years after exposure to sources of natural infection. This screening of resistant clones under natural condition continued until 1983.

After eight years of work on resistance testing of Korean pine in Korea, Hyun and Koo (1981a, 1981b) concluded that there was a high potential for developing blister rust resistant clones by rooting individuals from canker-free trees selected from young blister rust infected plantations. However, this promising project was discontinued in 1984 because of the drastic reduction of the blister rust incidence brought by the eradication of *Pedicularis* and removal of infected trees in Korean pine plantations. Currently, breeding of Korean pines for blister rust resistance is not being conducted in Korea.

Disease Management

Removal of infected trees

Since 1973, the removal of infected Korean pines and the eradication of *Pedicularis* are the two principal measures implemented for the control of Korean pine blister rust in Korea. Aecial blisters are most conspicuous on Korean pine from late April to the middle of May in Korea. *C. ribicola* grows rapidly on *P. koraiensis* in Korea and large cankers over 50 cm tall are common on young trees. Thus, infected trees are readily detected when aecial blisters are present. Swelling or distortion of the stem is rare on infected *P. koraiensis*, in contrast to the common swelling of stems and branches on blister rust infected *P. monticola* and *P. lambertiana* in North America. Intensive searches for infected trees in Korean pine stands are usually made in the middle of May. When infected trees are found, the cankered portions of the stems and branches are carefully wrapped first with vinyl sheets to prevent the scattering of aeciospores. The diseased stems and branches are then cut and gathered together, and either buried or burned. The entire tree is removed when the stem is infected, but if only branches are infected, then only the diseased branches are removed. Thorough removal of infected Korean pines in the stands before the release of aeciospores from the blisters has been very effective in preventing the dissemination of aeciospores to *Pedicularis.*

Eradication of alternate hosts

In addition to the removal of infected trees and branches of the pines, an intensive *Pedicularis* eradication program was also initiated in 1973. This eradication program is still in progress. *Pedicularis* eradication takes place during early August before the appearance of telial horns on *Pedicularis* which start to appear in late August. *Pedicularis* plants are generally removed using hand tools. Removal using a hoe and shovel is only practical for removing *Pedicularis* from small areas in Korean pine stands. Eradication of *Pedicularis* in large areas of steep Korean pine forests by hand would be cost prohibitive. A more cost effective method for larger areas that is now used is the application of foliar sprays of a two percent solution of the herbicide, glyphosate (Yi *et al.*, 1981). This herbicide has been used for large scale eradication of *Pedicularis*. Removal of *Pedicularis* in the planting sites is also practiced prior to planting seedlings of *P. koraiensis*. This practice is usually carried out during site preparation, and glyphosate application is widely used for this purpose.

It is believed that this continuing effort of eradicating *Pedicularis* over the last 35 years has not only contributed to the successful prevention of the spread of the disease, but also minimized the potential development of potentially more virulent races of *C. ribicola* in South Korea via migration from other countries. Korean pine is extensively distributed in North Korea, northeastern China and eastern portions of Russia, and blister rust is also known to occur in these regions (Shao, 1980). Removal of infected Korean pines greatly diminishes the local source of inoculum, but aeciospores of *C. ribicola* are constantly blown over from these regions to infect *Pedicularis* in South Korea. Therefore, consistent eradication of *Pedicularis* from South Korean forests is particularly important in preventing new infections of *Pedicularis* spp. by aeciospores of *C. ribicola* from neighboring countries.

Summary

If applied frequently and very extensively, eradication of *Pedicularis* and sanitation of infected Korean pine trees provides a very effective short-term solution to minimizing the mortality caused by white pine blister rust. However, *C. ribicola* is a permanent component of *P. koraiensis* forests in South Korea and increasing costs may make it more difficult in the future to continuously utilize eradication and sanitation as the primary method of reducing the effects of blister rust. A more effective, long-term solution would be the development of resistant *P. koraiensis* parents that could be placed into seed orchards (for seed production) or utilized via rooted cuttings to establish future *P. koraiensis* forests. Working together, pathologists and geneticists could investigate the potential for developing resistant *P. koraiensis*, including uncovering any limitations to genetic resistance, and a timeline for utilization of resistance in operational planting programs.

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Genetic Diversity in the Bulgarian Populations of *Pinus peuce* Grsb.

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Abstract – The paper presents results of a study on the genetic diversity in ten indigenous populations of the Balkan endemic *Pinus peuce* Grsb., one of the two five-needle pines in Europe. Fifty to sixty trees per populations were studied by means of allozyme gene markers. The within population diversity (*Ho*=0.12) was lower than the average figures reported for other five-needle pines and significant level of inbreeding was detected in some populations. Genetic differentiation measured by F-statsitics was 0.08 - well within the range typical for the outcrossing conifer species. The possible reasons for the observed results and the implications for the species conservation and use are discussed.

Key words: *Pinus peuce*, genetic diversity, differentiation, conservation

Introduction

Five-needle or white pines (subgenus *Strobus*) possess some particular characteristics that make them a special group within the genus *Pinus*. These include valuable timber, ecological tolerance, sometimes edible seeds, and other traits that make them important from economical and environmental point of view. Also, like most pines they occupy large territories and can influence the environment. Therefore, they are important also for maintaining the habitat characteristics (Critchfield & Little 1966, Mirov 1967, Vidakovic 1991). The group of five-needle pines has received special attention by the experts and has been a subject of extensive studies. However, there are some species that were studied to a lesser extent, mostly due to their limited distribution. One of these species is *Pinus peuce*, a Balkan endemic.

Usually Europe is generally poorer in coniferous species in comparison with North America and Eastern Asia, and this trend can be extended to five-needle pines too. There are only two white pines in Europe and their range of natural distribution does not exceed the continent's boundaries. Both species are distributed at higher elevation in the mountains and both are endemics to Europe – *Pinus cembra* occurs in Alps and Carpathians, and *Pinus peuce* – only in the high mountains of the Balkan Peninsula.

Pinus peuce was discovered by the German botanist August Grisebach in 1839 and in 1845 he published the description of the new species from Balkans (Grisebach 1845). It was considered initially that it is a small tree (up to 10 m in height in the classical locality) but later on it was found that *P. peuce* could reach 42 m in height and up to 1.5 m in diameter (Stefanoff 1934), and is used for timber and fuel wood by the local people.

In comparison to other pines, there are relatively few studies on the genetics of this species (Hagman and Mikkola 1963, Popnikola *et al.* 1978, Petrovska and Stamenkov 1987).

Dobrev (2002 a,b, 2005) studied in detail the inheritance of quantitative traits of *P. peuce* and found relatively high heritabilities for height- and diameter growth. He predicted that genetic gain will be large enough to allow the commercial use of the species as a source of valuable timber. Neutral markers still have not been applied to study the distribution of its genetic diversity. Most studies concerning the species were designed for studying the phylogeny of the genus (Bermann & Gillet 1997, Shurkhal *et al.* 1992, Liston *et al.* 1999, Wang *et al.* 1999; Gernandt *et al.* 2005). There were some occasional reports about the isozyme inheritance (Zhelev *et al.* 2002) and mating system (Zhelev *et al.* 2008), but the population structure and differentiation in natural populations were not studied so far. Therefore, the objective of the present study was to assess the distribution of genetic diversity and differentiation within and among Bulgarian populations of the species. The information could be useful for designing of proper management and conservation strategies.

Material and Methods

Sampling and electrophoresis

The populations studied originated from three mountain

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Table 1. Populations studied

Mountain ridge	Population (abbreviation)	Altitude (m)	Sample size
Rila	Malyovitza (MAL)	1900	60
	Semkovo (SEM)	1800	60
	Parangalitza (PAR)	1900	67
	Saragyol (SAR)	2000	44
	Panichishte (PAN)	1800	60
Pirin	Bansko (BAN)	2000	56
	Yavorov (YAV)	1900	62
	Bezbog (BEZ)	1900	56
	Kamenitza	1800	51
Stara planina	Tzarichina (TZ)	1600	60

ridges: Rila, Pirin and Stara planina (Table 1). Dormant winter buds were sampled from fifty to sixty individuals per populations, with the exception of Saragyol – 44 individuals.

The bud tissue was homogenized in Tris-HCl buffer pH 7.0, with 15 mg Dithiothreitol, 5 mg EDTA (Na_2-salt), 150 mg Polyvinil-pyrrholydone (PVP 40), 300 mg sucrose and 0.1 ml β-mercapto-ethanol added just before the homogenization.

Standard 12% horizontal starch gel-electrophoresis was applied in two buffer systems and eight enzyme systems were analyzed (Table 2). The electrophoretic and staining procedures followed those described by Cheliak and Pitel (1984) and Conkle *et al.* (1982), with slight modifications. For the Mendelian inheritance and linkage of allozymes see Zhelev *et al.* (2002).

Data analysis

Diploid genotypes were scored directly from electrophoregrams and the allele frequencies were determined based on diploid genotypes. The genotypic disequilibrium, heterozygote excess and deficiency were tested by using the software Genepop v. 3.3 (Raymond & Rousset 1995).

Genetic diversity within populations was measured by the mean allele number per locus, effective allele number, heterozygosity – expected (*He*) and direct-count (*Ho*), and inbreeding coefficient, expressed as: $F = 1 - Ho/He$. These parameters were calculated by using the software BIOSYS-1 (v. 1.7., Swofford & Selander 1989). The differentiation among populations was measured by Nei's genetic distance (Nei 1978) and by Wright's F-statistics (Wright 1965), as suggested by Weir & Cockerham (1984) and using the software F-STAT (Goudet 1995).

For better interpretation of the genetic distances, Principal Coordinate Analysis (Gower 1966) was applied, using the software SYN-TAX 5.0 (Podani 1993).

Results

Polymorphism and diversity

Allele frequencies are available from authors upon request. Like in most tree species the allozyme gene markers revealed "minor polymorphism" (Lewontin 1985), which means that the frequency of the predominant allele was higher than 0.5, a trend expressed in all populations.

There was no evidence for genotypic linkage disequilibrium among the loci analyzed (data not shown). Positive and in most cases significant values of inbreeding coefficient (F_{IS}) were found, indicating deficiency of heterozygotes relative to Hardy – Weinberg expectations.

The polymorphism and diversity characteristics (Table 3) showed that mean number of alleles varied from 1.8 (Kamenitza) to 2.4 (Yavorov). Effective allele number was within the range 1.105 (Panichishte) and 1.299 (Malyovitza), and the percent of polymorphic loci was relatively low, when 0.05 criterion was applied – from 25 to 50%. A clear elevation gradient was observed in the percent of loci polymorphic (p=0.001), expected (p=0.002) and observed (p=0.007) heterozygosity, all they

Table 2. Enzyme systems studied and buffer systems

Enzyme	E.C. code	Loci	Buffer system
Glutamate-oxaloacetate transaminase	2.6.1.1.	2 (GOT-1, GOT-2)	A
Leucine aminopeptidase	3.4.11.1.	2 (LAP-1, LAP-2)	A
Malate dehydrogenase	1.1.1.37	1 (MDH-4)	TC
Alcohol dehydrogenase	1.1.1.1.	1 (ADH-1)	TC
Phospho glucose isomerase	5.3.1.9.	1 (PGI-2)	A
6-phosphogluconate dehydrogenase	1.1.1.44.	1 (6PGD)	TC
Glutamate dehydrogenase	1.1.1.2	1(GDH)	A
Shikimate dehydrogenase	1.1.1.25.	2 (SKD-1; SKD-2)	TC

Legend: A – buffer system Lithium-borate (pH 8.1) + Tris-citrate (pH 8.1) according to Ashton & Braden (1961); TC – buffer system Tris-citrate (pH 7.0) according to Shaw & Prasad (1970)

Table 3. Polymorphism and diversity in the populations of *Pinus peuce*

Population	Sm	A	Ae	P (0.05)	Ho	He	F
MAL	59.5	1.9	1.299	37.5	0.102 (0.045)	0.121 (0.049)	0.157*
BAN	55.8	2.1	1.273	50	0.156 (0.050)	0.188 (0.066)	0.170*
SEM	60	2.1	1.210	37.5	0.108 (0.042)	0.120 (0.047)	0.100*
YAV	62	2.4	1.159	37.5	0.121 (0.045)	0.129 (0.052)	0.062
TZ	60	2	1.108	25	0.085 (0.043)	0.076 (0.036)	-0.118*
PAR	67	2	1.150	50	0.110 (0.056)	0.113 (0.053)	0.027
KAM	51	1.8	1.150	37.5	0.100 (0.054)	0.124 (0.06)	0.194*
BEZ	56	2	1.168	50	0.107 (0.049)	0.128 (0.056)	0.164*
SAR	44	1.9	1.195	50	0.162 (0.059)	0.158 (0.056)	-0.025
PAN	60	2.3	1.105	37.5	0.075 (0.018)	0.087 (0.02)	0.138*
Mean	57.5	2.05	1.182	41.25	0.112	0.124	0.087*

Legend: *Sm* – average individuals assessed per locus; *A* – mean allele number per locus; *Ae* – effective allele number (the reciprocal of homozygosity); *P* – percent of loci polymorphic (0.05 criterion); *Ho* – observed heterozygosity (standard error in parentheses); *He* – expected heterozygosity (standard error in parentheses); *F* – inbreeding coefficient.
*significantly different from zero at $p \leq 0.05$.

Table 4. Genetic distances between the population pairs (Nei 1978)

	MAL	BAN	SEM	YAV	TZ	PAR	KAM	BEZ	SAR
BAN	0.038	*****							
SEM	0.002	0.042	*****						
YAV	0.003	0.046	0.000	*****					
TZ	0.003	0.038	0.001	0.002	*****				
PAR	0.004	0.049	0.002	0.001	0.005	*****			
KAM	0.006	0.048	0.001	0.003	0.006	0.002	*****		
BEZ	0.005	0.055	0.002	0.002	0.006	0.001	0.001	*****	
SAR	0.010	0.057	0.005	0.006	0.009	0.005	0.003	0.002	*****
PAN	0.010	0.031	0.008	0.012	0.005	0.018	0.014	0.019	0.018

increasing with the increasing of altitude. The expected heterozygosity, i.e., the heterozygosity that should be if the populations meet Hardy-Weinberg expectations, was lower than the observed one in 8 of 10 populations. Respectively, the inbreeding coefficient was positive in 8 cases, and also overall across the populations, even though not always significantly different from zero (Table 3).

Population differentiation

The genetic distances were generally low, ranging from zero to 0.057 (Table 4). These values are typical for coniferous species characterized by extensive gene flow. The most distant population was Banderitza (Bansko), differing from the remaining populations to a considerable extent (Figure 1).

The overall genetic differentiation measured by Wright's F-statistics was 0.083 (Table 5), which means that about 8% of the total diversity should be attributed to the within-population differentiation.

Discussion

Diversity and differentiation

The polymorphism and diversity parameters fall well within the range reported for other tree species with similar life-history characteristics (Hamrick *et al*. 1992) however, at the lower tail. There were substantial differences between some populations indicating different demographic history in the past several centuries. We detected homozygote excess in most populations, which is not unprecedented in pines. Positive, even though not high inbreeding coefficients were reported for some loci in other white pines, like *Pinus strobus* (Epperson & Chung, 2001), *P. longaeva* (Lee *et al*. 2002), *P. sibirica* (Krutovskii *et al*. 1989; Politov & Krutovskii 2004), *P. monticola* (El-Kassaby *et al*. 1987), and *P. albicaulis* (Krakowski *et al*. 2003). Moderately high inbreeding was detected in allozyme studies of other white pines with relatively small and fragmented populations: *Pinus rzedowskii* (Delgado *et al*. 1999), *P. pinceana* and *P. lagunae* (Ledig *et al*. 2001; Delgado

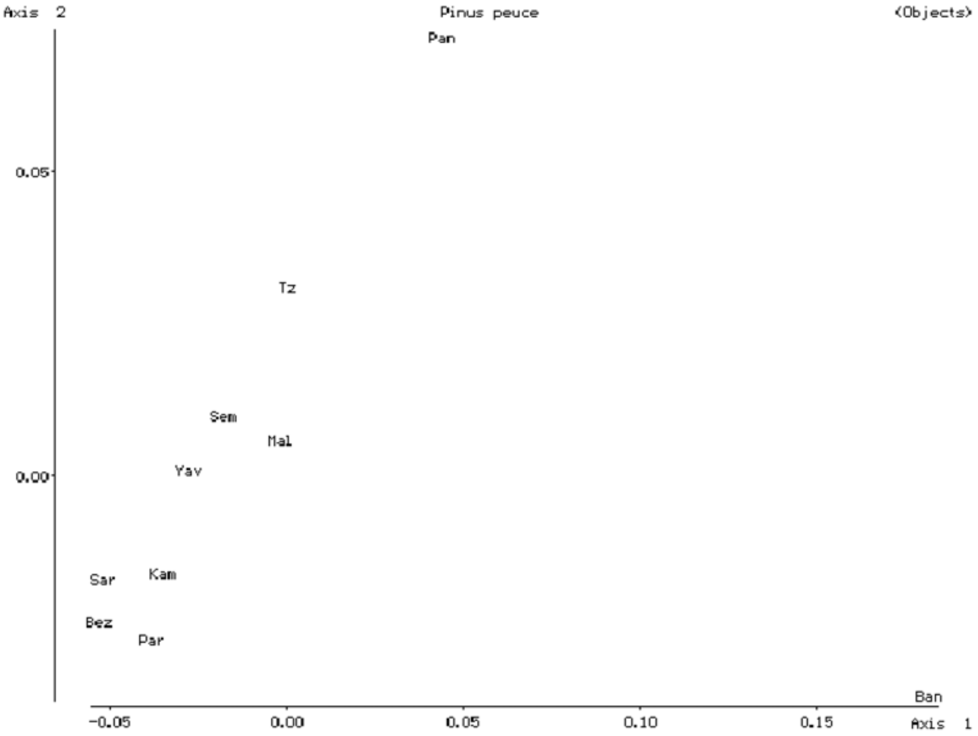

Figure 1. Principal Coordinate Analysis of the populations studied based on the genetic distances.

Table 5. Genetic differentiation among the populations (F-statistics)

Locus	F_{IS}	F_{IT}	F_{ST}
Got1	0.032	0.116	0.087
Got2	0.161	0.407	0.293
Pgi2	-0.054	-0.041	0.012
Gdh	0.29	0.302	0.017
Mdh4	0.223	0.263	0.052
6pg2	0.079	0.112	0.035
Skd1	0.085	0.128	0.047
Skd2	0.039	0.089	0.052
Mean	0.086	0.162	0.083

et al. 2008).

One of the explanations of inbreeding could be in the population demographic history. Some *P. peuce* populations could have originated from a few founders, probably early migrants from higher elevation refugia (Bozilova and Tonkov 2000). Following this hypothesis, we can expect departures from Hardy – Weinberg expectations, which will be related to the specific history of each subpopulation and to the migration processes (Whitlock and McCauley 1990; Whitlock 1992; Le Corre and Kremer 1998).

Pine species with large and continuous ranges generally exhibit low differentiation (Hamrick *et al.* 1992; Delgado *et al.* 1999), while the differentiation is expected to be higher for pine species whose ranges consist of small and disjunct populations (Delgado *et al.* 1999). The distribution pattern of *P. peuce* in Bulgaria allows predicting at least moderate inter-population differentiation, and the value estimated (F_{ST} = 0.083) is slightly higher as compared to other pines in Bulgaria, for example, *Pinus mugo* (F_{ST} = 0.041; Slavov & Zhelev 2004). There was no significant correlation between the pairwise genetic distances and the respective geographic distances, suggesting lack of significant isolation by distance among the populations sampled (data not shown). Low F_{ST} values could indicate extensive gene flow (Neigel 1997), but such interpretation should be taken with caution, because the current population status may be a result of the complex demographic history and nonequilibrium genetic structure (Bossart and Prowell 1998, Whitlock and McCauley 1999). The estimated number of migrants (*Nm*) per generation were 10 (private allele method) and 2.76 (F_{ST} method), respectively, which is not very high, but is theoretically considered enough to minimize the differences among populations due solely to genetic drift.

Implications for conservation

Most populations of *Pinus peuce* in Bulgaria are situated in protected areas, namely the national parks “Rila”, “Pirin” and “Central Balkan”. However, these territories were declared as protected in the 20th century and relatively little is known about their history before. The populations are considered indigenous, which does not necessarily mean unaffected by human pressure. For example, forest fires for releasing pastures were frequent practice until the end of 19th century (Yurukov & Zhelev 2001). Because most of the localities of *P. peuce* are situated near the alpine timber line, some of them might have undergone demographic changes leading to deviations of their expected genetic structure.

Conservation efforts are often more critical for tree species with limited distribution (Molina-Freaner *et al.* 2001) and growing under severe environmental conditions, like *P. peuce*. The allozyme differentiation among the populations included in this study was relatively low and no clear spatial trend was revealed. Differentiation for quantitative traits, however, can be substantially higher than differentiation for allozyme markers (Howe *et al.* 2003). Therefore, data on the genetic variation of adaptive traits in *P. peuce* can be used successfully to preserve the adaptive potential of the species. Even though the studies of Dobrev (2002ab, 2005) were focused on the breeding, they contain enough information to be used for assessment of the differentiation in the quantitative traits. This is an important point because sometimes the differentiation of quantitative traits could be different from one based on neutral molecular markers (Lewontin 1984; Lynch 1996).

The present study indicates that the differentiation among populations is relatively low and no particular population that deserves special measures for conservation could be outlined. Because all the populations of the species in Bulgaria are within protected territories, no special *in situ* measures can be recommended for their conservation. However, periodical monitoring on their demographic structure is desirable in order to take measures, if necessary, for increasing inter-population genetic diversity and to avoid the undesired changes in the plant communities, mostly due to anthropogenic influence and leading to decreasing the natural distribution area of *P. peuce*. Also, the studies initiated with application of DNA microsatellite markers (Paule *et al.*, in prep.) could bring additional insight to the population genetic structure of this Balkan endemic.

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Practical Study for *ex situ* Conservation of Endangered Species *Pinus armandii* Franch var. *amamiana* (Koidz.) Hatusima

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Abstract – *Pinus armandii* var. *amamiana* is a variety of pine that is endemic to only two islands in Japan: Yakushima Island and Tanegashima Island. The total number of trees has been estimated at less than 1,500 on Yakushima Island and around 200 on Tanegashima Island, and the species has been marked as "endangered" in the Red Data Book of Japan. To conserve its genetic resources and prevent extinction, *in situ* conservation is essential. Moreover, *ex situ* conservation is also very important because full seed and seedling production is rarely observed in nature. For the *ex situ* conservation in this study, we used the scions of more than 100 individuals collected from a wide area of natural distribution that had been grafted on *P. thunbergii* or *P. strobus* as rootstocks. Using the plants to produce vigorous seeds, we investigated the conditions required for pollen preservation, the flower bearing and flowering period of each clone, and the technique suitable for artificial crossing. As per the results, pollen preservation was the best by refrigeration or freezing. In the clones bearing only male strobili, only female strobili, or both, the ratios of flower bearing were around 20%, 25%, and 25%, respectively. The flowering period was from late April to mid May and differed among the clones, but later than that of two Japanese indigenous two-needle pines. The artificial crossing method used for *P. thunbergii* Parl., a two-needle Japanese pine, was applied for this variety. In the artificial crossing, the number of full seeds in a cone was ten times that of a naturally occurring var. *amamiana*. By the artificial crossing in the spring of 2003, we obtained more than 4,000 full seeds in the autumn of 2004. We sowed ca. 3,600 grains of them in the spring of 2005, which produced more than 1,300 seedlings in the winter of 2007. This number is nearly as much as the number of individuals existing in nature. These results will aid in successive *ex situ* conservation and may provide useful information to develop a conservation strategy for this endangered species.

Key words: *Pinus armandii* var. *amamiana*, endangered species, *ex situ* conservation, seed production, propagation

Introduction

Pinus armandii var. *amamiana* is an endemic variety of pine occurring on only two islands in Japan: Yakushima Island and Tanegashima Island (Fig. 1). Its wood has been used for fishing canoe and house construction. The total number of trees has been estimated at less than 1,500 on Yakushima Island (Yamamoto and Akasi 1994) and around 200 on Tanegashima Island (Kanetani *et al.* 2004a). On Yakushima Island, three populations–at Takahira, Hirauchi, and Hirase (cited as "Segire" in Kanetani *et al.* 2004b)–have been identified. On Tanegashima Island, only 51 individuals were known; however, a new population comprising 138 individuals has been found by Kanetani (Kanetani *et al.* 2004a). The number of trees on each island has been rapidly diminishing, and this variety has been marked as "endangered" in the Red Data Book of Japan (Environmental Agency 2000). The reasons for this decline include succes-

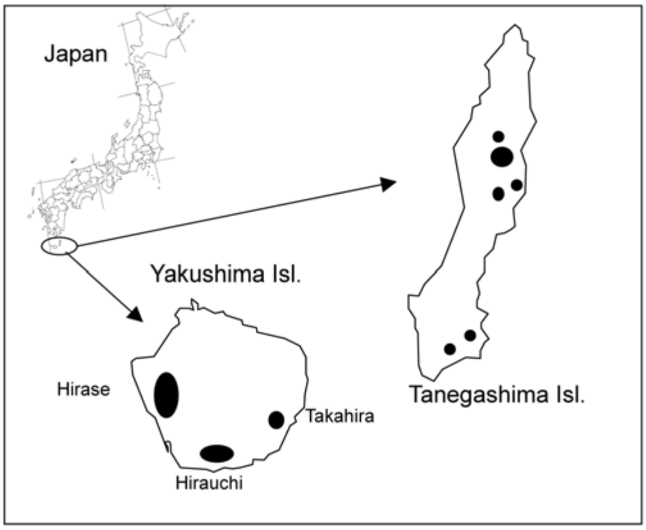

Figure 1. Natural distribution of *Pinus armandii* var. *amamiana*, with populations on each island.

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sion (Environmental Agency 2000), inbreeding depression (Hayashi 1988; Kanazashi *et al.* 1998), reduced natural regeneration (Chigira 1995; Kanetani *et al.* 1998), and pine wilt disease (Hayashi 1988; Yamamoto and Akashi 1994; Environmental Agency 2000; Nakamura *et al.* 2002).

On studying the genetic variation of this variety, Kanetani *et al.* (2004b) found that *P. armandii* var. *amamiana* maintains a more or less high genetic diversity with a fixation index of almost zero for all populations, although its distribution is limited. The genetic differences among this variety and the other two varieties – are as high as the species level, rather than at the conspecific level. Ishii *et al.* (2004) and Maruyama *et al.* (2007) developed a technique that makes it possible to regenerate the plants by tissue culture as explants from mature and immature embryos of full seeds. These studies seem to be highly valuable for *in situ* and *ex situ* conservation of this variety.

However, as already reported, in the natural forest, both seedlings and full seeds are rarely observed, which is the case with even the newly found young populations (Kanetani *et al.* 2004a). To conserve this variety not only for this generation but also for subsequent ones, it is essential to improve the techniques for mating, genetically-sound seed production, and healthy seedling production.

At the Kyushu Regional Breeding Office, we had conserved more than 100 clones of var. *amamiana* by grafting them as a clone bank and experimental seed orchard. The scions were collected from three populations of Yakushima Island and Tanegashima Island, and were grafted on rootstocks of *P. thunbergii* or *P. strobus*.

In this study, using the conserved clones, we investigated the flowering period, pollen preservation method, artificial crossing method, seed quality of the crossing seed, and seedling growth in order to develop a practical method for *ex situ* conservation.

Materials and Methods

Flowering period

Of the conserved clones at the Kyushu Regional Breeding Office, we selected 30 clones bearing male or female strobili, or both: 10 clones originated from Takahira, 10 from Hirauchi, and 10 from Hirase on Yakushima Island. From April 20th to May 26th, 2004, we observed the flowering procedure of the male and female strobili and recorded it according to the criteria in Table 1.

Pollen preservation

Of the conserved clones, we collected pollen from 4 clones in May 2002: they are native to Takahira (one clone), Hirase (two clones), and Hirauchi (one clone). They were preserved under the following conditions: (i) in desiccators with silica gel at room temperature, (ii) in a refrigerator (4 degree Celsius) packed in a plastic bag with silica gel, and (iii) in a freezer (-20 degree Celsius) packed in a plastic bag with silica gel after one week of refrigeration. After one year of preservation (i.e., in May 2003), we dispersed pollen on a pollen germination medium (1% agar and 5% sucrose) in three dishes per clone, as a single treatment. We incubated the dishes for 5 days at 30 degree Celsius in a dark condition and then counted the germinated pollen in each dish under light microscopy. As a control, we collected pollen in May 2003 from the same clones and immediately tested their germination.

Table 1. Criteria of the male and female strobili flowering procedure

Strobili	Flowering index	Flowering period	Status
Male	0	Before blooming	Strobiles covered with scales
	1	Beginning period	Microsporophyll extends, beginning of anthesis
	2	Middle period	Pollen dispersal
	3	Late period	Late stage of pollen dispersal
	4	After blooming	-
Female	0	Before blooming	Strobiles covered with scales
	1	Beginning period	Ovuliferous scale and bract scale emerge from surrounding scales
	2	Early period	Ovuliferous scale as long as bract scale
	3	Middle period	Ovuliferous scale grows and is twice as long as bract scale
	4	Late period	Ovuliferous scale thickens and is much longer than bract scale
	5	After blooming	Ovuliferous scales neighbor each other, bract scale invisible

Indexes 2 and 3 of the male strobili and indexes 2, 3, and 4 of the female strobili seem to be effective for pollination.

Artificial crossing method

We have much experience of artificial crossing in Japanese black pine (*Pinus thunbergii* Parl.). For *P. armandii* var. *amamiana*, we made attempt to apply the method for *P. thunbergii* with consideration of the procedure and its schedule.

Seed quality

In September of 2003, 2004, 2005 and 2006, a year and four months after crossing each, we collected cones. We dried the cones at room temperature, recovered all the seeds, and counted them. We then selected full seeds by using 100% ethanol and counted these. In March of the next years we sowed the seeds in a nursery. After three months, we counted the number of seedlings.

Seedling growth

In March 2006, a year after sowing seeds collected in 2004, we transplanted the seedlings in four blocks. In December 2007, we measured the seedling height and base diameter.

Results

Flowering period

Figure 2 shows the results of the flowering periods of all clones in 2004. Flowering began in late April. Male strobili dispersed pollen from early to mid May, and the dispersal was complete by late May. The flowering season was later than that of two Japanese indigenous two-needle pines, *Pinus thunbergii* Parl. (mid to late April) and *Pinus densiflora* Sieb. et Zucc. (late April to early May).

Flowering periods varied within each population. It seemed to be difficult for early flowering and late flowering clones to pollinate each other. Comparing the populations, Hirase had a flowering period that was a little later than that of the other two populations.

Of the 30 selected clones, 19 bore male strobili. Flowering began first in Takahira94-13 (anthesis on April 20th) and last in Hirauchi95-32 (anthesis ten days later on April 30th). Pollen dispersal occurred 10-14 days (average: 12.8 days) after anthesis. The period of dispersal (flowering indexes 2 and 3) ranged from the 4th to the 22nd of May (maximal dispersal from the 6th to the 12th; average period: 8.2 days).

Of the 30 selected clones, 24 bore female strobili. Their flowering periods were a little later than that of the male strobili. Takahira 96-29 was the first to begin flowering (emergence of ovuliferous scale and bract scale on April 24th). The last clone to begin flowering was Hirase 93-24 (the emergence of the scales started 16 days later on May 10th). The average durations from the start to each stage of flowering were 6.8 days until the early period (flowering index 2), 10.0 days until the middle period (flowering index 3), and 14.3 days until the late period (flowering index 4). The duration when the female strobili could pollinate (flowering indexes 2-4) was from the

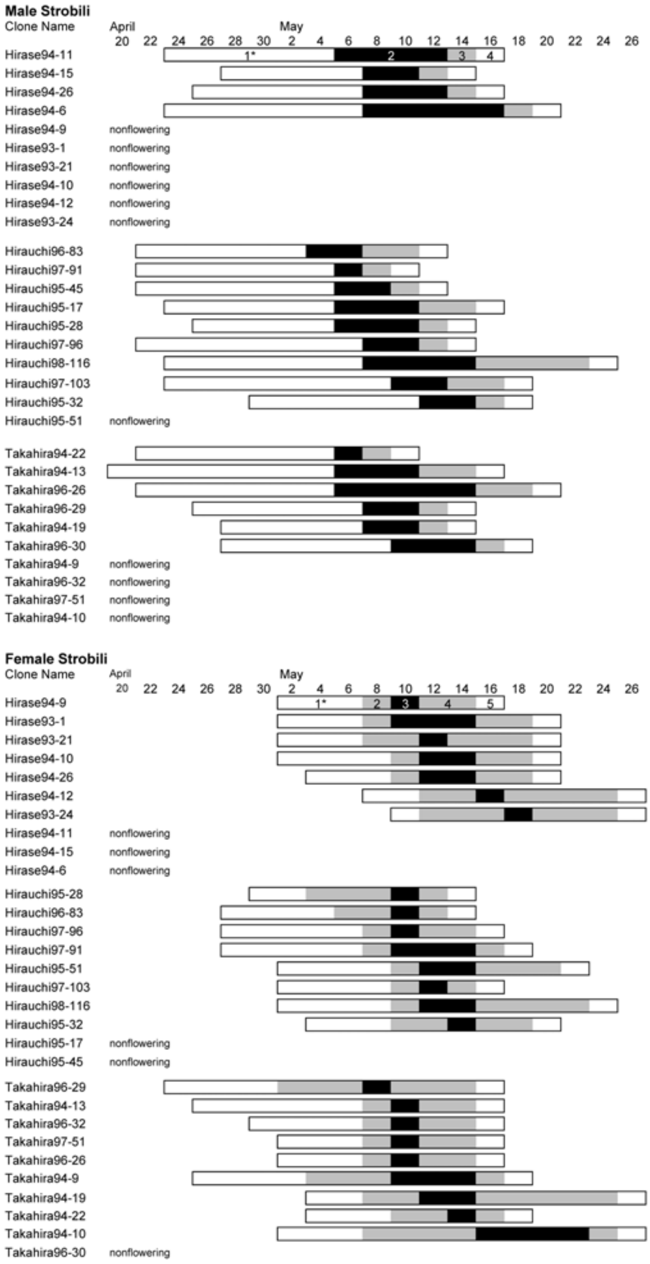

Figure 2. Flowering periods of *Pinus armandii* var. *amamiana* clones of the three populations on Yakushima Island in 2004. *Flowering indexes as per Table 1.

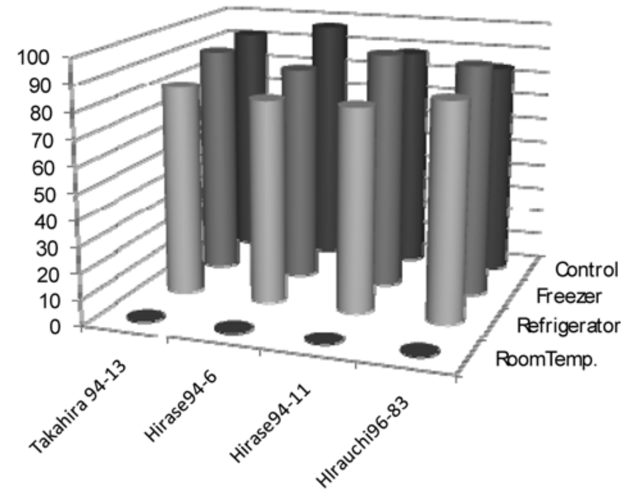

Figure 3. Pollen germination rate of *Pinus armandii* var. *amamiana*.

2nd to the 24th of May, of which the period from the 8th to the 18th (flowering index 3) was the most adequate for pollination.

Pollen preservation

Figure 3 shows the results of the germination rate per clone and per preservation method along with that of the controls. The rate was the least in the room temperature storage, with no pollen grain germination observed for any clone. On the contrary, high germination rates were observed in the refrigerator and freezer storage: more than 80% of the pollen grains from each clone germinated. In the statistical analysis, the germination rate in freezer storage was higher than that in refrigerator storage (at the 5% significant level). The germination rate after one year of freezer storage was similar to that of the controls.

Artificial crossing method

Because we were able to obtain high quality seeds shown in Table 2, the application of the method of *P. thunbergii* to *P. armandii* var. *amamiana* was successful. The schedule of the procedure of crossing was prepared by investigation of the flowering period. The procedure and its schedule were as follows: we covered female strobili-bearing branches with paper bags after removing nearby male strobili by hands in late April. In this duration, no pollen grain was dispersed and female strobili were covered with scales or a few ovuliferous scales and bract scales emerged. We collected male strobili-bearing branches in early May just before pollen dispersal. These were placed in paper bags and left to dry in a room.

Table 2. Seed quality on various types of crossing of *Pinus armandii* var. *amamiana*

Seed collection year	Type of crossing		Number of families	Number of seeds per cone	Full seed ratio among all seeds (%)	Number of full seeds per cone	Germination rate of full seeds (%)
2006	Artificial	Self	3	26.8	0.2	0.1	not sown3)
		Between populations	4	35.3	55.7	19.7	not sown3)
		Within a population	49	33.6	52.9	17.8	80.5
	Open pollinated at Kyushu Regional Breeding Office1)		31	7.4	22.9	1.7	73.7
2005	Artificial	Self	4	26.5	9.8	2.6	44.0
		Between populations	10	25.4	72.3	18.3	70.3
		Within a population	10	34.5	64.5	22.2	60.8
	Open pollinated at Kyushu Regional Breeding Office1)		17	15.1	31.8	4.8	71.5
2004	Artificial	Self	3	24.8	1.3	0.3	not sown3)
		Between populations	12	37.4	69.1	25.9	80.3
		Within a population	4	38.4	48.6	18.7	74.9
2003	Artificial	Self	4	25.6	12.2	3.1	not sown4)
		Between populations	8	33.1	55.8	18.5	not sown4)
		Within a population	7	39.8	59.2	23.5	not sown4)
	Open pollinated at Kyushu Regional Breeding Office1)		12	10.7	39.7	4.3	not sown4)
2002	Open pollinated at three populations of Yakushima Island2)		16	5.4	30.3	1.6	55.0
2001	Open pollinated at three populations of Yakushima Island2)		20	4.8	21.9	1.1	57.9

1) the seeds were collected from clone bank of Kyushu Regional Breeding Office,
2) the seeds were collected in natural forest in Yakushima Island,
3) because of very few seeds, 4) because of loss of activity caused by high temperature in drying

After drying, we collected pollen, cleaned it, and dried it again in desiccators with silica gel. The pollen was sprayed, by using a pollen gun, into the paper bags covering the female strobili twice or thrice between early to mid May (i.e., the early and middle periods of female strobili flowering). We removed the paper bags in early June, by when flowering was complete and no pollen dispersed.

Seed quality

Table 2 shows the results of the investigation of seed quality. The average number of seeds per cone in the artificial crossing was 24.8-39.8 grains. This number was more than that from the cones collected in the forest on Yakushima Island, and was also more than that of the open-pollinated family in the experimental seed orchard and clone bank at Kyushu Regional Breeding Office. There were no obvious differences in artificial crossing within populations and between populations. In the self-pollinated type, the number of seeds was low, but more than that from the cones collected in natural forest at Yakushima.

The average ratio of full seeds was high in the artificial crossing for both within populations and between populations. The ratio between populations was higher than within populations in 2004, but the ratios were more or less similar in the other three years (2003, 2005, and 2006).

The average number of full seeds per cone in the artificial crossing was 17.8-25.9, which was more than that from the cone collected in the forest at Yakushima. In the self-pollinated type, this number was very small – 3.1 grains at the most.

The average germination rate of full seeds was high in the artificial crossing, of which the rate between populations was higher than that within populations.

Seedling growth

Table 3 shows the numbers of transplanted individuals and survival rates one year and eight months after transplantation.

Table 3. Numbers of transplanted individuals and survival rates one year and eight months after transplantation

Male parent Female parent	Takahira 94-13	Hirauchi 96-83	Hirase 94-11	Hirase 94-6
Takahira94-13	-	62 (56%)*	144 (42%)	171 (20%)
Takahira94-22	-	144 (37%)	-	173 (6%)
Hirauchi96-72	225 (53%)	-	104 (34%)	195 (44%)
Hirauchi96-82	-	242 (57%)	175 (38%)	251 (45T)
Hirase94-9	247(74%)	342(80%)	174(32%)	133(26%)

*number of transplanting (survival rate)

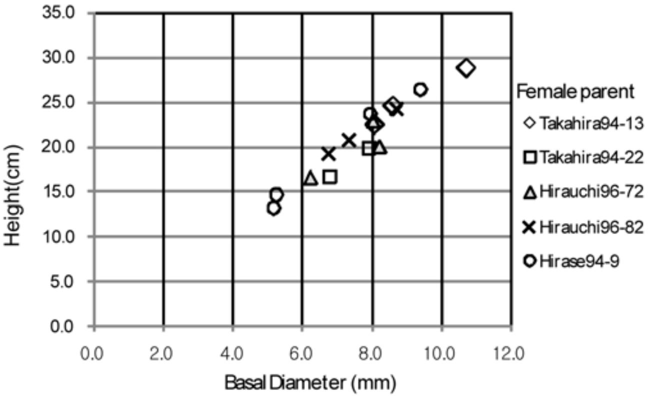

Figure 4. Seedling growth of *Pinus armandii* var. *amamiana* two years and eight months after sowing.

Totally, 1,333 of 2,887 individuals survived after transplantation. The average survival rate was 46%, which is a little less than that in the case of *Pinus thunbergii*. One-way analysis of variance using families as the factor showed that the difference among the families was at the 1% significant level. The survival rate of Takahira94-22×Hirase94-6 was 6%, which was the least among the families examined.

The average size of the seedlings two years and eight months after sowing (one year and eight months after transplantation) was 23.0 cm in height and 8.2 mm in basic diameter. This is much smaller than that of the same-age seedlings of *Pinus thunbergii*: 60.3 cm in height and 26 mm in basic diameter. In the seedling height and basic diameter, one-way analysis of variance using families as the factor showed that the difference among the families was at the 1% significant level. The tallest family was twice that of the smallest one. The families of which Takahira94-13 was female or male parent in crossing tended to be larger than the other families (Figure 4). This indicates that the parents have a genetic capacity for seedling growth.

Discussion

To conserve the genetic resources of endangered species and prevent extinction, *in situ* conservation is essential. In the case of *P. armandii* var. *amamiana*, because there are several populations genetically different from each other, all the populations need to be conserved. Many field investigations have been conducted by research, nonprofit, and governmental organizations. They have collaborated with each other for *in situ* conservation. As a result of this effort, the preservation of individuals of the current generation has progressed.

However, as has already been reported, in natural forest, the full seed ratio per cone is very low, as is the germination rate of seeds, and only few seedlings have been observed. These phenomena are thought to be caused by various factors, both individually and in combination. If the situation does not change, all trees of the current generation will die of old age and very few individuals of the next generation will be produced, resulting in the extinction of this variety in the future. To prevent such an occurrence, it is necessary to produce many individuals of the next and succeeding generations.

Our study makes it possible to produce genetically healthy seeds via *ex situ* conserved individuals. The seed production efficiency (per cone) of this method compared with that of the natural forest is 6-8 times greater, with 2-2.5 times more full seeds among all seeds and a total of 10-20 times more full seeds per cone. From the full seeds, we can grow healthy seedlings, the ratio of which is nearly 50%. This efficiency may reach the level of seedling production of forestry species for man-made forest, such as *P. thunbergii* or *P. densiflora*. By the artificial crossing in the spring of 2003, we obtained 4,393 full seeds in the autumn of 2004. We sowed 3,648 grains of them in the spring of 2005, which produced 2,887 seedlings in the spring of 2006. In the winter of 2007, 1,333 individuals of them still survived through transplantation. This number is nearly as much as the number of individuals existing in nature.

As for the genetic diversity, because our collection was obtained from all over representative populations (three populations from Yakushima and Tanagashima Islands), we probably secured a wide range of variation. However, our collection (100 or 150 clones) is less than one-tenth of that occurring in nature. Therefore, rare genes may not have been collected, which is an inevitable limitation of *ex situ* conservation. It is not practical to preserve all clones (more than 1,500) *ex situ*.

Our study will contribute to the progress in the conservation of *P. armandii* var. *amamiana*. One such contribution will be in improving the functioning of the seed orchard at the Kyushu Regional Breeding Office: open-pollinated seeds in this orchard are not of high quality. This may have been caused by a mismatch in the flowering period of neighbor clones, therefore the rate of self-pollination becomes rather high. If the clones are arranged according to adjacent flowering periods, the rate of outcrossing will increase and the seed quality will improve. Moreover, the time and cost involved in artificial crossing can be saved. This may also be applicable to the seed orchard established on Yakushima Island by the Kyushu Regional Forest Office of the Forestry Agency.

Our study also aimed at improving the resistance to pine wilt disease, which has resulted in a reduced number of individuals of *P. armandii* var. *amamiana* due to infection with the nematode of the disease. In this variety, the resistance to the nematode is not well known; however, according to Toda (2004), it is possible to select resistant individuals from seedlings after artificial inoculation of the nematode. After inoculating the nematode to the seedlings of crossed families, the surviving seedlings can be used as nematode-resistant individuals.

The benefit of our method is that it is not high-tech. The most complicated process is artificial crossing. The other process, such as collecting, selecting, and sowing seeds, and growing the seedlings, are usual techniques followed by any plant producer. Therefore, it can be carried out by schoolchildren, citizens, and nonprofit organizations after the brief technical training.

Although we consider our study to be fruitful, *ex situ* conservation is limited by the inability to collect a part of the genetic variation occurring in nature. Therefore, *in situ* conservation is very important. Both these methods of conservation have benefits and drawbacks, and therefore both should be applied to compensate each other effectively.

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Proceeding
Breeding and Genetic Resources of Five-Needle Pines:
Ecophysiology, Disease Resistance and Developmental Biology
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An Assessment of Genetic Variation of *Pinus albicaulis* Populations from Oregon and Washington in Relation to Height Increment, Phenology, and Form

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Abstract – It is desirable to ascertain patterns of genetic variation associated with provenance origin for any species that warrants intensive conservation efforts. Whitebark pine (*P. albicaulis*) is a species in the western United States that is currently experiencing a major dcline in health (accelerated mortality from cumulative effects of disease, bark beetles, and additional environmental stresses). Very little common garden data is available on whitebark pine from any part of its range (Mahalovich *et al.* 2006) or from Oregon and Washington provenances (Bower and Aitken 2008).

A complementary study to a planned white pine blister rust resistance evaluation experiment at the Dorena Genetic Resource Center was undertaken in order to evaluate genetic variation of height growth increment in whitebark pine (*P. albicaulis*). The study included a total of 100 families from 6 provenances located in Oregon and Washington with a variable number of parent trees (families) per provenance (Table 1). Wind-pollinated seed was sown in a container nursery in 2004 and then transplanted to a common garden raised bed for future assessment of disease resistance. Seedlings were inoculated with blister rust in September 2005, and height and phenology assessments were made from April 2006 - July 2006 before any adverse effects of the rust were noted. The experimental design was a randomized complete block with families planted in row-plots (up to 10 trees/family/plot). Phenology patterns, relative growth rates, total growth increment, and tree stem form were assessed and quantified.

Table 1. Provenance locations and sample size (# parent trees) in the common garden study

Provenance	Latitude (°N)	Longitude (°W)	Elevation (m)	Sample Size (N)
1. Mt. Rainier	46.9	121.6	1859-1939	19
2. Mt. Hood	45.3	121.7	1832-1898	19
3. Warm Springs	44.7	121.7	1676	7
4. Crater Lake	42.9	122.1	2146-2164	9
5. Malheur	44.7	118.6	2233-2405	19
6. Umatilla	44.7	118.6	2289-2332	27

The common garden locale was located near Cottage Grove, Oregon: 43.7°N latitude, 122.9°W longitude, 128 m in elevation.

Eight serial height measurements were taken throughout the third growing season in three of the six replications. Cumulative height increment was computed as well as percent of total seasonal height growth and percent of growth cessation (where growth cessation was equated to $\geq$ 95% of total seasonal height growth) at each serial measurement. A score (1 = single stem, 2 = forked, or 3 = numerous multi-stems/bush) was also given for the general stem form of the seedlings at the end of the growing season. Statistical analysis procedures were conducted using SAS (SAS Institute v. 9.1, 2007); provenance was considered as a fixed effect, while block, family within provenance, and associated interactions were considered as random effects in the general linear model.

Least square means for all serial height increments differed significantly ($P < .05$) among provenances, and there was significant variation among families within provenances at all serial measurement times. The cumulative height increment rank changed among provenances until the third periodic measure, after which the provenances' general rankings remained the same throughout the rest of the growing season (Figure 1). The northwestern provenances (Mt. Hood, Mt. Rainier, Warm

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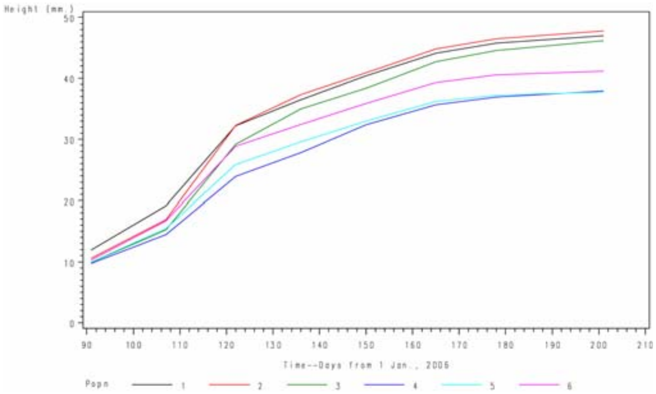

Figure 1. Cumulative height increment over time.

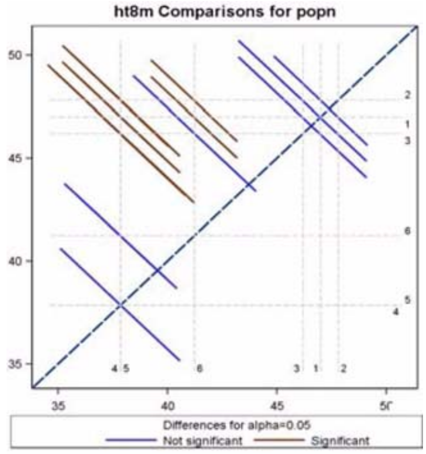

Figure 2. Statistical significance (P<.05) among provenance means for season's height increment. Height increment shown on vertical and horizontal axes (mm). Provenance (1…6) represent: Mt. Rainier=1, Mt. Hood=2, Warm Springs=3, Crater Lake=4, Malheur=5, Umatilla=6.

Springs) formed a general group with the maximum height. The Umatilla provenance was intermediate in height, followed by the Malheur and Crater Lake provenance grouping. Statistical comparisons among provenance means for total height increment is shown in the SAS - Proc Glimmix plot (Figure 2).

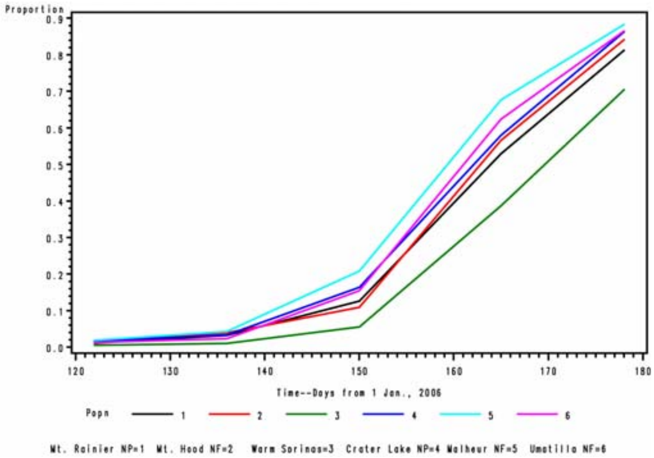

Figure 4. Proportion of growth cessation over time.

Individual tree heritability was 0.27 (family mean range = 25-64 mm for total height increment). The parent tree source environmental variables of elevation, longitude, latitude, and associated climatic means (average monthly high and low temperatures, precipitation) were assessed in a regression on total height increment. Precipitation (average amounts in September + February + November + May) explained 34% of the variation.

The percentage of the seasonal height growth completed (Figure 3a & b) and percentage of growth cessation (Figure 4) at each measurement date were also computed. Significant differences existed among provenances in the estimates of shoot elongation timing, where percent of growth completed at measurement times 1 and 2 was used as an indicator of initial growth (average family heritability=0.43 for the two measurement times). Twenty seven % of the variation was explained by temperature and latitude (average minimum temperature in December + $(\text{latitude})^2$ + average maximum temperature in January) in a regression on the second serial measurement (mean range=35-42% of total seasonal growth).

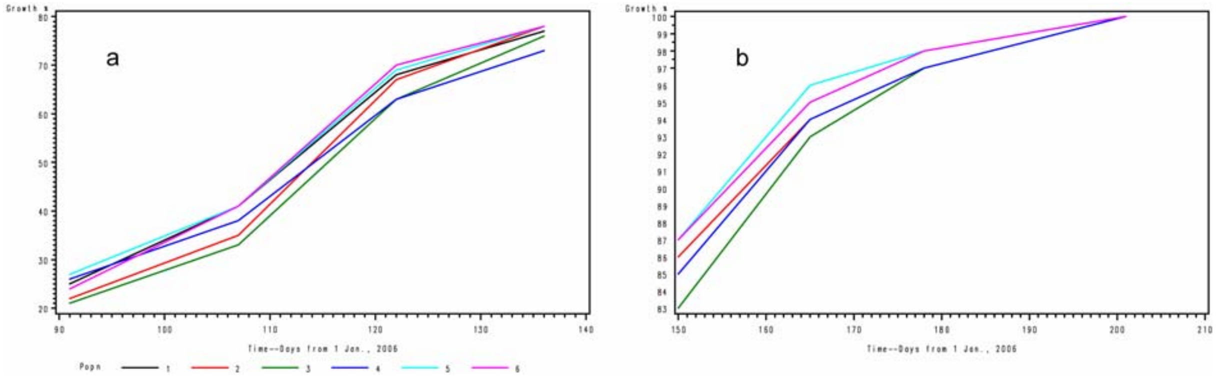

Figure 3. Percent of seasonal height increment completed over (a) early and (b) late season.

Significant differences existed among provenances in percentage of growth cessation at measurement times 5, 6, and 7 (average family heritability = 0.19). Twenty four % of the variation was explained by elevation + average maximum temperature in May for the sixth serial measurement (mean range = 39-67% cessation).

The least square means for stem form differed significantly among provenances. The rank order of the more desirable tree form class to the less desirable tree form class was closely aligned with the rank order (from tallest to shortest) of the final height per respective provenance.

Significant differences were noted among provenances in relation to height increment, relative percentage of growth completed and growth cessation during the season. These differences along with the low to moderate heritability estimates suggest a measurable degree of genetic adaptation across the sampled species range. These preliminary results indicate an adaptive response to the source elevation and latitude.

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Conservation Status and Breeding Work of Conifer Species in Vietnam with Reference to Pines

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Abstract – Among more than 50 conifer species found in Vietnam, the country has 33 native species which belong to 19 genera of six families. There are seven endemic species which include two species from Cupressaceae (*Cupressus torulosa* and *Calocedrus rupestris*), two species from Pinaceae (*P. dalatensis* and *P. krempfii*), two species from Taxaceae (*Amentotaxus hatuyenensis* and *A. poilanei*) and one species from Taxodiaceae (*Xanthocyparis vietnamensis*). Based on International Union for Conservation of Nature (IUCN) (2001) categories, the native conifer species in Vietnam can be classified as CR (3 species), EN (11), VU (11), NT (1), LC (4) and three species were not evaluated (NE) due to lack of information. Among three critically endangered species, *C. torulosa* has only some seedlings in the wild and 40 trees planted in Huu Lien Nature Reserve; *Glyptostrobus pensilis* has only two populations in one province, the first population has only 30 trees and the second population has 230 dying trees; and *Taxus wallichiana* has less than 250 mature individuals in one province in South Vietnam. Among six *Pinus* species in Vietnam, three are in the subgenus Strobus. Of these, *P. kwangtungensis* is classified as EN C2a due to its limited distribution range and low number of mature individuals while *P. krempfii* is classified as VU A1cd due to its limited distribution range and poor natural regeneration. *P. dalatensis* is considered as NT due to fragmentation in its natural distribution range. All of them will require significant effort to ensure their future. Breeding work commenced with species trials established for some exotic and native pines and provenance trials established for *P. kesiya, P. merkusii* and *P. caribaea*. A species trial established in 1980 which included 18 species showed that among exotics, only three species namely *P. caribaea, P. oocarpa* and *P. elliottii* were suitable for planting. The provenance trial for *P. kesiya* showed that promising provenances are Simao (China) and Tarlac (Philippine) for the Ba Vi site (lowland, North Vietnam), while Doi Suthep (Thailand) and Prenn Waterfall (Vietnam) showed promise for the Lang Hanh site (highland, South Vietnam). Plus tree selection for growth was applied for *P. kesiya* and *P. massoniana* while micro-chipping technique was applied for selecting high resin yielding plus trees and establishing seed orchards of *P. merkusii*. Four hundred and nine candidate plus trees were selected for *P. merkusii* for high resin yield, then 275 trees (67.2%) were chosen for both growth and resin yield and only the 40 best trees were selected for seed orchard establishment.

Key words: Conifer, white pines, conservation, breeding, Vietnam

Introduction

Conifer species and coniferous forests are very popular forest resources in many areas of the world. With more than 600 conifer species along with 250,000 species of angiosperms, they still play an important role in the economy and landscapes of many countries from America, Europe, and Asia to Australia and New Zealand.

Conifers and coniferous forests are also beautiful and are typical landscapes across Vietnam. They are usually distributed on higher elevations, such as *Pinus kesyia, P. krempfii, P. dalatensis, Dacrydium elatum, Podocarpus imbricatus* and *Fokienia hodginsii* which grow at more than 1500 m above sea level (a.s.l.) in Da Lat city. Some species can be planted at lower elevation, such as *P. merkusii* and *P. massoniana* in North Vietnam. Some other conifer species have been introduced into Vietnam such as *Platycladus occidentalis, Araucaria* and other pine species for planting as ornamental and bonsai plants and they have become as popular as native pines.

Conifers are generally considered as having an ancient origin, evolving about 300 million years ago. Some Vietnamese conifers are also ancient species, such as *Calocedrus macrolepis, Glyptostrobus pensilis* and *Pinus krempfii*, but other species such as *Pinus kesiya* are considered as newly evolved conifers (Nguyen and Thomas, 2004). *Glyptostrobus pensilis* is known to have fossils in Dong Giao (Ninh Binh province)

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and North-west provinces of Vietnam and fossils of the species were also discovered in Belgium in Europe. The species is currently only found in Vietnam and China. With high scientific value, old origin and hiddened threats, the conservation of conifer species in Vietnam requires imperative action. This paper gives an overview of the native and exotic conifers of Vietnam, with a special reference to the three native pines of subgenus Strobus.

Conifer species in Vietnam

There are more than 50 conifer species found in Vietnam, and the country has 33 native species which belong to 19 genera of 6 families. The two species *P. massoniana* and *Cunninghamia lanceolata* which were reported to be introduced many years ago from South China and are widely planted in

Table 1. Native conifer species of Vietnam and their endangeredment according to IUCN (2001) categories (modified from Nguyen, 2004)

	Species and synonyms	Family	Endangeredment
1	*Cephalotaxus mannii* Hook.f. *Cephalotaxus oliveri* Mast. *Cephalotaxus hainanensis* H.L.Li *Cephalotaxus drupacea* Sieb. et Zucc.	Cephalotaxaceae	EN C2a***
2	*Calocedrus macrolepis* Kurz *Libocedrus macrolepis* (Kurz) Benth.	Cupressaceae	VU A1cd
3	Calocedrus rupestris sp.nov.*	Cupressaceae	EN D
4	*Cupressus* sp* *Cupressus torulosa* D.Don	Cupressaceae	CR D
5	*Fokienia hodginsii* Henry & Thomas	Cupressaceae	VU A1cd
6	*Abies delavayi* sp *fansipanensis* Rushforth *Abies fansipanensis* Q.P.Xiang *Abies delavayi* var. *nukiangensis*	Pinaceae	EN D
7	*Keteleeria davidiana* (Bertr.) Beissn. *Keteleeria davidiana* var. *calcarea* Silba *Keteleeria calcarea* W.C.Cheng & L.K.Fu	Pinaceae	EN D
8	*Keteleeria evelyniana* Masters *Keteleeria roullettii* (A.Chev) Flous *Keteleeria davidiana* auct. Non Beiss. Hick.	Pinaceae	VU A1cd
9	*Pinus dalatensis* de Ferre*	Pinaceae	NT
10	*Pinus kesiya* Royle ex Gordon *Pinus khasya* Royle ex Hook.f. *Pinus insularis* Endl.	Pinaceae	LC
11	*Pinus krempfii* H.Lecomte * *Ducampopinus krempfii* (Lec) A.Chev.	Pinaceae	VU A1cd
12	*Pinus kwangtungensis* Chun ex Tsiang *P. kwangtungensis* var. *varifolia* Li & Zhong *P. fenzeliana* Hand-Mazz	Pinaceae	EN C2a
13	*Pinus massoniana* D.Don**	Pinaceae	LC
14	*Pinus merkusii* Jungh. & de Vries *Pinus merkusana* Cooling & Gauss. *Pinus lateri* Mast.	Pinaceae	LC
15	*Pseudotsuga brevifolia* Cheng & Fu *Pseudotsuga sinensis* Dode var. *brevifolia* *Pseudotsuga sinensis* Dode	Pinaceae	VU A1cd
16	*Tsuga sinensis* var. *sinensis* Pritzel ex Diels *Tsuga sinensis* (Franchet) Pritzel ex Diels *Tsuga dumosa* (D.Don) Eichle *Abies chinensis* Franch.	Pinaceae	EN D
17	*Dacrycarpus imbricatus* (Blume) de Laub. *Podocarpus imbricatus* Blume	Podocarpaceae	VU A1cd
18	*Dacrydium elatum* (Roxb.) Wall *Dacrydium pierrei* Hickel	Podocarpaceae	VU A1cd

Table 1. Continued

	Species and synonyms	Family	Endangeredment
19	*Nageia fleuryi* (Hickel) de Laubenfels *Podocarpus fleuryi* Hickel *Decussocarpus fleuryi* (Hickel) de Laub.	Podocarpaceae	EN B1
20	*Nageia wallichiana* (Presl.) Kuntze *Podocarpus wallichianus* Presl	Podocarpaceae	VU A1cd
21	*Podocarpus neriifolius* D.Don *Podocarpus annamiensis* N.Gray	Podocarpaceae	VU A1cd
22	*Podocarpus pilgeri* Foxworthy *Podocarpus brevifolius* (Stapf) Foxw *Podocarpus neriifolius* var *brevifolius* Stapf	Podocarpaceae	VU A1cd
23	*Amentotaxus argotaenia* (Hance) Pilger	Taxaceae	NE
24	*Amentotaxus hatuyenensis* N.T.Hiep*	Taxaceae	EN D
25	*Amentotaxus poilanei* D.K.Ferguson*	Taxaceae	NE
26	*Amentotaxus yunnanensis* H.L.Li	Taxaceae	NE
27	*Taxus chinensis* Pilger *T. baccata* sp. *cuspidata* var. *chinensis* Pilg *T. celebrica* auct. Non (Warb.) H.L.Li	Taxaceae	EN D
28	*Taxus wallichiana* Zucc. *T. baccata* sp. *wallichiana* (Zucc.) Pilg.	Taxaceae	CR C2a
29	*Cunninghamia konishi* Hataya *Cunninghamia kawakami* Hataya *Cunninghamia lanceolata* var *konishi* Fujita	Taxodiaceae	VU A1cd
30	*Cunninghamia lanceolata* (Lamb) Hook.f.**	Taxodiaceae	LC
31	*Glyptostrobus pensilis* (Staunton) K.Koch	Taxodiaceae	CR D
32	*Taiwania cryptomerioides* Hataya	Taxodiaceae	EN D
33	*Xanthocyparis vietnamensis* Farjon & Hiep*	Taxodiaceae	EN D

*Considered as endemic
**Considered as “native” or “localized”
***CR: critically endanged; EN: endanged; VU: vulnerable; NT: near threatened; LC: less concerned; NE: not evaluated.

North Vietnam can be considered as “naturalized” or “localized” species.

There are seven endemic species of Vietnam which include two species from Cupressaceae (*C. torulosa* and *Calocedrus rupestris*), two species from Pinaceae (*P. dalatensis* and *P. krempfii*), two species from Taxaceae (*Amentotaxus hatuyenensis* and *A. poilanei*) and one species from Taxodiaceae (*Xanthocyparis vietnamensis*).

Based on IUCN (2001) categories, the native conifer species in Vietnam can be classified as CR (3 species), EN (11), VU (11), NT (1), LC (4) (Table 1) and three species were not evaluated (NE) due to lack of information. Among six *Pinus* species in Vietnam, there are three from subgenus Strobus of particular interest to this IUFRO working group: *P. kwangtungensis* is classified as EN C2a due to its limited distribution range and low number of mature individuals while *P. krempfii* is classified as VU A1cd due to its limited distribution range and poor natural regeneration. *P. dalatensis* is considered as NT due to a high level of fragmentation in its natural distribution range and poor natural regeneration.

There are many conifer species introduced into Vietnam for trials and planting (Table 2), however about 20 species are still found in plantations or collections. Some of them have become important planting species, such as *P. caribaea*, and many of them have become ornamental plants. Most of them can be found in Da Lat city (Lam Dong province) as ornamental plants or in the pine collection of the Lam Dong Silviculture Centre.

Species and Provenance Trials

Species trial

In the late 1970s and early 1980s, dozens of pine species had been imported for planting trials in Vietnam. The trial established in Dai Lai (Vinh Phuc province, North Vietnam) in 1980 included *P. caribaea, P. cubensis, P. douglasiana, P.*

Table 2. Introduced conifer species in Vietnam (Nguyen, 2004)

	Scientific name	Family
1	*Agathis australis* Salisb.	Araucariaceae
2	*Araucaria columnaris* (G.Forst.) Hook *Araucaria cookii* R.Br.	Araucariaceae
3	*Araucaria cunninghamii* D.Don	Araucariaceae
4	*Callistris obtusa* L.	Cupressaceae
5	*Cupressus funebris* Endl.	Cupressaceae
6	*Cupressus lusitanica* Mill var. *lindleyi*	Cupressaceae
7	*Cupressus sempervirens* L.	Cupressaceae
8	*Cupressus* spp.	Cupressaceae
9	*Juniperus chinensis* L. *Sabina chinensis* (L.) Antoine	Cupressaceae
10	*Juniperus squamata* Buch. - Ham.	Cupressaceae
11	*Platycladus orientalis* (L.) Franco *Biota orientalis* (L.) Endl. *Thuja orientalis* L.	Cupressaceae
12	*Pinus caribaea* Morelet	Pinaceae
13	*Pinus elliottii* Engelm.	Pinaceae
14	*Pinus oocarpa* Schiede ex Schltdl.	Pinaceae
15	*Pinus patula* Schiede & Deppe *Pinus subpatula* Roezl ex Gordon	Pinaceae
16	*Pinus rigida* L.	Pinaceae
17	*Pinus taeda* L.	Pinaceae
18	*Podocarpus chinensis* Wall ex Forbes	Podocarpaceae
19	*Cryptomeria japonica* (L.) D.Don.	Taxodiaceae
20	*Taxodium disticum* (L.) Rich.	Taxodiaceae
21	*Ginkgo biloba* L. *	Ginkgoaceae

*Newly introduced gymnosperm

Table 3. Conifer species of Vietnam in comparison with the world (Nguyen, 2004)

Name and No. of families in Vietnam	No. of genus/ species in the world	No. of genus in Vietnam	No. of species/ endemics in Vietnam
Araucariaceae	3/41	0	0/0
Cephalotaxaceae	1/5-11	1	1/0
Cupressaceae	30/135	3	4/2
Taxodiaceae		4	5/1
Phyllocladaceae		0	0/0
Pinaceae	11/225	5	11/2
Podocarpaceae	18/190	4	6/0
Sciadopityaceae	1/1	0	0/0
Taxaceae	5/23	2	6/2
6/9	70/635	19	33/7

elliottii, P. greggi, P. halepensis, P. massoniana, P. mastrensis, P. merkusi, P. occidentalis, P. oocarpa, P. patula, P. peuce, P. pinea, P. pseudostrobus, P. radiata, P. taeda and *P. tropicalis* (Phi 1989). *P. halepensis* and *P. pinea*, died in the first three months in the nursery. After 15 months, seedlings of *P. oocarpa* showed the best growth, followed by *P. caribaea*. Table 4 illustrates the growth in this trial after 20 years. Other trials and plantations also showed that these two species performed best.

Provenance trials for *P. kesiya*

In 1976 and 1977, two small provenance trials were established for *P. kesiya* in North Vietnam which included only four provenances (Tables 5 and 6). The best results were obtained from the provenances from Zambia (Zambia landrace) and Hoang Su Phi. The native provenance (Da Lat) originated from the highlands so it could not show good growth in this lowland trial.

Provenance trial for *P. merkusii*

P. merkusii is planted widely in Vietnam for resin. It can be seen that growth of the species is very slow (Table 7), however trees can grow on very eroded areas and barren hills where other species can not be planted successfully.

Table 4. Growth of some pine species and provenances tested in Dai Lai after 20 years (Phan, 2002)

Species	Origin	D1.3 (cm)	H (m)
Pinus caribaea var *hondurensis*	Honduras	26.5	18.2
P. massoniana	Tam Dao (Vietnam)	25.6	16.4
P. oocarpa	unknown	25.5	16.6
P. massoniana	China	23.9	16.2
P.massoniana	Yen Lap (Vietnam)	22.8	16.3
Pinus caribaea var caribaea	Cu Ba	22.4	16.5
P. massoniana	Loc Binh (Vietnam)	22.2	16.7
P. merkusii	Ha Trung (Vietnam)	21.3	11.0
P. merkusii	Hue (Vietnam)	20.5	10.9
P. elliottii	USA	20.2	14.4
P. merkusii	Da Lat (Vietnam)	18.4	9.7

Table 5. Provenance trial of *P. kesiya* established in Dai Lai (Vinh Phuc province, North Vietnam) in 1976 and 1977 (Phi 1989; RCFTI 2003)

Provenance	Dai Lai, 1976 (10 years old)			Dai Lai, 1977 (9 years old)		
	D (cm)	H (m)	V (dm^3)	D (cm)	H (m)	V (dm^3)
Zambia	12.9	8.49	68.7	11.1	7.96	42.2
Philippine	12.0	8.70	49.9	10.2	7.22	41.8
HoangSuPhi (Vietnam)	12.7	9.10	58.8	10.6	8.49	40.7
Da Lat (Vietnam)	11.5	8.37	48.8	9.2	6.85	31.0

Table 6. Provenance trials of *P. kesiya* in Lang Hanh (Lam Dong province, South Vietnam), planted in 1991 and Ba Vi (Ha Tay province, North Vietnam), planted in 1993, measured in 1998 (RCFTI, 2003)

Provenance	Lang Hanh, 1991 - 1998			Ba Vi, 1993 - 1998		
	D (cm)	H (m)	V (dm^3)	D (cm)	H (m)	V (dm^3)
Benquet, Philippine	13.9	8.1	64	9.3	4.4	21
Tarlac, Philippine	15.0	7.6	70	11.0	5.0	29
Coto Mines, Philippine	14.7	7.9	74	10.0	4.1	22
Xuan Tho, Vietnam	14.9	7.2	68	11.2	4.5	28
Prenn Waterfall, Vietnam	14.9	8.9	78	10.3	4.4	25
Lang Hanh, Vietnam	15.1	8.4	76	10.7	4.6	27
Nong Krating, Thailand	15.4	8.2	80	9.9	4.1	22
Doi Suthep, Thailand	15.9	8.3	85	11.2	4.6	28
Doi Inthanon, Thailand	13.3	6.9	53	11.0	4.6	28
Phu Kradung, Thailand	13.7	6.9	55	10.5	4.3	25
Nam Now, Thailand	13.7	6.7	55	11.1	4.6	28
Wat Chan, Thailand	14.2	7.3	61	10.7	4.4	25
Simao, China	15.3	8.8	78	11.1	4.9	29
Jingdung Arb., China	12.6	7.8	61	10.4	4.1	24
Zokhua, Myanmar	11.3	8.2	68	10.0	3.5	20
Aungban, Myanmar	14.4	8.2	67	9.5	3.8	20

Table 7. Provenance trial of *P. merkusii* established in Dong Ha in 1982 (7 years old) (Phi, 1989)

Provenance (province)	Information about seed source			Dong Ha, 1982-1989	
	Latitude	Longitude	Altitude	D (cm)	H (m)
Ria (Ninh Binh)	20°30	105°55	20-50	6.5	2.8
Ha Trung (Thanh Hoa)	20°20	105°55	20-50 m	6.8	2.9
Hoang Mai (Nghe An)	19°20	105°35	50	6.7	2.9
Nam Dan (Nghe An)	18°45	106°30	100	6.5	2.7
Bo Trach (Quang Binh)	17°45	106°25	50-70	6.1	2.53
Hue (Thua Thien Hue)	16°35	107°35	50	6.4	2.6

Breeding Work for Pines

Plus tree selection

Plus tree selection for growth was applied for *P. kesiya* and *P. massoniana* while micro-chipping technique was applied for selecting high resin yielding plus trees and establishing seed orchards of *P. merkusii*. Four hundred and nine candidate plus trees were selected for *P. merkusii* for high resin yield, then 275 trees (67.2%) were chosen for both growth and resin yield and only the 40 best trees were selected for seed orchard establishment. Plus trees of *P. kesiya* were also selected for establishment of progeny trials.

Vegetative propagation

Vegetative propagation is also widely applied for pines. Grafting was successfully used for producing grafted trees

Table 8. Seedling demand and available seed stands for the period 2006-2015 (Forestry Department, 2007)

Species	Seedling demand (million)	Demand for seed stand (ha)	Available seed stands (ha)
P. kesiya	16.2	114	1222.5
P. caribaea	14.0	118	194
P. massoniana	16.2	72	229
P. merkusii	25.2	170	531.7

Table 9. Seed orchards (SO) requirement for the period 2016-2020 (Forestry Department, 2007)

Species	SO requirement (ha)	Available SOs	New SOs (ha) needed
P. kesiya	102	29.1	72.9
P. caribaea	117	52.1	64.9
P. massoniana	77	4	73
P. merkusii	123	57.9	65.1

from plus trees for establishment of seed orchards such as *P. merkusii. P. massoniana* and *P. kesiya*. Recently, cutting propagation was also applied for some pine species such as *P. kesiya* and *P. caribaea*, mainly for young seedlings.

Two Vietnamese *Taxus* species (*T. wallichiana* and *T. chinensis*) can be easily propagated by cuttings at large-scale, however they maintain topophysis so that branch cuttings can not be taken from vertical leading shoots (Nguyen and Tran, 1996). Other conifer species propagated successfully by cuttings are *Calocedrus macrolepis, Fokienia hodginsii*, *Podocarpus neriifolius*, and *Nageia fleuryi* (Nguyen and Tran, 2002). Only *Glyptostrobus pensilis* was difficult to propagate by cutting. Hundreds of cuttings of the species were put in vegetative propagation but only 10 rooted cuttings were obtained.

Seed stands and seed orchards

According to Forestry Department (2007), the demand for seedlings and current status of seed stands for four pine species for the periods 2006-2015 and 2016-2020 are shown in Tables 8 and 9.

Pines of Vietnam with Reference to Three White Pines of Subgenus Strobus

Pinus krempfii

Scientific name: *Pinus krempfii* H.Lec.

Synonym: *Ducampopinus krempfii* (Lec.) A.Chev.

Description: An evergreen, large-sized tree, up to 30 m tall (or more), 150-160 cm in diameter (sometime up to 2 m). Crown quite spreading, dark and fan-shaped which can be very easily recognized. Bole is high, without branches. Trunk cylindrical, straight.

Needles are flat, not round. Seedlings have the first 10-13 cotyledons, 2-3 cm long, curved as sickles. At early age (5-20 years old) needles 10-15 cm long and wide, longer and wider than needles of mature trees, arranged as scissors, grouped at tip of the twigs. When trees become older, needles smaller and shorter, only 4-5 cm long, dark green, so that crown becomes dark and dense.

Cones unisexual. Female cones solitary, occur from April to May, mature from July to September. Seeds light brown and winged. After ripening, cones can remain on trees for sometime.

Distribution: This is an ancient, endemic pine species with typical flat needles, only found in Vietnam. They can be found in some areas of Lam Dong, Dac Lac, Khanh Hoa and Ninh Thuan provinces at elevation from 1000 to 2000 m a.s.l. They are found with some broadleaved species of moist tropical forests such as species of Fagaceae, Lauraceae, Magnoliaceae and some important conifer species such as *Fokienia hodginsii, Dacrydium elatum, P. dalatensis, Podocarpus wallichianus* and *Podocarpus imbricatus*. The species is considered as VU A1cd due to its limited distribution, fragmentation and poor natural regeneration (Nguyen, 1993, 2000, 2004). Important natural populations are now in Bidoup National Park and Cong Troi Protected Forest (Lam Dong province) and Chu Yang Sinh National Park (Dac Lac), and they are in a safe condition. Thousand of seedlings have been planted in Da Lat city (at an elevation of 1500 m a.s.l.), however only 30 survived, therefore requirements of the species in cultivation are not known.

Formerly the species was put into a separate genus Ducampopinus, but some experts thought that the species could be a close relative of Keteleeria or Pseudolarix genus due to their similar morphology and anatomy. Results from Wang, Szmidt and Nguyen (2000) showed that the species clearly belongs to Pinus genus (Strobus subgenus) and it was not necessary to form a new genus as Ducampopinus. The results also showed that the species has no relation to Keteleeria and Pseodolarix.

Uses: Timber light yellow, quite hard, can be used as many other pines. This is a very valuable pine species from a scientific point of view because it is an endemic for Vietnam.

Pinus kwangtungensis

Scientific name : *Pinus kwangtungensis* Chen

Synonym : *P. kwangtungensis* var. *varifolia* Nan Li & Y.C. Zhong

P. fenzeliana Hand.-Mazz.

Description: An evergreen, large-sized tree up to 20 m tall, 70 cm in diameter, however they usually grow on limestone mountains in North Vietnam so that their height is often 10-15 m. Bark brown, scaly and rough, peeling in small plates.

Needles in fascicles of 2-5 (generally five) in sheaths, 3-7 cm long (needles of young trees can be 10 cm long), clustered towards tip of twigs. Branches large.

Female cones cylindrical or ovoid, solitary or in pairs, up to 8 cm long and 6 cm wide, opening and releasing seeds while on the tree. Seed ovoid, wing 2-3 cm long.

Distribution: The species has a natural distribution in northern provinces of Vietnam such as Hoa Binh, Son La, Ha Giang, and Cao Bang with an altitudinal range from (600) 900 to 1400 (1600) m a.s.l. They grow in very harsh conditions on limestone mountains in a tropical monsoon climate with cold winters, mean annual temperature of 14-20^{o}C, and rainfall above 1200 mm. The species associates with other conifers such as *Taxus chinensi* and, *Podocarpus pilgeri*. Natural regeneration very poor. Cones ripen in August but are difficult to collect due to access problems. In Vietnam the species is considered as EN C2 due to small, fragmented populations and danger of illegal logging (Nguyen, 2000, 2004). Pham, Zuidema, and Nguyen (2008) also studied the demography of the species and gave some guidelines for conservation. The populations in Vietnam represent the southern-most distribution of the species; therefore they require effort for conservation. The species is safely protected in Hang Kia- Pa Co Nature Reserve (Mai Chau district, Hoa Binh province) and Thang Heng Nature Reserve (Tra Linh district, Cao Bang province).

Uses: Timber used for house construction.

Pinus dalatensis

Scientific name : *Pinus dalatensis* de Ferre
Synonym : *P. wallichiana* A.B.Jacks var. *dalatensis* (Ferre) Silba

Description: An evergreen, large-sized tree about 20 m tall (sometimes more), 70-90 cm in diameter. Due to large size and great form, together with other conifer species, they form the dominant layer of natural forest.

Needles in fascicles of five in sheaths, terminal, 10-11 cm long. Needles light green in colour, when dry change to reddish brown. Crown open, branches slender. They grow in mixed forest with flat needled pine (*P. krempfii*) and they can be distinguished from *P. krempfii* by the shape of their dense crown. Bark fissured but not as deep as *P. kesiya* and *P. merkusii*.

Cones unisexual. Cones mature from August to September, each kg of seed has about 50.000 seeds. When planted in Mang Linh (Lam Dong province), they grow slowly in comparison to other pines.

Distribution: This is one of two 5-needled pines of Vietnam. This is an endemic pine of Vietnam which has a very limited distribution range, occurring only on some high mountains of the Western Highlands in Lam Dong, Dac Lac, Gia Lai and Kon Tum provinces, from elevation of 1500 m a.s.l. above. They are usually found in dense, moist evergreen subtropical forest with monsoon tropical climate, mean annual temperature 16-21^{o}C, and rainfall above 1800 mm. Associated with *P. krempfii, Fokienia hodginsii, Dacrydium elatum* and other broadleaved species. The species was categorized as NT due to fragmentation and bad regeneration. They can be well protected in Bidoup National Park and Cong Troi Reserve (Lam Dong province), Chu Yang Sinh National Park (Dac Lac) and Kon Ka Kinh National Park (Gia Lai) in South Vietnam (Nguyen, 1994, 2000).

Uses: Wood is soft and light, usually used as other common pines.

Due to the small native ranges of these three pine species and their geographic remoteness, no molecular genetic studies or common garden studies have been undertaken yet to examine patterns of genetic variation. Little or no *ex situ* conservation has been undertaken to date.

Pest and Disease Problems for Pine

Pests and diseases could not be found on three native white pine species, namely *P. krempfii, P. dalatensis* and *P. kwangtungensis*, due to the fact that they are found scattered through broadleaved forest, and do not form pure populations.

Pest and disease problems were found only on native plantation pine species, namely *P. merkusii, P. massoniana* and *P. kesiya*. At a large scale, *Dendrolimus punctatus* could cause significant damage for *P. merkusii* and *P. massoniana* plantations in Northern and Central Vietnam while *Dasychira axutha* damage *P. massoniana* only in northern provinces.

Some rust fungi were also found in young *P. kesiya* plantations in South Vietnam. They are Needle rust (*Coleosporium plectranthi* Barclay), Blister rust (*Peridermium* sp., aecial state) and Gall rust (*Cronatium orientale* S.Kaneko). The fungi were first found in Vietnam in 2007 (Shigery, Thu, and Yasuyuki, 2007).

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Proceeding
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Factors Affecting Seed Production in *Pinus monticola* and *P. albicaulis*

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Abstract − With the development of breeding programs for five-needle pines, there will be a need for breeding orchards and seed orchards consisting of selected rust-resistant trees or clones for seed production. Many seed orchards have already been established and seed production will be a primary goal for orchard managers. For most five needle pines we know little about their reproductive biology and the factors that influence cone and seed production in natural stands and especially in seed orchards. This paper describes: for western white pine (*Pinus monticola*) the factors that affect cone and seed production in natural stands and in seed orchards based on studies done from the time of pollination to cone and seed maturity; and, in whitebark pine (*Pinus albicaulis*) based on studies of mature seed cones in natural stands. Both species have the typical pine life cycle in which pollination occurs in the spring but fertilization does not occur until about one year later, followed in the next 3 to 4 months by cone and seed maturation. Cone loss may result from low temperatures or abrasion at and shortly after pollination, inadequate pollination, and by insects during later cone development. Most seeds are lost as a result of inadequate pollination, self-pollination resulting in early ovule abortion soon after the time of pollination, or early embryo abortion soon after fertilization. Fewer subsequent seed losses may occur during seed and embryo development following the time of fertilization. Various "seed types" (empty, aborted, etc.) are described and the time and cause of seed losses are given for each. In general, *P. monticola* and *P. albicaulis* have a high seed potential per cone (about 100-200, respectively) and a high percentage of filled seeds (seed efficiency) (59% and 74%, respectively). The data indicate that both species are very good seed producers and *P. monticola* is well suited to seed orchard conditions where cone and seed production can be influenced. We have no experience with *P. albicaulis* in seed orchards but we believe, based on the data available from natural stands, that it also may be well suited for seed orchard cone and seed production following the methods used for *P. monticola*. Some of these conclusions and generalizations may also apply to other five-needle pines.

Key words: Seed production, 5-needle pines, *Pinus monticola*, western white pine, *Pinus albicaulis*, whitebark pine

Introduction

There have been relatively few detailed developmental or experimental studies of seed production in 5-needle pines that were designed to determine the cone production, seed production and the various factors that cause cone loss and affect filled seed production during the long reproductive cycle. Five-needle pines have a 2-year reproductive cycle (Owens 2004, Owens and Fernando 2007), as do most other pines (Owens *et al.* 2005; Owens 2006), that includes pollination and early pollen-tube growth during the first growing season, followed by winter dormancy, then fertilization the next spring, and embryo and seed development during the rest of the second growing season (Figure 1C). In most species this extends over about 16 months, usually from about June of the first year to about September of the second year. During this time cones, may be lost from abortion due to low temperatures, abrasion or predators mostly at the time of pollination (Figure 1F-O) but also during the entire first growing season. Filled seeds also may be lost due to many causes, again most often at the time of pollination and during the first growing season, but also due to insects, disease and predators during the second growing season. Little may be done to decrease these losses thus increase the cone and seed production in natural stands but there are many ways to prevent these losses and increase cone and seed production in seed orchards.

This report combines studies of pollination, cone and seed production in natural stands and in seed orchards for western white pine (*Pinus monticola* Dougl.) in British Columbia, Canada (Owens 2004, Owens and Fernando 2007) and in natural stands for whitebark pine (*P. albicaulis*) in the Northwest of the United States. *Pinus monticola* belongs to the subsection

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Strobi Laud., the white pines (Little and Critchfield 1969) and is distributed from the west coast to the central British Columbia (BC) interior and south into California and Montana through the Cascade and Rocky Mountains (Critchfield and Little 1966, Hosie 1979). It bears large, long, woody seed cones that can produce over 200 seeds each (Figure 1A). Detailed developmental and experimental studies of *P. monticola* have been done for cone-bud intiation and development (Owens and Molder 1977), pollination (Owens *et al.* 2001), gametophyte development (Owens and Bruns 2000), fertilization and cytoplasmic inheritance (Bruns and Owens 2000), reproductive biology and seed and cone production (Owens and Molder 1975, Owens and Fernando 2007) and causes of male and female sterility (Wilson and Owens 2003). These studies have been summarized in a manual entitled “The reproductive biology of western white pine” (Owens 2004).

P. albicaulis (whitebark pine) (Figure 1B) is a five-needle pine that belongs to the subsection Cembrae Laud. (Little and Critchfield 1969). It has an extensive but very scattered distribution at high elevations from 1000 m in coastal areas of BC to 1700-2200 m in the Cascade Mountains of Oregon and Washington up to 3600 m in the Sierra Nevada Mountains of Northern California to 2200 m in elevation in the interior of BC and 1900-2300 m in western Alberta and 1900-3300 m in the inland Empire forest regions of eastern Washington, Northern Idaho, western Montana and northwestern Wyoming (Critchfield and Little 1966, Hosie 1979). Its cones are indehiscent and deciduous at maturity (Figure 1B). Studies of reproduction and seed production for *P. albicaulis* given here are limited to a study of mature cones and seeds from natural stands in the Inland Empire in the Northwestern U.S. (Owens *et al.* 2007). Other studies have dealt with seed dispersal by birds (Lanner 1982) and the related issue of soil seed banks (Tillman-Sutella *et al.* 2008) for *P. albicaulis*, which are unusual for conifers.

This paper reports on the various aspects of reproductive biology that affect cone and seed production and includes studies of coastal natural stands, in a clonal interior seed orchard and a coastal seedling seed orchard of *P. monticola* in British Columbia over several years and from studies of mature cones collected from *P. albicaulis* at two high elevation sites in Idaho and Nevada in September, 2004.

The paper will show that the potential for seed production per cone for these two species is very high, compared to most conifers, and that, in well managed seed orchards of *P. monticola* cone and filled seed production can be increased using standard, simple and inexpensive techniques. Based on the titles of papers from the last 5-needle pine conferences, it is evident that breeding and seed orchard development are advancing or in the planning stage in many parts of the world. Many of the generalization discussed here may be applicable to the five-needle pines in these other regions.

Materials and Methods

Studies of mature *P. monticola* trees in natural stands were done over several years in the Malahat area about 50 km north of Victoria, British Columbia at an elevation of about 200 m on Vancouver Island. Seed orchard studies were done over several years in a coastal seedling seed orchard, 5 to 15 years old in Central Saanich, about 15 km north of Victoria B.C. on Vancouver Island (Saanich Seed Orchard) and at an interior clonal seed orchard, about 5 years old, located 13 km south of Vernon, B.C. (Vernon Seed Orchard at Bailey Rd.). Seed orchard studies involved open pollinations, open plus supplemental mass pollinations and control pollinations using varying amounts of pollen and variable times of pollination coupled with studies of pollination success (amount of pollen on each ovule tip and amount of pollen taken into each ovule in fresh dissected cones (see Figure 1E)) and fertilization success (proportion of ovules in which fertilization had occurred). Because of the long reproductive cycle Figure 1C), collection and dissection of cones, ovules and developing seeds were done at: pre-pollination; pollination; post-pollination; dormancy; fertilization following dormancy; early embryo development; late embryo development; and, at cone maturity as indicated by the red dots shown on Figure 1C. Ovules and developing seeds were dissected then observed and photographed using a dissecting photo-microscope. Or, for some stages they were embedded in paraffin, serially sectioned, sections mounted on microscope slides and stained then studied anatomically and photographed using a dissecting or compound microscope. The purpose was to determine the stage of development at the time of collection, if development was normal or not, and if the ovule or seed was aborting, and then determine the possible the cause of the abnormality or abortion. At cone maturity samples of cones were collected from several trees or for several treatments and their controls and the total number of scales counted, number of fertile scales (those bearing fertile ovules) counted, then all seeds were extracted counted and determinations were made of the seed potential (number of fertile scales times two), total seeds of all kinds. Then all seeds were dissected, their contents observed, then the seeds were categorized as: filled (normal, healthy with a mature embryo); small aborted ovules due to a lack of pollination; ovules aborted soon after pollination, ovules aborted at the time of fertilization leaving a collapsed megagametophyte with no embryo usually resulting from early self-incompatibility and often called "empty seeds"; late aborted seeds with small retarded embryos usually resulting from late-acting self-incompatibility; and, insect or disease-damaged seeds. The seed efficiency (percent of normal, healthy filled seeds) was then calculated (see Table 1). For all categories of seeds, careful consideration was given regarding the possible

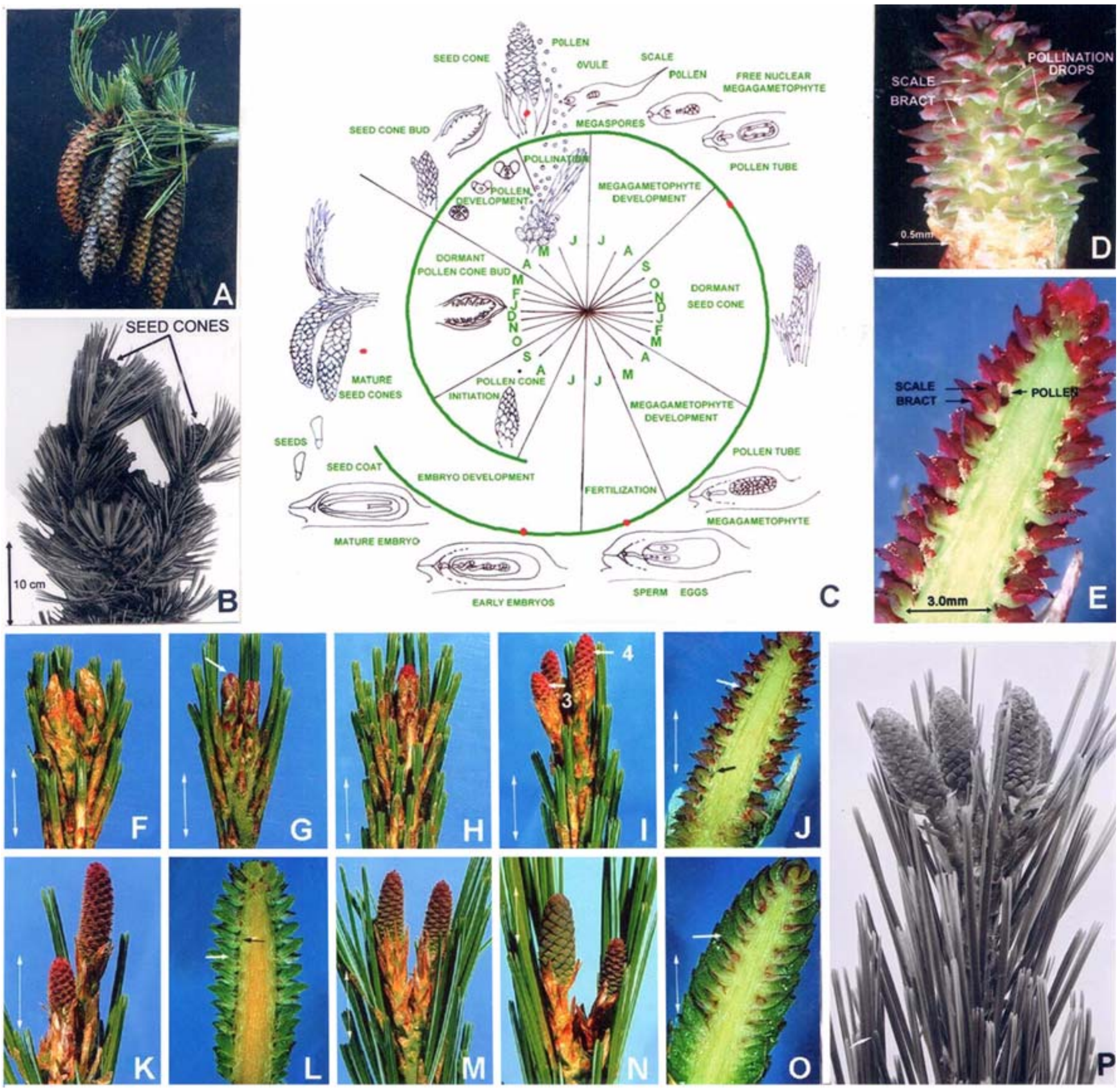

Figure 1. **A.** Mature seed cones of western white pine. **B.** Mature seed cones of whitebark pine. **C.** The reproductive cycle of western white pine in British Columbia, Canada. Red dots indicate time of main cone or seed observations. Modified from Owens (2004). **D.** Five-needle pine cone at pollination showing bracts, scales and pollination drops. **E.** Western white pine seed cone at pollination sliced longitudinally to show bracts, scales and white pollen on the micropylar arms. This cone was supplemental pollinated and contains an average of over 10 pollen grains per ovule tip. **F-O.** Stages of western white pine seed cones from pre-pollination through the pollination period. **F.** Two seed-cone buds at Stage 0, just before bud burst, with a vegetative terminal-long-shoot-bud between, about one week before cone-bud burst. **G.** The start of Stage 1, showing a seed-cone bud starting to burst (arrow). **H.** Seed-cone bud bursting (Stage 2). Distal ovules are receptive. **I.** Receptive seed cones at Stages 3 and 4 showing red scales with thin space between to allow pollen to enter. Cones are most receptive at Stages 3 and 4. **J.** Pollinated Stage 4 seed cone sliced down the center to show green ovule tips with white pollen attached (See enlargement in Figure 1E). **K.** Stage 5 seed cones when scales thicken and start to prevent further pollen from entering the cone thus causing the cones to become less receptive. **L.** Stage 5 seed cone sliced down the center to show that spaces between the scales are nearly closed. Stage 5 is too late for effective supplemental pollinations even though externally they may appear receptive. **M.** Stage 6 seed cones in which the scales have thickened, closing and sealing the cones as the margins of the scales bend upwards. **N.** Stage 7 seed cone with tightly sealed scales allowing no more pollen to enter the cone. **O.** Stage 7 seed cone tightly sealed scales about one week after the Stage 3 cones shown above. Small cone is aborting. Vertical arrows on figures **F-I** and **K, M, N** = 1 cm and on figures **J, L, O**=0.5 cm. **P.** Post-pollination dormant seed cones that were pollinated. They over-winter after the pollen has germinated and pollen tubes grown part way through the nucellus, and the megagametophytes is at the free nuclear stage.

causes for that type of seed. For details of pollination methods and determinations of pollination success and cone and seed set (Owens and Molder 1977b; Owens and Bruns 2000; Bruns and Owens 2000; Owens 2004; Owens *et al.* 2005; Owens and Fernando 2007).

Because the collection sites in natural stands of *P. albicaulis* were at very high-elevations and difficult to access, periodic collections of cones at various stages of development were not possible, therefore, seed cones were only collected at cone maturity. There were no pollination experiments or dissections at various stages of cone development. Cone and seed analyses were done only for mature cones in the same manner as for *P. monticola* (see Table 1) (Owens *et al.* 2007).

Results

The pollination mechanism in pines

There are five pollination mechanisms found in living conifers. That found in pines is the most common and is also found in several other genera within the Pinaceae (ie. *Picea*). Seed-cone buds are large and erect (Figure 1F-H) and are found distally on vigorous shoots in upper regions of the crown. The seed cones are erect at pollination (Figures. 1I, K, M), the two ovules found on each scale are inverted (Figure 1E) and the ovule tip bears two arms or prongs with the micropyle between. At pollination the cones elongate and the spaces between the scales widen allowing the airborne pollen to enter the cone and settle on various cone surfaces. The ovule arms secrete tiny lipid droplets on the surface of the cell to which pollen grains adhere. Midway through the week-long receptive period for each cone, a pollination drop is secreted out of the micropyle and fills the space between the arms of each ovule (Figure 1D). The saccate pollen enters the pollination drop and, because the sacci are large and air-filled, they cause the pollen to float up into the drop then through the microple and into the micropylar canal. At the same time, the pollen entering the pollination drops appears to alter the surface tension of the drop and the drop dries, thus decreasing the volume of the of the drop causing it to recede back through the micropyle and into the micropylar canal. The pollen then settles on the surface of the nucellus within the ovule where it germinates within a few days. A pollen tube then forms and penetrates into the nucellus carrying the sperm to the archegonia that contain the eggs. Many pollen grains may adhere to the micropylar arms (Figure 1E) and several pollen grains are usually taken into each ovule, but only one is required to fertilize an egg within that ovule. If no viable pollen is taken into an ovule the ovule will abort within one or two weeks. Non-viable pollen will not stimulate the ovule to develop into a seed. In pines the pollen tube stops elongating in mid-summer and the small cone becomes dormant (Figures. 1C and P). Cone growth resumes the next spring and fertilization occurs within a few weeks (Figure 1C).

Stages of seed cone receptivity during the pollination period

Pollination in *P. monticola* has been studied extensively for several years at the coastal seedling seed orchard and for 2 yrs at the interior clonal seed orchard (Owens *et al.* 2001; Owens 2004; Owens and Fernando 2007). Pollination in an orchard occurs during about 2 wks, but cones may be at various stages on different trees and it takes only about 1wk for a single cone to burst become receptive than cease to be receptive. Stages of seed cone bud development, receptivity and post-receptivity are shown in Figures 1F-L. Several seed-cone buds may be found just below the terminal long-shoot bud on a branch (Figure 1F). Seed cones elongate within the bud scales (Stage 0, Figure 1F) then the cone tip extends beyond the bud scales (Stage 1, Figure 1G, arrow). Seed cones continue to elongate and at Stage 2 are about one-third emerged from the bud scales but are not yet receptive (Stage 2, Figure 1H). By Stage 3 (Figure 1I) cones are about two-thirds emerged and the cone axis begins to rapidly elongate, widening the spaces between the bract-scale complexes. This opens spaces between the bract-scale complexes and the cones begin to be receptive-some pollen can enter at the tip of the cone. The cone axis continues to elongate and the spaces become very wide, so the white ovules and cone axis can be seen. This is Stage 4 when cones are most receptive (Figures 1D, E, I, J). Cones remain at this most-receptive stage for several days. This is the best time to make supplemental pollinations or control crosses. Elongation of the cone axis then slows and the scales thicken narrowing the spaces between bract-scale complexes (Stage 5, Figures 1K, L). Stage 5 cones are less receptive that Stage 4 cones based on counts of pollen borne on the ovules (See Figure 1E) after control pollinations with measured amounts of pollen at different stages (Owens *et al.* 2001, Owens and Fernando 2007). Scales continue to thicken and seal the cone closed (Stage 6, Figure 1M) and the cones are no longer receptive. Seed cones then become broader and remain tightly sealed (Stage 7, Figures. 1N, O). Seed cones remain erect, broaden but elongate very little before becoming dormant in mid-summer (Figure 1P). Seed cones are most receptive for only about 4 days at Stage 4 (Figures 8D, E, I).

Pollination success can be measured after pollination is mostly completed, at the end of Stage 4, by sampling of cones then slicing the fresh cones longitudinally and observing the ovule using a dissecting and counting the number of pollen grains per ovule tip (Figure 1E). In general if there are no pollen grains on an ovule tip, the ovule will abort within 1-2 wks, if there are fewer than five pollen grains per ovule tip supplemental pollination would help prevent ovule abortion and increase filled seed production. If there are five to 10 pollen grains per ovule tip, then supplemental pollination may increase ovule survival, filled seed production and possibly cone survival. If there are 10 or more pollen grains per ovule tip then supplemental pollinations will likely be of little or no benefit weighed against the cost of the supplemental pollination. Supplemental pollinations after Stage 5 also will be of little or no benefit. As a general guideline, the earlier the supplemental pollination the better but be sure that the pollinations overlap Stage 4 cones. Rarely will all ovules in a cone receive equal amounts of pollen but if there is an average of five to ten pollen grains for most ovules good filled seed set should occur assuming the pollen is of good quality (over 90% viability). For greater details the references by Owens *et al.* (2001), Owens (2004) and Owens and Fernando (2007) should be consulted. Similar experiments with other 5-needle pines would not be difficult if reproductive trees are accessible and of small size and seed cones reachable by ladders. Because of the similarities in pollen, cone structure and pollination mechanism in *P. albicaulis* and *P. monticola*, the above generalizations will likely also apply to both species and other five-needle pines.

Pollination effects on seed and cone development

Pollination is the first and most important step in seed production. Unlike many other conifers, in pines, if an ovule is not pollinated by at least one fertile pollen grain, the ovule will abort within about 2 wks after cones cease to be receptive. In pines if a seed cone is not pollinated, if too few ovules in a cone are pollinated, or if cones are pollinated with enviable pollen, the unpollinated or enviably pollinated ovules abort and if many ovules abort, the seed cone will start to abort within about 2 wks after pollination. Cone abortion at this stage begins near the cone axis then extends out to the visible scales and within another 2 wks the cone has aborted, turned brown and usually falls from the branch. This early cone abortion occurs at about the same time and appears the same as abortion due to low temperatures.

The number of ovules that must be pollinated in order for cones to continue to develop varies with the pine species and cone morphology, specifically the proportion of fertile scales (scales bearing fertile ovules) in the cone. In western white pine about 86% (108) of the average total scales (126) are fertile and our studies have shown that 50-70% of the fertile ovules must be pollinated for the cone to be retained. This means that about 100 to 150 fertile ovules had to be pollinated by one or more fertile pollen grains for the cone to be retained (Owens and Fernando 2007). No pollination studies were done for whitebark pine (Owens *et al.* 2007) so we can only estimate that about 48 to 67 fertile ovules must be pollinated by one or more fertile pollen grains for cones to be retained. In some hard pines, such as lodgepole pine (*P. contota*), about 80% of fertile ovules must be pollinated by one or more fertile pollen grains for the cones to be retained (Owens *et al.* 2005). The reason for the higher percentage required in lodgepole pine is because about 85% of the scales are sterile (all scales in the basal two-thirds of the cone), whereas, in *P. sylvestris,*

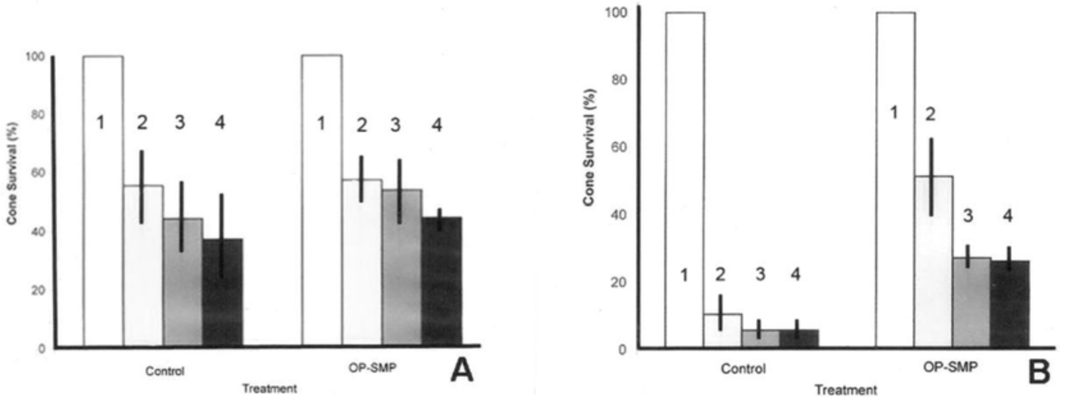

Figure 2. A and B. Histograms showing the percent of western white pine cone survival at: **1.** pollination; **2**. just before winter dormancy; **3**. fertilization; and, **4**. cone maturity. **A.** Older coastal, seedling seed orchard which had abundant pollen-cone and seed-cone production. **B**. Young interior, clonal seed orchard where few seed cones and very few pollen cones were produced. In both figures, the histogram on the left is the control (no supplemental pollination) and the histogram on the right is the treatment where supplemental pollination was given. Error bars are shown.

which has few sterile scales, only about 20% of the ovules in a cone must be pollinated for cone survival (Sarvas 1962). Therefore, if supplemental mass pollinations are done in seed orchards using pollen having high viability when seed cones are most receptive, this may not only increase filled seed production per cone but also increase the percentage of cones surviving.

Cone survival at two seed orchards

Similar experiments were done using *P. monticola* at the coastal Saanich and interior Bailey Road seed orchards in British Columbia. Branches on several trees were flagged and the number of seed cones present at pollination was counted. Seed cones were left for Open Pollination (OP) or given Operational Supplemental Mass Pollination (OP-SMP) three times. Cones on the same branches were counted 2 wks after the last pollinations to determine the cone loss due primarily to inadequate pollination, low temperatures and cone abrasion. Cones were counted again after winter dormancy, at the time of fertilization, and at cone maturity (see Figure 1C). Figures 2A and 2B, respectively, show the results obtained in the older coastal seed orchard where there was abundant natural pollen flight and also OP-SMP on half of the trees (the other half had cones bagged during OP-SMP), and in the young interior orchard where there was very little natural pollen produced and, again, half of the trees had OP-SMP and the controls had no OP-SMP. Results show in Figure 2A that, in the older seed orchard where there was abundant natural pollination (all trees produced pollen cones) there was no difference in the cone survival after pollination (Figure 2A2), at fertilization (3) or at cone maturity (4). However in the young seed orchard (Figure 2B), where there were some seed cones but very few pollen cones (only 8% of trees produced a few pollen cones), cone survival was very low (about 10%) following open pollination (Figure 2B2) which indicates that a lack of pollinated ovules caused about 90% of the young seed cones to quickly abort but there was only a small amount of seed cone loss during the next year (3), until cone maturity (4). Also, in the young seed orchard, OP-SMP resulted in only about a 50% cone loss as a result of inadequate pollinations (cones only received one OP-SMP at peak receptivity), and about half of the remaining cones (Figure 2B2) aborted from post-pollination until the time of fertilization (3) and cone maturity (4). From these results, we estimate that sexually mature trees in natural stands would likely follow a similar pattern as the trees in the older coastal seed orchard, with about 60% of cones being lost due to various causes during the 14-15 mo. from the time of pollination to cone maturity.

There is another factor that is of considerable importance in seed orchard breeding programs but of uncertain importance in natural stands. The erect seed cones that are borne just below the long-shoot terminal bud in five-needle pines (Figure 2, I and P) are very susceptible to abrasion from adjacent branches, pollinaton bags applied during breeding and insect bags applied thereafter. In two experiments using seed-orchard-grown western white pine and lodgepole pine (*P. contorta*). Cones that were not bagged were control pollinated, as were comparable numbers of bagged cones in which a plastic frame (Figure 3K) was placed over the branch, or not placed over the branch, and the pollination bag and insect bag were than place over the plastic frame (Figures 3L). It is important that a light weight stick (in our case bamboo) was attached to the base of the frame and attached tightly to the base of the branch using twist ties. If this is not done, the pollination and insect bags sag pull the frame down over the cones causing contact between the bag and the cones. The frame with the stick attached to the frame and the branch largely prevented any contact of the bags with the cones at pollination and for the following 3 mo. At the interior *P. monticola* seed orchard, 93% of the cones covered by frames and pollination bags and then frames and insect bags survived 3 mo after pollination. On the same trees, 80% of the cones survived if covered by and pollination bags and insect bags but no frames. About 74% of cones survived for the 3 mo if no frames and no pollination bags covered the cones (Owens and Fernando 2007). The frames did not improve cone survival in lodgepole pine where seed cones at pollination are small and usually located further down the branch. In some species cone abortion, from contact with pollination bags, especially when there has been heavy rains and strong winds during pollination, can cause abortion of nearly all cones in a breeding project and destroy the project or invalidate the statistical methods intended to be used.

Types of seeds and their possible causes

During the 15 months from pollination to cone and seed maturity in pines (Figure 1C) ovules, embryos and seeds may abort or be destroyed leaving the remains of the “seed” at the stage at which it ceased development. If you have a good understanding of the reproductive cycle of a species, when all seeds are removed from mature seed cones and observed then dissected and observed again using a dissecting microscope, the time and possible causes for the seed loss can be determined. Here, the causes are separated into: 1. Early abortion of ovules and seeds (from pre-pollination through fertilization about one year later); and, 2: Later seed abortion due to abortion of early embryos forming “empty seeds”, embryo abortion at mid-embryo stages, insect-damaged and diseased seeds.

This is followed by a description of mature and healthy seeds.

Early abortion of ovules and seeds

In pre-pollination seed cones, some ovules do not fully develop and remain as small flat ovules at the base of the seed wing in mature cones of *P. monticola* (Figure 3A) and in *P. albicaulis*, which lacks seed wings, they remain as tiny flattened ovules at the base of the scale with a small apparently rudimentary seed wing that never developed. These are most

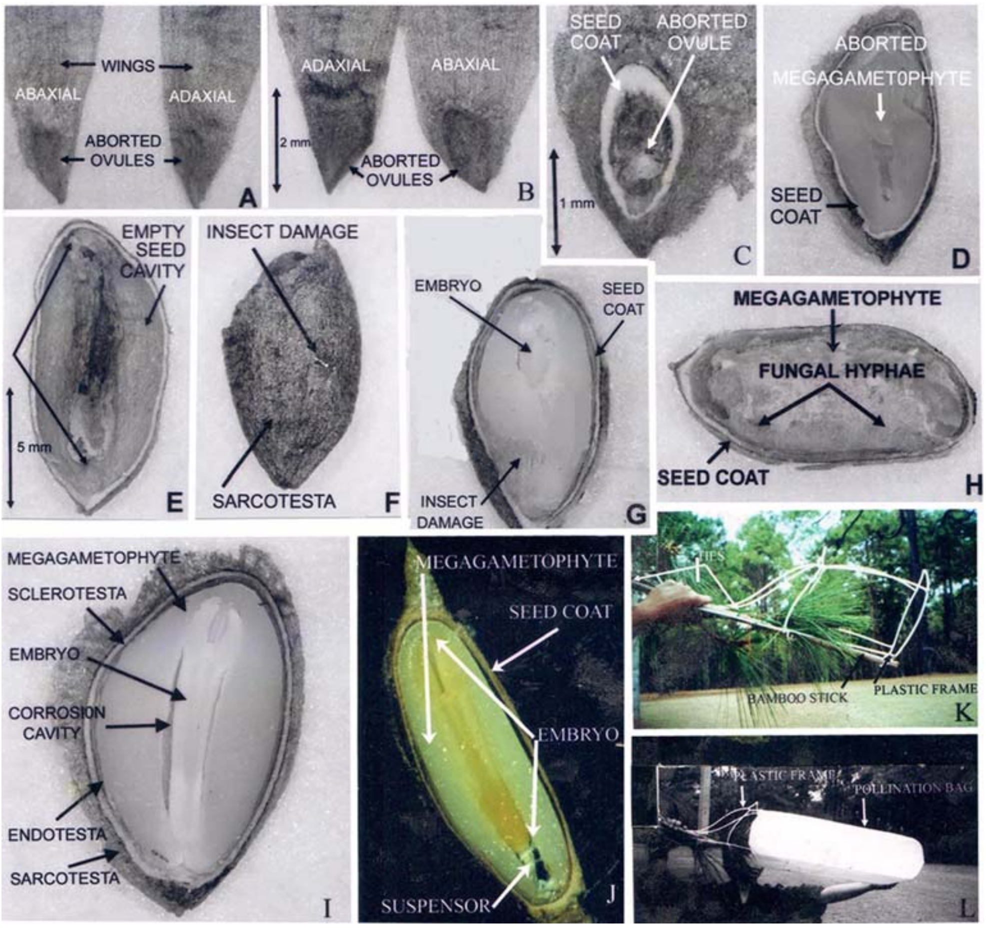

Figure 3. A. Some ovules aborted before pollination, thus are never pollinated and remain as flat ovules at the base of the seed wing in western white pine. In whitebark pine these seeds were attached to small rudimentary wings. **B.** Unpollinated western white pine ovules that aborted soon after the time of pollination leaving a small ovule (rudimentary seed) at the base of the seed wing. **3C-E.** Western white pine seeds sliced longitudinally and viewed using a dissecting microscope. **C.** Early self-incompatibility causes seeds to abort soon after pollination resulting in a slightly larger rudimentary seed containing collapsed tissues. **D**. A seed in which the embryo has aborted and the megagametophyte, which is partially filled with solid storage products, is starting to abort, probably as a result of late acting self-incompatibility or insect damage, such as from *Leptoglossus* feeding. **E**. Many seeds abort at the time of fertilization and proembryo development, when the megagametophyte consists of large fluid-filled cells and no solid lipid and protein food reserves. The megagametophyte collapses forming a brown empty sac (long arrows) in the seed cavity. These are often called "empty seeds" but similar-appearing seeds may result from *Letoglossus* feeding at that same stage of develpment. **F.** Insects, such as *Leptoglossus*, may damage the seed coat but damage is small and difficult to observe. **G**. Insect feeding, as shown in Figure 3F, leaves small scars on the seed coat and may damage the megagametophyte but not destroy the embryo. **H**. Fungi may infect some seeds and may be introduced by insects feeding on seeds. **I**. Mature dehydrated, healthy western white pine seed sliced longitudinally to show the three layers of the seed coat, the megagametophyte and embryo in the corrosion cavity. **J**. Mature hydrated, healthy western white pine seed showing the light yellow embryo fitting tightly within the corrosion cavity and the cream-colored megagametophyte fitting tightly within the seed coat. Magnification of **FF-J** are the same as indicated in **E**. **K and L.** Structure of frames designed to reduce cone abortion caused by abrasion from contact of young cones with pollination bags and insect bags. **K.** The frame was made from 4 mm diameter polyethylene welding rod and fused with heat. It is fitted over the branch and fastened in place by a bamboo stick and twist ties so that the branch does not bend downward and the cones are held in the center of the frame. **L**. Pollination bags and later insect bags can be slid over the frame and held in place with twist ties.

Table 1. Cone and seed analysis for whitebark pine (*P. albicaulis*) and western white pine (*P. monticola*).

Species*/ No. trees**/ No. cones***	Cone Length (cm)	Total Scales	Sterile Scales	Fertile Scales	% Fertile Scales	Seed Potential	Total Seeds	Filled Seeds	Early Aborted Seeds (No pollination)	Late Aborted Seeds (Embryo abortion)	Insect-and Disease-Damaged Seeds	Seed Efficiency
WBP* 9**/45***	6	62	20	48	77	96	81	48	1	23	2	59
WWP* 5**/20***	13	128	18	108	86	216	155	115	67	27	7	4

***Several mature cones from wild WBP trees were completely destroyed by insects and could not be used for cone analysis. This was not true for WWP orchard trees where insecticides were used.

common at the base and tip of the seed cones but may occur on scales in other regions of the cones. They represent only a few percent of the seed potential in both species (Table 1). Ovules that develop normally up to the time of pollination, at which time they were undergoing meiosis to form the four megaspores or entering early free nuclear division, then aborted due to a lack of pollination, formed small rounded ovules (rudimentary seeds) in both species (Figures 3B). They may have a thick seed coat but contain a small amount of dry, shrunken tissue (Figure 3C). In *P. monticola* these represented about 30% of the seed potential but less than 1% of the seed potential in *P. albicaulis* (Table 1).

Later seed abortion

The next major time of seed loss is at fertilization or very early embryo development, about one year after pollination (Figure 1C). In mature cones, it is difficult to separate seeds that aborted soon after pollination from those that aborted during very early embryo development. However, the cause for both seems to be from self-fertilization resulting from self-pollination. Self-pollen germinates and forms pollen tubes that grow to the archegonia, but the sperm may fail to fertilize the egg within the archegonium or if fertilization does occur, the free-nuclear or few-cell proembryo may abort. Either of these causes result in the collapse of the megagametophyte, which at this stage is a large sac filled with watery cytoplasm and many free nuclei. The collapsed megagametophyte remains as a dry brown wrinkled sac inside the thick seed coat (Figure 3E) have often been referred to as "empty seeds" because the collapsed megagametophyte is not visible using x-rays for seed analysis. This usage is unfortunately inaccurate because there can be several causes for "empty seeds". This is especially true for seeds that have been insect damaged at an early stage of development, in which all the rater fluid seed contents have been removed leaving only a seed coat. Using x-ray techniques, these are usually indistinguishable form empty seeds resulting from self-fertilization. About 17% and 20% of potential seeds were lost at this time in *P. monticola* and *P. albicaulis*, respectively. We believe that most of these losses resulted from self-fertilization. However, using x-ray seed analysis, it is difficult to distinguish seed losses from self-fertilization, from seed losses from the western cone bug (*Leptoglossus*) which sucks the contents from the seeds with its proboscis leaving a damaged or partially collapsed megagametophyte (Figure 3G). The distinction can more accurately and easily be made using dissected seeds because *Leptoglossus* may damage seeds at any time from pre-fertilization, when the megagametophyte is still at the free-nuclear stage through early embryo development when the megagametophyte is at the cellular stage with varying amounts of storage lipids and proteins that don't begin to accumulate until after fertilization (Figure 3D). Seeds damaged after the accumulation of these more-solid storage products do not totally collapse (Figure 3G). Many seeds on which *Leptoglossus* feeds become filled with variously colored fluids which appear to result from bacteria or fungi entering the seed during the insect feeding. These can be easily detected when mature seeds are sliced open. *Leptoglossus* damaged seeds may be abundant in seed orchards in years when the insect populations are high and they may destroy a large proportion of the seeds in many cones (Owens *et al.* 2005).

It should be mentioned here that much of the confusion over "empty seeds" results from seed analysis being done using radiographs which although expensive are no faster and are less accurate than seed dissection. By the time you have removed, cleaned and arranged the seeds and taken the radiogram you could have stuck all of the seeds to tape on a board, then sliced and analyzed all the seeds using a dissecting microscope. Also, it should be mentioned that seed x-ray machines are very expensive, each radiogram is also expensive, and radiograms are only valid for dry seeds and cannot be used for imbibed seeds. A good seed manual such as Kolotelo (1997) or Kolotelo *et al.* (2001) should be consulted if seed analyses are to be accurately done.

Other insect- and disease-damaged seeds

The damage from other insects is usually more obvious. Entire cones may be damaged and the insect larva may remain in the seed cavity, the cavity may be filled with fras, or an exit whole may be evident. Cones that have high insect damage may show obvious deformation of the cones and abundant fras on the outside of the cone. These cones will bear few or no filled seeds and should not be collected. Each conifer species may be infested by several cone and seed insects and insects new to a conifer may appear when trees are placed in seed orchards near orchards containing other conifer species. In all cases a good cone and seed insect manual should be consulted for correct identification and control measures such as Hedlin *et al.* (1980).

There are few diseases that affect pine cones and seeds but these may increase in some seed orchards where trees are grown outside their natural range and in association with other conifer species or fallen cones are not cleared away. A few seeds from *P. monticola* were found to contain a collapsed megagametophyte and fungal hyphae (Figure 3H) but it was not determined if the seed was destroyed by the fungus or more likely damaged by an insect that introduced the fungal spore into the seed. Again, a good reference on cone and seed diseases should be consulted, such as Sutherland *et al.* (1987), for identification of diseases and control methods.

Insect- and disease-damaged cones and seeds were few in these studies because for *P. monticola*, many studies were done in seed orchards where there was good management or cones were collected from natural stands and cones that were obviously damaged were not collected because they would have few or no filled seeds. As a result, in the cone and seed analysis, less than 1% of seeds were classed as insect- or disease-damaged but some nearly totally destroyed cones were present but seeds from them could not be analyzed.

Frost damage can occur to seed cones at the time of pollination. This does not appear to be a major problem in either the coastal or interior seed orchards of *P. monticola*. We have no information for *P. albicaulis* but we would assume that such a high elevation species may suffer from low temperatures at some sites in some years but this is less likely to be a problem when the trees are moved to low elevation seed orchards. Seed orchard managers should be warned not to use orchard overhead sprinkling excessively to control phenology and avoid outside pollen because in Douglas-fir this has been shown to increase the growth of microorganisms in the receptive cone which can cause cone drop at pollination (Colangela *et al.* 1990).

Filled and healthy seeds

Fortunately *P. monticola* and *P. albicaulis* have no serious impediments to a high proportion of seed production per cone which we calculate as Seed Efficiency (SEF), the percentage of filled seeds compared to total seeds. In our cone and seed analysis, *P. monticola* had a SEF of 59% and *P. albicaulis* a SEF of 74% (Table 1). A more common SEF for species of Pinaceae is about 35% (Owens *et al.* 1991; Owens *et al.* 2005). Filled and healthy seeds should have a cream-colored megagametophyte that fills the seed cavity but in dehydrated seeds this may pull away slightly from the seed coat and the mature embryo should be about 90% the length of the megagametophyte (Figure 3I). Seeds with embryos filling only about 50% or less the length of the megagametophyte will probably not be viable. The slightly yellow to white embryo may pull away from the megagametophyte leaving space in the corrosion cavity around the embryo in dry seeds (Figure 3I) but in hydrated seeds (Figure 3J) the embryo fits tightly within the megagametophyte and the megagametophyte fits tightly within the seed coat. Discoloring of the megagametophyte or embryo may result in an enviable seed (Kolotelo1997, Kolotelo *et al.* 2001).

Concluding remarks

Results from the studies of many aspects of reproduction of *P. monticola* and seed and cone analysis for *P. albicaulis* are compared here and demonstrate that these two five-needle pines have a very impressive seed-production capacity. It is especially impressive when you consider that *P. monticola* may occupy harsh high elevation sites and *P. albicaulis* always occupies harsh high elevation sites in western North America. It is also important to recognize that *P. monticola* can be easily managed in seed orchards, which indicates that other five-needle pines may be equally amenable to cone and seed production in seed orchards. This will be important in future breeding for rust resistance. All five-needle pines apparently have similar reproductive cycles to that described here for *P. monticola* which may be typical of the group, however the phenology may vary with the site and the species.

All species will have the same sensitive or fragile stages in reproduction, where something may go wrong, resulting in reduced cone or seed production. These stages include: 1. Cone initiation, which was not discussed here, but for which the time of seed cone differentiation (in the spring) differs form that in hard pines (Owens 2006). Fortunately, these two pines tend to initiate frequent cone crops and produce abundant pollen; 2. Pollination in the spring is very critical since inadequate pollination may result in cone abortion soon after pollination or low filled seed thereafter; 3. Self-pollination results in self-fertilization leading to failure of sperm to fertilize the eggs one year after pollination. This results in abortion of proembryos soon after fertilization or abortion at later stages of embryo development in the 3 to 4 mo after fertilization which leads to different kinds of unviable seeds; and, 4. Cone insects and diseases may destroy whole cones or destroy or render seeds unviable. Fortunately, results from the detailed work already done with *P. monticola*, indicates that many of the problems which might arise at these critical reproductive stages can be avoided, solved or minimized in well managed seed orchards making it possible to develop successful breeding programs and hopefully, someday, the production of abundant rust-resistant seeds for reforestation. Other five-needle pines may have other problems of which we are not yet aware. This paper does not give all the answers but hopefully some of the ideas will serve as guidelines for cone and seed production for five-needle pines.

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Effect of Ectomycorrhizal Fungi on the Growth of Korean Pine

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Abstract – To study the effects of ectomycorrhizal fungi on growth of Korean pine (*Pinus koraiensis* Sieb. et Zucc.) seedlings, the combinations of different ectomycorrhizal fungi strains were screened by dual culture method based on previous research. Three–year old seedlings of Korean pine were then inoculated with different single strains and different combinations using lister inoculating method in the field, and those inoculated by spell out first what PD stands for (PD) liquid culture medium were designed as control. The growth characteristics of 3-year old seedlings were observed 100 days after inoculation. Strain 010 and strain 025 promoted seedlings growth significantly as compared with the control; the average height increases of seedlings inoculated with strain 010 and strain 025 were 19.6% and 17.0% and the average collar diameters were increased 13.6% and 15.9%, Respectively. The average heights of the seedlings inoculated with combination of GT001/GT005 was increased when compared over those with single strain, The growth of seedlings treated with other combinations of strains rather inhibited the growth as compared to each single strain. The average collar diameters of the seedlings inoculated with combination 044/009 was between that inoculated with strain 044 and strain 009, and that with other three combinations were lower than that inoculated with each single strain. In conclusion, strain 010 and strain 025 significantly promoted the seedling growth of Korean pine. Inoculation by the mixture of highly effective strains and other single strain did not promote growth of seedlings.

Key words: Korean pine, ectomycorrhizal fungi, single-inoculation, mixed-inoculation, growth-promoting effect

Introduction

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is native to eastern Asia, Manchuria, Russia Far East, Korea, and central Japan. It is one of the major tree species in the timber-lands of northeastern China (Editorial Committee of Forest in China, 2000). The timber is important source and used for construction, aviation, papermaking, and musical instrument as the stem texture is light, soft, and grows straight from top to bottom. Korean pine is also one of the major tree species as landscape use for its graceful appearance. Therefore, Korean pine is the important species with high economic and ecological value in China (Editorial Committee of Forest in China, 2000). A lot of research work on biological and ecological characters, population ecology, structure and function of ecological system, and genetic ecology about Korean pine in China and many other countries has been carried out. However, research work on ectomycorrhiza did not receive attentions in the past.

The research work indicated that stabilization of forest community, tree regeneration and growth are directly associated with the type and diversity of ectomycorrhiza (Molina *et al*. 1992; Newman, 1988; Perry *et al.* 1987, 1989). In general, trees that need ectomycorrhiza can form symbiotes with many ectomycorrhizal fungi for seedling establishment and better growth. The trees in forest are related closely in time and space through sharing consensus ectomycorrhizal fungi (Wang, 1986; Read, 1993). Selection from the diverse and the utilization of right ectomycorrhiza can make trees use resources more effectively, and renew the trees which. In disturbed site conditions, efficient and rapid formation of ectomycorrhiza is very important for seedlings to survive under unfavorable environments (Schoenberger, 1982; Smith *et al*. 1977; Trappe, 1977; Harley, 1989).

Information on the use of ectomycorrhiza to investigate how they affect the growth of Korean pine except resources and distribution of ectomycorrhizal fungi are not available readily (Lee *et al*. 1987; Meng *et al*. 2001; Kikuchi *et al*. 2003; Hirose *et al*. 2004; Choi *et al*. 2005, 2006). There are many problems that are to be investigated, such as how many and which ectomycorrhizal fungi can form symbiots with Korean pine at every period of growth and regeneration, the effect of ectomycorrhizal fungi on the survival and growth of Korean pine, the types and diversity of ectomycorrhiza related to the different ages of Korean pine at various growing conditions, the effect of potential ectomycorrhizal fungi inoculated on Korean pine seedlings, and adaptability of ectomycorrhizal fungi after afforestation with ectomycorrhizal seedlings in every site. To

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Table 1. Pairing combinations

No.	Pairing strain 1/ Pairing strain 2	No.	Pairing strain 1/ Pairing strain 2	No.	Pairing strain 1/ Pairing strain 2
1	LH004/009	9	009/GT001	17	010/044
2	LH004/025	10	009/044	18	010/GT005
3	LH004/010	11	009/GT005	19	GT001/044
4	LH004/035	12	025/010	20	004/GT005
5	LH004/GT005	13	025/GT001	21	035/009
6	LH004/044	14	025/044	22	GT001/GT005
7	009/025	15	025/GT005		
8	009/010	16	004/GT001		

understand these problems, following research on the understanding the biological and ecological characters of Korean pine, stability maintenance mechanisms of forest ecological systems, relationship between Korean pine and other tree species in time and space, and screening good ectomycorrhizal fungi for growth of Korean pine seedling, and rapidly renew the forest vegetation should be carried out.

The objectives of this research are to understand the effect of ectomycorrhizal fungi on the growth of Korean pine seedlings, to screen highly-effective ectomycorrhizal fungi and their combinations for Korean pine seedlings growth, and to perform field inoculation with ectomycorrhizal fungi which have been identified to form ectomycorrhiza with Korean pine seedlings by inoculation.

Materials and Methods

Ectomycorrhizal fungi

Ectomycorrhizal fungi strains 004, 009, 010 (*Agaricus silvaticus*), 025 (*Cortinarius collinitus*), 035 (*Suillus granulatus*), 044, LH004, GT001 and GT005 were collected from the Xinlin Forestry Bureau of Great Xingan Mountain and Xinglong Forestry Bureau, Heilongjiang Province of China. These nine strains were proved to form ectomycorrhiza with Korean pine seedlings following inoculations.

Amplify culture of ectomycorrhizal fungi

Potato dextrose (PD) medium was used to amplify culture for ectomycorrhizal fungi. The strain was inoculated on the plate (diameter is 90 mm) of PD agar (PDA) medium A at 25 for 7 days. Three pieces colonies of diameter of 0.5 cm were cut and placed on culture flask (500 ml) which contains 250 ml PD liquid culture medium, placed on a shaker at 120 rmp/min for 15 days at 25^{o}C.

Screening combinations of ectomycorrhizal fungi for mixed-inoculation

The aim of this test is to seek out the best combination which can be used for mixed-inoculation. Discs of 0.6 cm diameter from every two ectomycorrhizal fungi strains were cut and cultured on PDA at 25^{o}C. The distance between two strains was 3 cm. Diameters of 0.6 cm discs of single strain cultured at center of PDA served as control. Twenty-two pairing combinations of two strains were prepared as stated in Table 1. The colony radius was measured at given time intervals. In pairing culture test, the directional radiuses of two colonies were used to determine for pairing growth (Song *et al.* 2001). The experiments were repeated 5 times.

Inhibititon ratio was expressed as (colony radius of control-directional radius of colony)/colony radius of control × 100%.

The best combinations were selected according to inhibited rate. When the inhibited rate was <0, tere was no inhibition between two paired strains, and it is considered that the combination is the best one showing promoting effects. When the inhibited rate was 0, there were no inhibiting effects between the two strains and the combination was considered good. There was inhibition between the two strains when inhibited rate was >0.

Testing of field inoculation

Three-year old seedlings of Korean pine transplanted in Yanshou Forestry Nursery of Heilongjiang Province were inoculated with ectomycorrhizal fungi strains 004, 009, 010, 025, 035, 044, LH004, GT001, GT005 and combination in pairs 009/044, GT001/044, 025/044, GT001/GT005 with lister inoculation method on June 1, 2004. The seedling bed is 1 × 20 m, the seedlings on one seedling bed were inoculated with one strain or one combination of two strains. Seedlings in one line were inoculated with 200 ml liquid inoculum. The PD liquid culture medium was designed as control.

The growth characteristics of 3-year old seedlings were observed 100 days after inoculation. Thirty seedlings were collected with the method of diagonal sampling to measure seedlings height and seedlings collar diameter at the ground level.

Results and Discussion

Screening combinations of ectomycorrhizal fungi for mixed-inoculation

The results (Figure 1) showed that the best combinations are 009/044, GT001/044, 025/044, GT001/GT005. To these four combinations, one strain's inhibited rate is 0, and the other's is smaller than 0, suggesting coexistence phenomenon which indicates that one species of the organism is benefited from the other and the other can not be affected (Song *et al.* 2001). Other combinations are not suited for mixed-inoculation as there are inhibiting between each other.

Field inoculation with single strains

The results in this section (Table 2, Figure 2) showed that, with the exception of strains GT001 and GT005, all other strains, especially strains of 009, 010, 025 have promoting effect on the growth of Korean pine seedlings. Comparing with the control, the average heights of the seedlings for 100 days inoculated by strain 010 were increased 19.57%, and those inoculated by strain 009 and strain 025 were increased, respectively, 17.04% and 13.15%. Except for strain 009, all other strains have promoting effects on the growth of collar diameter growth of seedlings. The promoting effects of 5 strains (010, 025, 035, 044, LH004) are notable. The average collar diameters of seedlings inoculated by strain 035 and strain LH004 for 100 days were both 20.46% as compared to the control, and those inoculated by strain 010 and strain 025 were, respectively, increased by 13.64% and 15.91%. In conclusion, strain 010 and strain 025 are the best for promoting Korean pine seedlings growth.

Table 2. Growth of Korea Pine seedlings 100days after inoculation

	Seedlings height		Seedlings collar diameter	
Strains (Combinations)	Seedlings height (cm)	Percentage increase (%)	Seedlings collar diameter (cm)	Percentage increase (%)
004	10.75	4.67	0.47	5.91
009	12.02*	17.04*	0.43	-2.27
010	12.28*	19.57*	0.50*	13.64*
025	11.62*	13.15*	0.51*	15.91*
035	10.43	1.56	0.53*	20.46*
044	10.28	0.010	0.49*	12.27*
GT001	10.05	-2.14	0.47	6.82
GT005	10.25	-0.195	0.46	4.55
LH004	11.13	8.37	0.53*	20.46*
044/025	10.16	-1.07	0.47	6.82
044/009	10.09	-1.75	0.48	9.10
GT001/044	9.80	-4.58	0.47	6.82
GT001/GT005	10.66	3.80	0.44	0
control	10.27		0.44	

*Signicant as compared to the control at =0.01, F-test.

Except the promoting effect on seedlings growth, strain 010 and strain 025 still have self-growth advantage. The self-growth advantage maybe shown on both ways: one way is forming definite beneficial relationship with some indigenous flora, some indigenous flora can promote the growth of strain 010 and strain 025; the other way is forming definite synergism effect with some indigenous flora, and promoting seedlings growth together. However, this still need to be validated through experiment.

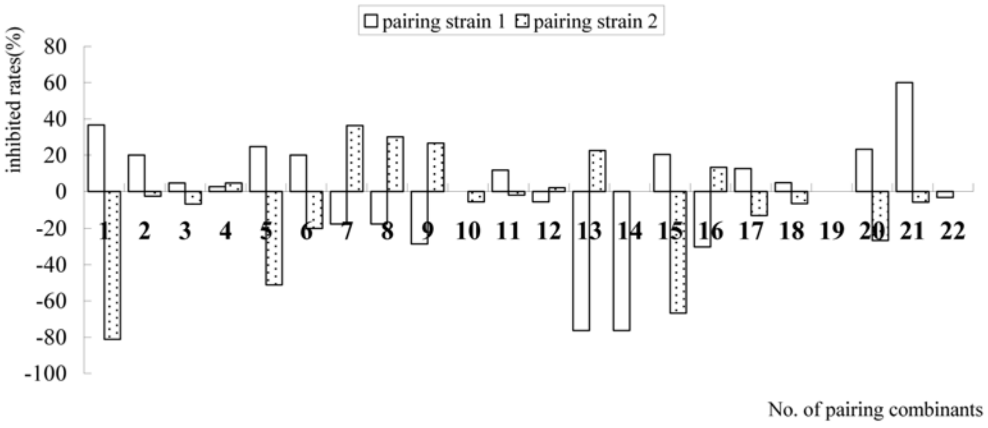

Figure 1. Inhibitted-rates of ectomyrrhizal fungal strains.

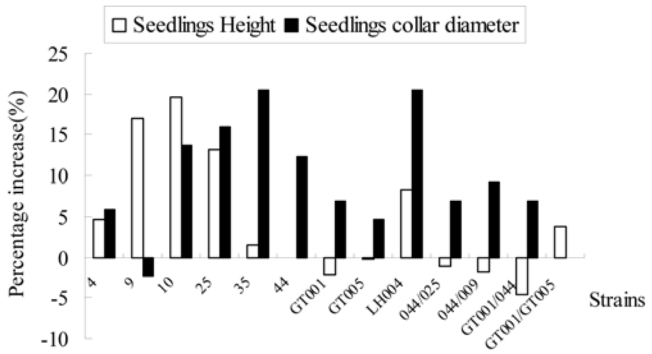

Figure 2. Percentage increase in parameters of seedlings 100 days after inoculation (%).

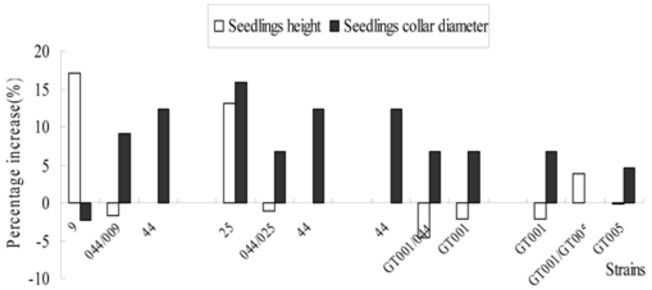

Figure 3. Percentage increase in parameters of seedlings 100 days after inoculation (%).

Field inoculation with combination of strains

Only combination of GT001/GT005 strains showed promoting effect on the height growth of Korean pine seedlings (Table 2, Figure 2). However, 100 days after inoculation the average heights of the seedlings inoculated by GT001/GT005 was only increased 3.80% as compared with the control, Other combinations all inhibited seedlings height growth.

Except GT001/GT005, all other three combinations had promoting effects on seedlings collar diameter growth slightly although it is not significant.

Comparison of inoculation by single and combinations of strains

The average heights of the seedlings inoculated by combination of GT001/GT005 strains were taller than that inoculated by each single strain. The average heights of the seedlings inoculated by combination 044/009 was shorter than that those inoculated by each single strain, the same as the seedlings inoculated by 044/025 and 044/GT001 (Figure 3). The average collar diameters of the seedlings inoculated by combination of 044/009 strains was between that inoculated by strain 044 and strain 009, and that inoculated by combination of 044/025 was smaller than that inoculated by each of single strains, the same as the seedlings inoculated by 044/GT001, and GT001/GT005.

The promoting-effects of combination treatments of two strains were less than those of the effect observed by single strain treatment. Thus, inoculation by the combination of high efficient strain and common strain does not elevate the effect of high efficient strain to promote the growth of Korean pine seedlings.

Conclusion

Agaricus silvaticus strain 010 is the best strain to promote the growth of Korean pine seedlings; increasing the height of seedlings 19.57% and collar diameter 13.64% in 100 days. *Cortinarius collinitus* strain 025 can also be regarded as an effective strain. The effect of inoculation by combining two of highly effective strains of ectomycorrhizal fungi is not recommended to promote seedlings growth.

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Interspecific Hybridization as a Factor of Reticulate Evolution in 5-Needle Pines of Northern and Eastern Asia

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Abstract – Phenotypic and genetic diversity in 5-needle pines of Northern and Eastern Asia was analyzed. *P. pumila* is intermediate between *P. sibirica* and *P. parviflora* and *P. koraiensis* was intermediate between *P. sibirica* and *P. armandii* on complex of morphological, anatomical and genetic traits. We suppose that stone pines (subsection *Cembrae*) are not monophyletic. Along with some other species of subsection *Strobi* stone pines rather comprise a complex phylogenetic group that might have originated in consequence of reticulate evolution. This process was mediated by climate dependent rotation of divergence and hybridization of the species. Genetic processes in a contemporary hybrid zone between *P. sibirica* X *P. pumila* in the Lake Baikal region were studied. Natural hybrids are identified throuout the introgression zone (Khentei-Chikoi and Stanovoje Highlands, Khamar-Daban Range) at the average of about five plants per hectare. Most hybrids were intermediate between the parent species on morphological and physiological traits. The hybrids have ability for vegetative reproduction. They canoccupy ecological niches at second storey of the mixed stands but have a lower fitness and fertility as compared with pure species. Significant contribution of hybrids in total mixed stand effective pollen pool especially in fertilization of hybrid plant ovules is evidence of the phenomenon evolutionary perspectives.

Introduction

Already in the first scientific classification of the genus *Pinus,* subgenus *Haploxylon* included subsection *Cembra* subdivided into two groups - *Strobi* and *Cembrae* (Shaw, 1914). In the later classifications were changed only some taxon rank or their names (Price *et al.* 1998). Division of 5-needle pines into two groups was made by seed cone structure: dehiscent in *Strobi* and indehiscent in *Cembrae*. Why is it so important? R. Lanner (1990) wrote: "*Cembrae* seed cone is unique; it cannot arise twice in the course of evolution". We think it is true. Does it mean that 4-5 *Cembrae* species and 17-18 *Strobi* species are two natural (monophyletic) groups? The main goal of our paper is to analyze structure of diversity in northern and eastern Asia 5-needle pines so as to understand their phylogeny and patterns of evolution. The target species are subarctic and subalpine *P. pumila*, boreal *P. sibirica*, nemoral *P. koraiensis* and *P. parviflora*, and subtropic mountain *P. armandii*.

Species Comparison

We examined the diversity of traits that are veryimportant in reconstruction of taxonomy for this species. Seed cone structure. Three *Cembrae* species (*P. sibirica*, *P. koraiensis*, and *P. pumila*) have indehiscent cones. In the base of scale, there is special abscission layer of brittle tissue. Birds turn off scale and take off seeds. Two *Strobi* species (*P. parviflora* and *P. armandi*) have dehiscent cones. Really in *P. armandi*, chink between scales is not very wide. The Eurasian nutcrackers (*Nucifraga caryocatactes* L.) extract seeds through this chink by means of their bill using it as tweezers. *Cembrae* species are not quite equal with respect to integrity of mature cones. Mature *P. sibirica* cones never have even thin chink between scales. In *P. koraiensis* and especially in *P. pumila*, this chink is not rare and cones seem to be loose (Figure 1).

Seed shape and structure: *P. armandi* and *P. parviflora* seeds have rudiment wing while *Cembrae* species lack it. *Strobi* pine seeds are more flattened, *P. sibirica* seeds are rather round in transversal cross-section. whereas *P. koraiensis* and *P. pumila* occupy intermediate position (Table 1).

Pollen grains of *P. sibirica*, *P. pumila* and *P. parviflora* have a few difference; in *P. koraiensis* and *P. armandii* pollen grains are significantly larger and have slightly another structure

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Table 1. Seed shape in five 5-needle pine species

Traits of seed	*P. armandi*	*P. koraiensis*	*P. sibirica*	*P. pumila*	*P. parviflora*
Length, mm	5,3 c*	7,4 a	6,2 b	4,3 d	4,3 d
Width, mm	7,7 b	10,1 a	7,5 b	5,5 d	6,4 c
Length/width ratio	0,69 c	0,73 b	0,87 à	0,78 b	0,68 c

*Numbers with different letters (a, b, c, d) within a line differed significantly (P = 0.95)

Figure 1. Indehiscent (left) and semidehiscent (right) seed cones of *P. pumila*

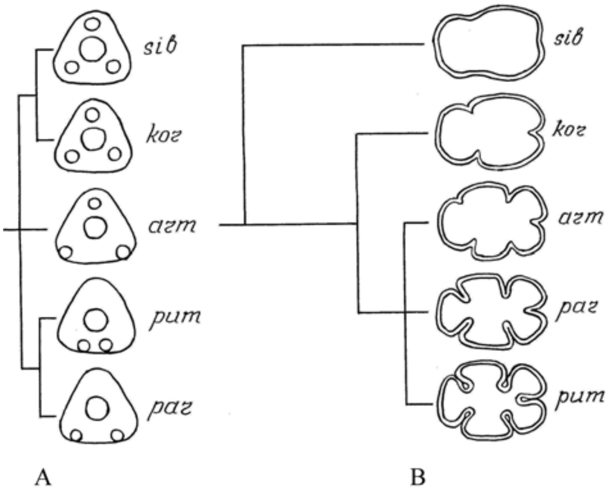

Figure 2. Needle anatomy in five 5-needle pine species: resin ducts location (A) and shape of mesophyll cells (B).

(Kuprijanova, Litvintseva, 1974).

Juvenile shoot structure: At first, all seedlings have only hypocotyl and cotyledons. Then juvenile shoot with single green needles is initiated and elongated. Finally transition to first mature shoot with scale leaves and axillary 5-needle fascicles is occurred. Duration of juvenile shoot development is one or two years. Juvenile shoot may have or have not axillary 5-needle fascicles. Concerning juvenile shoot structure, *P. sibirica* is distant from other species (there is no 5-needle fascicles). The latter are subdivided into the same two pairs: *P. pumila* + *P. parviflora* and *P. koraiensis* + *P. armandii* (they have 2- or 1-year cycle of juvenile shoot development, respectively).

Needle anatomy: *P. sibirica* and *P. koraiensis* have 3 resin ducts located inside of mesophyll (Figure 2). *P. armandii* has 3 resin ducts as well, but two abaxial ducts are adjacent to hypoderm. The rest species have only 2 resin ducts which are adjacent to hypoderm. In respect of mesophyll cells shape *P. sibirica* is significantly differ from other species. Wall of mesophyll cells have not any plications in this species. Four other species have typical plicate mesophyll. There are few superficial wrinkles in *P. koraiensis* mesophyll but other species have more wrinkles which depth increased from *P. armandi* to *P. pumila*.

Molecular markers: Species classifications based on modern methods of biochemical and molecular genetics are the same conflicting as morphological classifications. Isozyme-based phylogeny did not show clear separation of the section *Strobi* onto two clusters corresponding to traditional scheme, since genetic distances between some representatives of the two nominative subsections are similar with those observed within the subsections (Belokon *et al*, 1998; Politov & Krutovskii 2004; Politov, 2007). Classifications based on DNA-markers dates are inconsistent. For example, one of them (based on chloroplast DNA sequences) opposes *P. parviflora* to four other species (Gernandt *et al.* 2005); another (based on nuclear (LEA)-like gene polymorphism) does the same for *P. armandii* (Syring *et al.* 2007).

Taking into account all premises, we can subdivide the five studied species into three groups:

(1) typical *Cembrae,* which includes *P. sibirica* only;

(2) typical *Strobi*, *P. parviflora* and *P. armandii*;

(3) species combining *Cembrae* and *Strobi* traits or having intermediate traits, *P. pumila* and *P. koraiensis*.

The schemes of evolution based on different traits seem to be intricate. We think that this complexity is mainly caused by reticular evolution of these species group. Investigation of hybrid speciation has relatively long history. As early as in 18-th century K. Linnaeus believed that a new species can originate via hybridization between two “old” species. A. Kerner

(1894, 1895) in the end of 19-th century described over 1000 natural interspecific hybrids only in not very rich European flora. In the first quarter of 20-th century, J. Lotsy (1916) published the book "Evolution by means of hybridization". Its main idea was that species are constant, and hybridization is the main if not the only factor of evolution. Than T. Dobzhansky (1937) introduced the term "reticular evolution". V. Grant (1981) discerned natural interspecific hybridization as one of many speciation mechanisms. He intended essentially classic slowly speciation. B. McKlintock (1950, 1965) discovered mobile genetic elements and genome instability. M.G. Kidwell and co-authors first described hybrid disgenesis, which is a genetic mechanism of specific genome transformation after hybridization causing "momentary" reproductive isolation between hybrids and parent species, so-called hybrid speciation (Kidwell *et al*. 1977).

Hereby we formulated a hypotheses that: *(1) P. pumila* and *P. koraiensis* are of hybrid origin and are the products of reticular evolution; *(2)* there are quick speciation processes in the modern zones of 5-needle pines interspecific hybridization. The proofs for the first hypothesis were adduced above. Below we discuss the evidences for the second one.

P. sibirica and *P. pumila* hybrid zone

In Baikal and Trans-Baikal area the ranges of *P. sibirica* and *P. pumila* are overlapped. There are many types of plant associations where the two species occur. For the last ten years we did research work in various parts of this region.

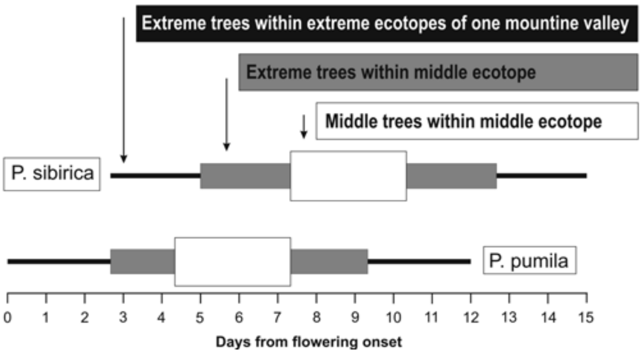

Figure 3. Flowering period of *P. sibirica* and *P. pumila* in their hybrid zone.

Flowering phenology of the species was studied in some areas in 3 sites across the hybridization zone. Middle (typical) tree of *P. pumila* starts flowering 2-3 days earlier than average tree of *P. sibirica*. Total duration of flowering for both species within one mountain valley is about 15 days, including 12 days when cross-pollination between the species is possible (Figure 3).

Identification of species and hybrids was based on two traits - habit and color of maturing cones. *P. sibirica* has vertical trunk, while *P. pumila* and hybrids have not. *P. pumila* cones are green, *P. sibirica* and hybrids cones are violet. Hybrids were founded in all investigated sites: from one tenth to 5 specimens per hectare. In general, hybrids occurrence decreased from southwest to northeast of the hybridization zone.

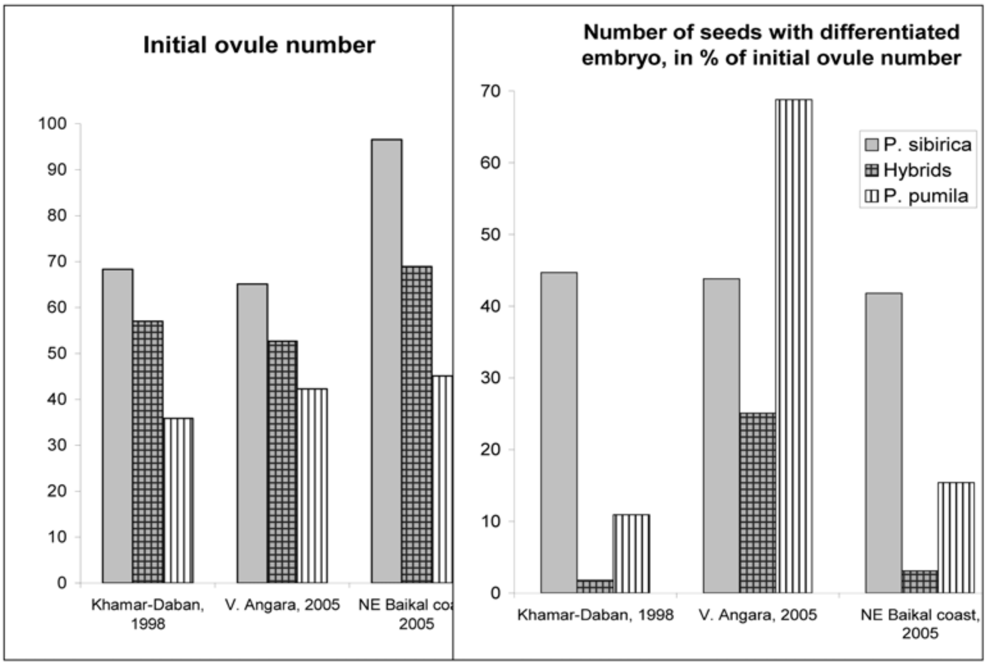

Figure 4. Seed production in *P. sibirica*, *P. pumila*, and their natural hybrids.

Genetic evidence of the hybrid origin of a single specimen of a putative hybrid was obtained by allozyme loci (Politov *et al*. 1999). Politov (2007) revealed and genetically proved a number of natural hybrids in several localities along the eastern Baikal coast.

Hybrids are intermediate between parental species in all studied morphological traits. For the whole period of ontogeny, *P. sibirica* has one direct vertical trunk. *P. pumila* has many unified trunks-branches from the beginning, and the number of such trunks-branches increased during the whole period of ontogeny. Hybrids have a single trunk from the beginning. Transition from a single-trunk to a multi-trunk status happens not so early as in *P. pumila*, but also not so late as in *P. sibirica*. Hybrid crown is situated above the snow cover, and its saber-shape trunks-branches have no snow breakage resistance. Such trunks-branches are fallen down, contacted with a mossy-lichen cover, stroked roots, and rejuvenated. As *P. pumila* clones, hybrid clones are virtually not subjected to ageing and have no internal limits of size and period for growing.

Mature cones from *P. sibirica*, *P. pumila* and their putative hybrids were collected to estimate variation of morphological traits and seed quality in three sites – the Northern Khamar-Daban, the delta of the Verhnaya Angara River (North Baikal shore) and Davsha Bight (Nothern-Eastern Baikal shore) (Figure 4). Cone size and initial number of ovules in hybrids were intermediate between the parent species. At all developmental stages, ovules and embryo abortion were several times higher in hybrids. But in some regions hybrids demonstrate normal seed production, and under favorite weather conditions approximately 25% of ovules generated a full seed with differentiated embryo.

In order to determine paternal parents of embryos from natural hybrids seeds we tried to answer the question through our investigation of mixed *P. pumila* - *P. sibirica* forest growing at marshland in the delta of the Verhnaya Angara river. In this population the ratio of fertile *P. pumila*, *P. sibirica* and their natural hybrids is 100/3/1. Species and hybrids ratio in population pollen production was determined through counting of pollen weight per one pollen cone, pollen cone number per average tree, and tree number per hectare. Participation of species and hybrids pollen in embryos generation was determined through isoenzyme analyzes of endosperms and corresponding embryos. Ratio of hybrids in composition to general population pollen pool is not significant (0.3%). Ratio of hybrid pollen in cross-pollinated embryos of hybrids is 40 times more (11,8%) (Petrova *et al*. 2007). Therefore, if all things being equal, hybrids of the next generations take chance to appear 40 times more often then any backcrosses!

DNA-polymorphism of *P. pumila*, *P. sibirica* and their natural hybrids from Northern Khamar-Daban mixed population was studied using the ISSR-PCR technique. Fractions 860, 840, 680, 560, and 400bp were detected. Some of them were monomorphic in the three groups, but other were absent in two of three groups. Thus, separation between species and hybrids was true and reliable. In comparison with parent species, hybrids demonstrate new monomorphic fractions of DNA as well as absence of fractions that were typical for one or both parent species. Cluster analysis reveals three strictly differentiated clusters, where difference between species is smaller than between each of the species and natural hybrids (Figure 5). We supposed that it is result of hybrid genome stress under coupling of two independent genomes and is a manifestation of hybrid disgenesis. Existence of mobile genetic elements in conifers and their role in insertion polymorphism appearance are known (Bennetzen, 1996; Freisen *et al*. 2000). So, one of possible explanations of the specific character of the *P. pumila*

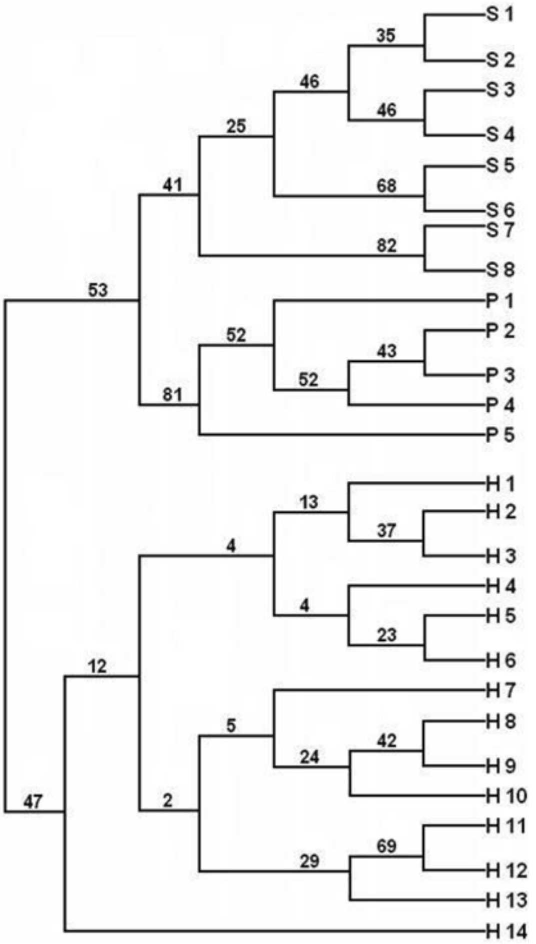

Figure 5. The dendrogram of studied samples similarity generated from a maximum parsimony analysis. S - *P. sibirica*, P - *P. pumila*, H - hybrids. Bootstrap replicates (1000) are indicated as percentage values above the branches.

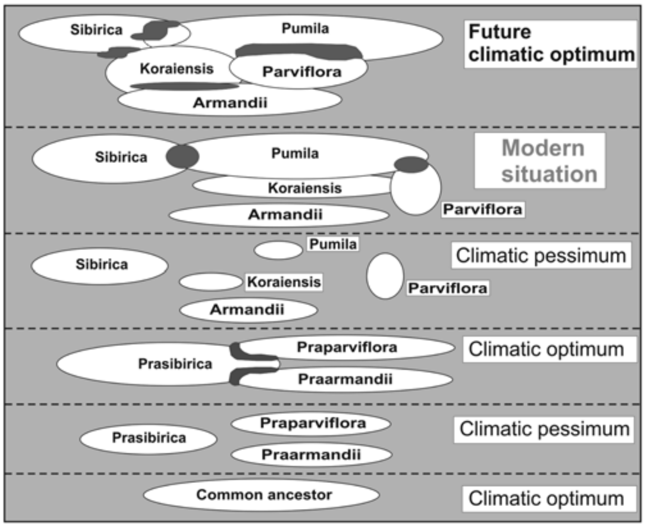

Figure 6. Hypothetic scheme of Asian 5-needle pines reticulate evolution. Six time "cuts" are situated bottom-up. Each cut is schematic geographic map of species ranges (white areas). Black spots are hybridization zones or areas of hybrid speciation.

× *P. sibirica* hybrids genome is activation of mobile genetic elements under hybridization.

Thus, stone pines (subsection *Cembrae*) are rather not monophyletic species group but together with some white pine species (subsection *Strobi*) constitute united phylogenetic system. The system is a product of reticulate evolution that is climatically caused cyclic alternation of species divergence and interspecific hybridization. The hypothetic scheme of this process is described below. Once a long time ago during climatic pessimum period, a common ancestor was broken up into 3 species: *prasibirica*, *praarmandi, praparviflora*. During the next climatic optimum their ranges overlapped and hybrid speciation was happened. During the next climatic pessimum united range was broken up again, but species number increased to 5: *P. pumila* and *P. koraiensis* appeared. Modern climate is not optimal for 5-needle pines on the vast territory. That is why their natural ranges are separated. But one of the new species, namely *P. pumila*, was founded as "child prodigy". Because of unusual growth form, the species quickly extended its natural range and penetrated into the parent species natural ranges. As a result, possibility for hybridization and hybrid speciation appeared. In the future, such possibility will appear for other species too if climatic conditions will be favorable.

Conclusion

1. Taking into account all traits which are important for taxonomy, we can subdivide 5-needle pines of Northern and Eastern Asia into 3 groups: (1) typical *Cembrae*, it is only *P. sibirica* and *P. cembra*; (2) typical *Strobi*, *P. parviflora* and *P. armandii*; (3) species combining *Cembrae* and *Strobi* traits or having intermediate traits, *P. pumila* and *P. koraiensis*. The third group presumably has a hybrid origin and the species are products of reticulate evolution.

2. Natural hybridization of *P. sibirica* and *P. pumila* takes a place within the whole area of its ranges' overlapping. By many morphological characters, most of natural hybrids occupy strictly transitional position between the parent species. In some plant associations hybrids occupies a relatively free ecological niche on the second tree layer. Fertility of hybrids is significantly lower than that of each of the parental species. However, under favorable weather conditions up to 25% of hybrid ovules generate sound seeds with differentiated embryo. In course of hybridization, the genome structure of all the first generation hybrids has significant and relatively unified changes. That is why under otherwise equal conditions, the first generation hybrids more often generate hybrids of the next generations than back-crosses of parental types. So we can regard these hybrids as "embryo" of new species.

3. Stone pines (subsection *Cembrae*) are not monophyletic species group but together with some white pine species (subsection *Strobi*) constitute united phylogenetic system. The system is product of reticular evolution that is climatically caused cyclic alternation of species divergence and interspecific hybridization.

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Tracking the Footsteps of an Invasive Plant-Pathogen: Intercontinental Phylogeographic Structure of the White-pine-blister-rust Fungus, *Cronartium ribicola*

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Abstract – Presently, little is known about the worldwide genetic structure, diversity, or evolutionary relationships of the white-pine-blister-rust fungus, *Cronartium ribicola*. A collaborative international effort is underway to determine the phylogeographic relationships among Asian, European, and North American sources of *C. ribicola* and closely related taxa. Here, we present preliminary information on phylogenetic relationships among selected Eurasian and North American populations of *C. ribicola* using DNA sequences from four nuclear loci totaling over 2,100 bp. Geographic regions included eastern and western North America, northern Germany, Korea, Japan, and northeastern China. Phylogenetic and network analyses suggest *C. ribicola* comprises at least three distinct clades. Isolates from Korea and China formed one clade, and Japanese isolates formed a second clade that was intermediate the third clade, which comprised isolates from USA and Germany. Identifying the evolutionary relationships and potential origin(s) of *C. ribicola* that spread through Eurasia and North America, and determining the phylogenetic relationships of its hosts are critical toward understanding this pathogen's native ecology. Such information will help evaluate risks of cryptic introductions, contribute to the development of biological controls, identify sources of host resistance and develop appropriate regulatory practices.

Introduction

Invasive plant pathogens pose a worldwide threat to natural and agricultural ecosystems, yet little is known about their origins or how they interact with other biotic and abiotic components of their native ecosystems. Phylogenetic and population genetic research is a critical first step in mitigating impacts of invasive plant pathogens through better understanding of their biology, epidemiology and ecology. This research could elucidate potential sources of invasive plant pathogens, which can improve measures to prevent introductions, identify potential biological control agents, and determine sources of host resistance.

Cronartium ribicola, the white-pine-blister-rust pathogen, was first reported in eastern Europe during the 1850s before spreading to western Europe and subsequently to North America in the late 1800's to early 1900's (Spaulding 1922, McDonald and Hoff 2001). Before these records, the occurrence and movement of blister rust is unknown, but origins of eastern Asia have been speculated (Leppik 1970). Today outside of North America, incidence of *C. ribicola* has been mainly found in plantations of introduced eastern white pine (*Pinus strobus*) in northern Europe. In Asia, white pine blister rust has been reported on Korean white pine (*P. koraiensis*) in the Korean peninsula and northeastern China, Japanese stone pine (*P. pumila*) in Japan, eastern Siberia and Kamchatka, Siberian pine (*P. sibirica*) in northcentral Asia and Siberia, and Himalayan blue pine (*P. wallichiana*) and Chinese white pine

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(*P. armandii*) in the Himalayas and central China (reviewed in McDonald *et al.* 2005). Varying alternate hosts have been reported among these regions, including 1) predominately *Pedicularis* spp. in South Korea, hereafter referred to as Korea (La and Yi 1995, Stephan and Hyun 1983); 2) *Pedicularis*, *Castilleja* and *Ribes* spp. in Siberia (Azbukina 1995, Kakishima *et al.* 1995); and 3) *Ribes* spp. in northern India (Bagshee 1950). Across much of Asia, blister rust disease incidence is usually minor, suggesting this pathosystem is highly coevolved in these regions.

In this study, we used four nuclear loci, over 2,100 bp of DNA sequence data, to elucidate the genetic relationships among *C. ribicola* that occurs in different continents and regions. We examined isolates collected from *Pedicularis* in Japan, *P. koraiensis* in Korea and China, *Ribes* from Germany, *P. strobus* in the eastern USA, and *P. albicaulis* in the western USA. The objectives of this study are to assess the phylogenetic relationships among *C. ribicola* from diverse geographic regions, and evaluate the potential origin(s) of populations that are extant in North America and western Europe.

Material and Methods

Rust isolates were collected either from the *Ribes* / *Pedicularis* (telial) or five-needled, white pine (aecial) hosts, placed in desiccant for shipment following the permit guidelines of the USDA Animal and Plant Health Inspection Service. DNA was extracted from aeciospores following the protocol of Zambino (2002). For telial host infections, three to four telial columns were placed directly into 50 μL of Lyse-N-Go™ reagent (Thermo Scientific) and processed according to the manufacturer's thermocycling protocol. PCR reagents were prepared with the following concentrations: 0.2 mM dNTPs, 4 mM $MgCl_2$, 0.5 μM of DNA primers, 10X PCR buffer, 1 U DNA polymerase, and 1 μL of the DNA template (i.e., Lyse-N-Go mixture™ diluted 10X with dH_2O) in a total volume of 30 μL. PCR was performed using fungal specific primers 5.8SR and LR7R for the large subunit (LSU, 28S) of the ribosomal DNA (Moncalvo *et al.* 2000) with the following thermocycling parameters: 95°C for 1 min and 35 cycles of 94°C for 30 sec, 49°C for 45 sec and 72°C for 1.5 min. A nested primer (5'-TTAAAAAGCAAAGGAGTG) was then used for the 5' sequencing reaction. Previously developed primers and thermocycling reactions were used for the other three loci: Dcon03, Dcon10, Dcon57 that showed highest homology to elongation factor 1-α, glutamine synthetase, and cytochrome P-450 monooxygenase, respectively (Joly *et al.* 2005). DNA sequencing was performed at the University of Wisconsin Biotechnology Center with an Applied Biosystems 3700 automated sequencer using both forward and reverse primers.

Sequence alignments were compiled with SEQUENCHER ver. 4.5. A partition-homogeneity test was performed with PAUP* 4.0b10 (Sinauer Publishing, Sunderland, MA) using 1,000 heuristic searches and TBR branch swapping to evaluate the concordance the four loci. Statistical parsimony

Table 1. Geographic location and host information of *Cronartium ribicola* isolates in this study

Sample name	Location	Host	Longitude	Latitude
Ch1	Jiaohe, China	*Pinus koraiensis*	127°28	42°10
Ch12	Jiaohe, China	*P. koraiensis*	127°28	42°10
Ch3	Jiaohe, China	*P. koraiensis*	127°28	42°10
Ger2	Walsieverdorf, Germany	*Ribes nigrum*	14°03	52°32
Ger6	Walsieverdorf, Germany	*R. nigrum*	14°03	52°32
JJ127_56	Jinbu, Korea	*P. koraiensis*	128°31	37°29
JJ127_6	Jinbu, Korea	*P. koraiensis*	128°31	37°29
LJ13	Jinbu, Korea	*P. koraiensis*	128°06	37°29
LJ14	Jinbu, Korea	*P. koraiensis*	128°06	37°29
Mt_K_B	Mt. Kisokomagatake, Japan	*Pedicularis* spp.	137°48	35°46
Mt_K_C2	Mt. Kisokomagatake, Japan	*Pedicularis* spp.	137°48	35°46
Mt_N_A	Mt. Norikura, Japan	*Pedicularis* spp.	137°48	35°46
Mt_N_D	Mt. Norikura, Japan	*Pedicularis* spp.	137°48	35°46
MN2-1B	Minnesota, USA	*P. strobus*	-95°33	44°40
NH1-1B	New Hampshire, USA	*P. strobus*	71°57	44°02
RN32	Idaho, USA	*P. albicaulis*	-116°34	48°37
WI4-1B	Wisconsin, USA	*P. strobus*	-88°51	45°33
*Cronartium occidentale**	Idaho, USA	*P. monophylla*	-113°43	43°05

* = outgroup

haplotype network analysis was conducted with TCS 1.21 (Clement *et al.* 2000). The interspecific outgroup, *Cronartium occidentale*, was excluded in this analysis. Gaps were treated as a 5th character state. Maximum parsimony phylogenetic analysis was performed with PAUP* using the same parameters as above, with gaps coded as missing and using the MULPARS option. Node support was evaluated with 1,000 bootstrap replicates with random addition. Model Test 3.7 (Posada and Crandall 1998) was used to determine the most suitable nucleotide substitution model using Akaike information criterion (AIC) for the combined dataset. The best-fit model was the general time reversal (GTR) model. Bayesian phylogenetic analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo search of 100,000 generations. Maximum likelihood was performed with PAUP using the same search parameters as parsimony analysis. Node support was evaluated with 100 bootstrap replicates using the full heuristic search.

Results and Discussion

DNA sequencing produced 395 characters for Dcon03, 441 characters for Dcon10, and 271 characters for Dcon57 with three, seven, and five intraspecific, single-nucleotide polymorphisms (SNPs), respectively. A portion of the LSU totaled 1,039 characters with nine SNPs. The partition homogeneity test results showed that all four loci could be combined (p = 0.35). The combined DNA sequence data totaled 2,146 base pairs, with 24 polymorphic sites.

The network analysis recovered 10 haplotypes from 17 isolates of *C. ribicola*. Three distinct clusters of isolates were found within the network that included 1) Korea/China, 2) Japan, and 3) Germany/North America Isolates from Japan were intermediate to Korea/China and Germany separated by five mutation steps from both clusters (Fig. 1). Five and three haplotypes, separated by one mutation, were found within Korea/China and Japan, respectively. Only two isolates were found within the Germany cluster. The Wisconsin isolate was separated by two mutation events from the other Germany isolates.

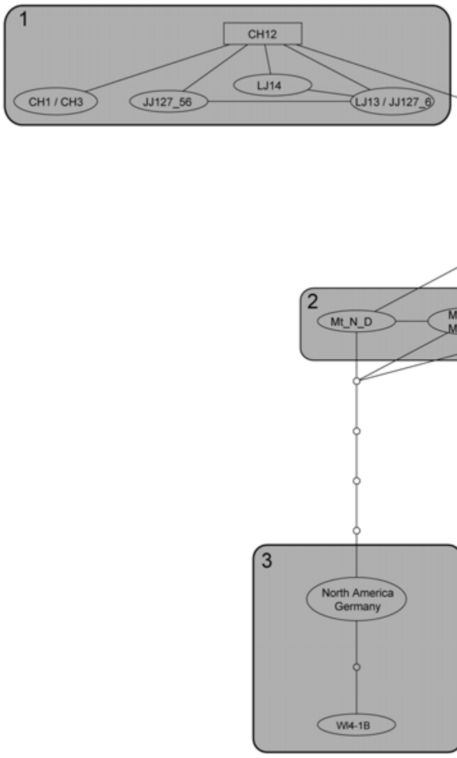

Figure 1. A statistical parsimony haplotype network analysis of 17 *Cronartium ribicola* isolates. The lines connecting isolates represent one mutation event and the small circles are unsampled or extinct haplotypes. The gray boxes signify the major groups in the network: 1) eastern Asia (Korea/China), 2) Japan and 3) North America/Germany*. Isolates of *C. ribicola* are described in Table 1. *The North America/Germany node is represented by the following isolates: Ger2, Ger6, NH1-1B, RN32 and MN2-1B.

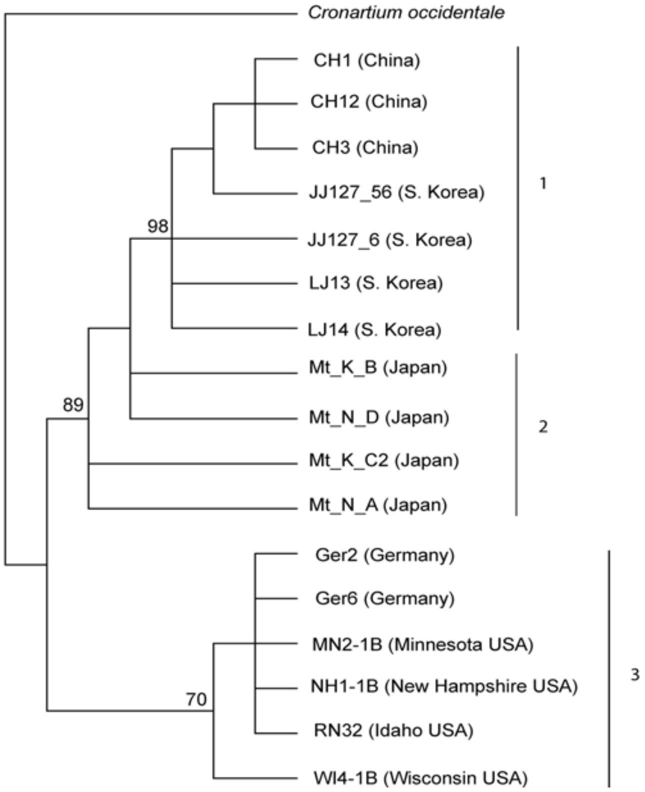

Figure 2. Maximum-likelihood topology generated from DNA sequences of four nuclear loci implementing a general time reversal model. Isolates of *Cronatirum ribicola* are described in Table 1. Bootstrap replicate values of 70 or greater are shown. Three major clades (1, 2 and 3) representing geographical regions (China/Korea, Japan, North America and Europe) are apparent with high bootstrap support.

All three phylogenetic analyses, maximum likelihood, Bayesian, and maximum parsimony, produced similar topologies. Given the redundancy among phylogenetic analyses, we present the results from maximum likelihood. Results from phylogenetic analyses resembled the results from the network analysis, with three well-supported clades. Similar relationships were also observed, with the Korea/China clade most distant and the Japan clade intermediate in relation to the Germany/North America clade (Figure 2). All three clades were supported with a high bootstrap support (>70). *Cronartium occidentale* was selected as a outgroup. *C. occidentale* had 20 SNPs and 10 gaps among the four DNA sequences when compared to *C. ribicola*.

The phylogeographic patterns of *C. ribicola* show that at least three distinct lineages exist: 1) Korea/China, 2) Japan, and 3) Germany/North America. The similarity of Germany isolates corroborates the historical records of white pine blister rust spread in western Europe in the late 1800's with subsequent introductions into North America from *P. strobus* nursery stock grown in France and Germany (reviewed in McDonald and Hoff 2001). Based on the current samples, the source(s) of the European and North American epidemic remains unclear. Our preliminary results suggest that our sampled locations in Japan, Korea, and China do not represent the source of *C. ribicola* that spread through Eurasia and North America; however, it cannot be ruled out that this genetic source could be present at low frequency at the sampled locations or from other geographic areas within one of these countries. Furthermore, these data and varying infection of different telial host species by artificial inoculation (e.g., Stephan and Hyun 1983, Yokota and Hama 1981) suggest that this pathogen has ecological races in Asia.

Further study is needed to determine the genetic relationships among populations of *C. ribicola* that occur in northeastern Siberia/Kamchatka, northern India and Pakistan, and other regions of Eurasia. Further sampling of eco-regions occupied by five-needled, white pines and *C. ribicola* should help elucidate the phylogenetic relationships and origins of the western European and North American epidemic. In addition to the intraspecific relationships in this taxon, related microcyclic species, such as *Endocronartium sahoanum*, extant in Japan and the Kamchatka region of Russia (Imazu *et al.* 2000), and another potential pine-stem rust that infects *P. armandii* in central China (Hei *et al.* 2003) warrants further phylogenetic characterization.

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PROCEEDINGS

EXTENDED ABSTRACT

Proceeding
Breeding and Genetic Resources of Five-Needle Pines:
Ecophysiology, Disease Resistance and Developmental Biology
Yangyang, Korea 2008

Sustaining *Pinus flexilis* Ecosystems of the Southern Rocky Mountains (USA) in the Presence of *Cronartium ribicola* and *Dendroctonus ponderosae* in a Changing Climate

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Limber pine, *Pinus flexilis* James, is characterized by a patchy distribution that displays metapopulation dynamics and spans a broad latitudinal and elevational range in North America (Webster and Johnson 2000). In the southern Rocky Mountains limber pine grows from below the forest-grassland ecotone up to the forest-alpine ecotone, from ~1600 m above sea level in the short grass steppe to > 3300 m at the continental divide (Schoettle and Rochelle 2000). In this region, limber pine's altitudinal range is wider than any of its co-occurring tree species. Limber pine ecosystems serve a variety of important ecological roles, such as (1) occupying and stabilizing dry habitats, (2) defining ecosystem boundaries (treelines), (3) being among the first tree species to colonize a site after fire, (4) facilitating the establishment of late successional species and (5) providing diet and habitat for animals (Schoettle 2004).

Limber pine appears to have very broad environmental tolerances (Schoettle and Rochelle 2000) and may therefore be expected to adjust to a changing climate via migration of populations or acclimation within populations. Assuming full dispersal, some models (McKinney and others 2007) suggest that limber pine will increase its area of distribution yet populations will shift northward and, although not modeled, presumably to higher elevations (Aitken and others 2008). These projections however only consider direct impacts of climate on the species' distribution and do not account for novel stresses such the presence of *Cronartium ribicola*, the non-native fungus that causes white pine blister rust (WPBR), or the warming trend driven expansion of the distribution of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), both of which will further affect population sustainability.

Within species genetic variation is the foundation for survival and evolution in the face of threats such as WPBR, mountain pine beetle (MPB) and climate change. The full suite of adaptive and life history traits and biotic interactions need to be considered when developing management options to sustain this ecologically important species into the future. Unfortunately, range-wide common garden studies have not been done to evaluate genetic variation in limber pine, but indications of geographic variation have been noted (Jorgensen and others 2002; Mitton and others 2000; Steinhoff and Andresen 1971). Provisional seed transfer guidelines for limber pine define five zones in the Interior West and recommend limiting the elevational movement within each zone (Mahalovich 2006).

Sustaining limber pine populations in the face of novel stresses will require maintenance of high genetic diversity and a functioning regeneration cycle (Figure 1). Proactive management to position the ecosystem and the regeneration cycle for resiliency will enable the ecosystem to continue to function and provide opportunity for the populations to adapt. Stimulating natural regeneration will increase the number of genetic combinations on the landscape, providing a higher probability of unique combinations that prove to be adaptive. In the case of WPBR, increasing the frequency of resistance traits in the pine population prior to rust invasion will uphold the regeneration cycle as the resistant seedlings will mature before the susceptible mature trees die therefore ensuring the presence of seed-bearing trees even during the period of maximum mortality (Schoettle and Sniezko 2007). The presence of MPB imposes an additional stress as the beetles preferentially kill the mature seed-bearing trees in the populations. Infusing the

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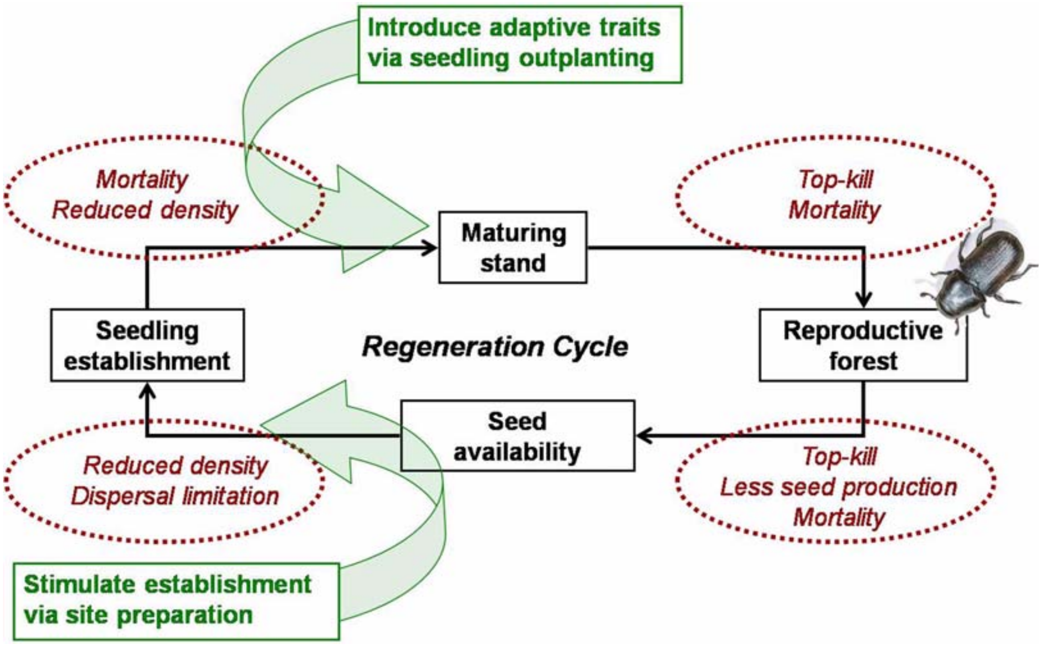

Figure 1. Forest regeneration cycle. White pine blister rust can cause impacts at all stage (ovals) and the mountain pine beetle preferentially kills larger trees. Broad arrows depict intervention options for increasing population resiliency under the threat of novel stressors. (Redrawn from Schoettle and Sniezko 2007).

regeneration cycle with adaptive traits and accelerating generation times will increase the capacity of these slow growing long-lived species to adapt (Schoettle and Sniezko 2007) and help increase the resiliency of populations to the rust and other novel stresses such as those that will accompany climate change (Figure 1).

Resistance to WPBR in limber pine is essential to the future of limber pine populations given the lethality of the disease and its continued spread. As in several other native white pine species, limber pine most likely possesses several types of rust resistance. Screening for complete resistance (canker-free trait) is rapid (~2 yrs) compared to the assessment of partial resistance traits (~7 yrs). Geographic variation in complete resistance, which leads to moderate levels of canker-free seedlings, has been observed in several species (Kinloch and Davis 1996; Kinloch and others 2003; Sniezko, unpublished data; Sniezko and others 2007) suggesting a possible correlation with other adaptive traits or evolutionary legacies. Partial resistance mechanisms in whitebark pine (*P. albicaulis*) have also been shown to vary geographically (Mahalovich and others 2006; Sniezko and others 2007) and in one study their frequency has been noted to be inversely related to cold hardiness, supporting the hypothesis that rust resistance may not be independent of other adaptive traits (Mahalovich and others 2006). Evidence for an association between disease resistance and adaptive traits has been shown in other pathosystems (e.g. Tauer 1978; Powers and Matthews 1980).

Complete resistance to WPBR in limber pine has been evaluated in a limited number of sites and varies among sites from a frequency of 1 to 29%; the data suggest a greater frequency of resistance in higher than lower elevation populations and higher frequencies of rust resistance in populations in Northern Colorado than elsewhere in the Southern Rockies (Schoettle, Sniezko and Burns, unpublished data). Assessment of the frequency of the partial resistance mechanisms in limber pine is underway (Schoettle, Sniezko and Pineda in process). Ultimately, the frequency of resistance to WPBR and its distribution will provide further guidelines for effective conservation and restoration.

Regrettably, the same populations where rust resistance was found are also experiencing a MPB epidemic. It is estimated that MPB infestations will put most of these populations at risk (Gibson and others 2008). *In situ* and *ex situ* genetic conservation methods are being used to ensure that a mosaic of populations in the Southern Rockies with rust resistance survives the MPB epidemic thus providing opportunities for adaptation to climate change in the future. Each of these three stressors justify urgency for intervention, but the coincidence of the MPB epidemic in populations with rust resistance adds further necessity for expedient technology development.

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The Impact of White Pine Blister Rust and Distribution of Native Resistance: The Observations of "Field Resistance" in Provenance and Other Trials

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White pine blister rust caused by *Cronartium ribicola* (J.C. Fisch. in Rabh.), has been devastating to the North American native white pine species since its introduction a century ago, but although an exotic disease, significant native genetic resistance has been found. Reviewed here are results of what we know about the distribution of this genetic resistance from observations of "field resistance" based on survival in long-term field trials, primarily in western white pine *Pinus monticola* (Dougl. ex D. Don), but also some of the other western species where work is currently being carried out. Provenance research has always been an important feature in understanding the background genetic variability of our forest species. Western white pine has one of the most comprehensive series of provenance tests for native North American 5-needle pines in both in the US and Canada. This review summarises results from these provenance trials now that white pine blister rust has impacted them and draws some conclusions to the native geographic variability for resistance as well as summarises some of the key findings from the operational screening programs.

Although WPBR is an exotic pathosystem in North America, several important inheritable forms of resistance have been noted in our populations: major gene (R gene) and multigenic partial resistance or slow rusting resistance are categories that are broadly recognized (Kinloch 2003). Another identified heritable form of resistance has been through recessive genes particularly for some of the traits (e.g. needle shed) selected for in the Idaho western white pine populations (Hoff and McDonald 1971). These categories may not always be distinguishable in the observed phenotypic distribution of resistance and recessive gene inheritance especially is not easily picked up in the open-pollinated type of screening that has been part of most of the western operational screening programs.

The distribution of the major gene resistance found in both sugar pine (*P. lambertiana* Dougl.) and western white pine, identified as *Cr-1* and *Cr-2* respectively, and the virulence that has arisen in *C. ribicola* to these genes has been extensively reported (Kinloch *et al.* 2004). This work shows that these R genes have higher distribution in the south of these species ranges, and in western white pine the *Cr-2* gene is not even present in the northern part of the species distribution (Kinloch *et al.* 2003).

Most of the trials reported here deal with partial resistance for western white pine. Reported here is a brief summary of the provenance trials reporting the distribution of resistance in random population samples (Steinhoff *et al.* 1982) with the following features:

- The Northern Populations (North and Central Cascades and Interior Populations) are superior for growth and resistance over Southern Populations (Sierra Nevada and California).
- Interior populations are in general more susceptible but are good overall for vigor and growth.
- On the Coast the North Central Cascades are some of the most resistant populations but taking out high elevation the North Coast (Olympic Mountains and Vancouver Island) are as good.
- Some recent results in whitebark pine (*P. albicaulis* Engelm) also indicate a similar trend to the distribution of native partial resistance (Mahalovich *et al.* 2006; Sniezko *et al.* 2007).

More striking than these slight differences in the natural distribution of resistance, especially in western white pine however is that:

- Selected populations (those that have been selected and screened in operational programs) are significantly more resistant than this background variability. Over 100% more resistant on average.

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Figure 1. Surviving full-sib block at Grass Creek trial, Umpqua National Forest, Oregon. Showing marked full-sib effects (Sniezko *et al*. 2004).

Another feature that has emerged from the observation of long-term field trials with full-sib families is an often marked phenotypic resistance effect in certain specific crosses (Figure 1, Sniezko *et al.* 2004, Kinloch *et al.* 2008). This points to the expression of specific combining ability (perhaps a demonstration of recessive genes?).

In summary results from the provenance trials of western white pine show geographic trends to the native partial resistance with north and coastal populations having higher levels of resistance – similar results are shown in whitebark pine. But the progress from the operational screening is more striking indicating significant progress over wild trees decreasing infections by one half. Site hazard or "rust environment" however is still a significant and unfortunately unpredictable factor for partial resistance.

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White Pine Blister Rust Resistance and Genetic Conservation of the Nine Five-Needle Pine Species of the United States

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Nine species of five-needle pines (*Pinus* L. subgenus *Strobus* Lemm.) are native to the United States: *Pinus monticola*, *P. strobus*, *P. lambertiana*, *P. albicaulis*, *P. flexilis*, *P. strobiformis*, *P. aristata*, *P. longaeva*, and *P. balfouriana*. To varying degrees, these species are important in maintaining ecosystem health and in providing commercial products and scenic beauty. All nine species of pines are susceptible to a non-native, invasive pathogen, *Cronartium ribicola*, the cause of white pine blister rust. Blister rust is now known to occur on eight species in natural forests or reforested stands (rust has not yet been documented on *P. longaeva* in native forests). Very high levels of infection and mortality occur in some parts of the ranges of several of these species, and there is little likelihood that current levels of natural regeneration will maintain these species in the areas of highest rust hazard (Harvey *et al*. 2008, Samman *et al*. 2003, Schwandt 2006). In the western United States, where eight of the nine species occur (all except *P. strobus*), blister rust continues to expand its geographic range.

Harnessing the genetic resistance to blister rust will be a key to retaining or restoring these species in their ecosystems or utilizing them in reforestation (Samman *et al*. 2003, Schwandt 2006). Fortunately, some level of genetic resistance to the rust exists in all of our native species (Bingham 1972; Hoff *et al*. 1980; Kegley and Sniezko 2004; Sniezko *et al*. 2008a, 2008b). Operational programs to develop genetic resistance have been underway for decades for *P. monticola*, *P. lambertiana*, and *P. strobus* (e.g. McDonald *et al.* 2004, Sniezko 2006), but continued efforts will be needed to increase the level of resistance and retain genetic diversity as well as to utilize resistant seedlings in reforestation and restoration. Recently, additional concerns have been raised about the future viability of the high elevation five-needle pine species (Aubry *et al*. 2008, Samman *et al.* 2003, Schwandt 2006). Concerted efforts to evaluate the level of genetic resistance in *P. albicaulis* (whitebark pine) have now begun (Mahalovich *et al*. 2006, Sniezko *et al.* 2007), and smaller scale efforts to evaluate the baseline level of rust resistance in the other high elevation species are just beginning (Schoettle pers. comm.; Sniezko *et al*. 2008a, 2008b). In addition to resistance to the rust, the maintenance of genetic diversity and adaptability are also key elements to ensure successful restoration of these species. Additional studies of molecular and adaptive genetic diversity have been recently completed or are underway (for a summary of work with whitebark pine see Aubry *et al*. 2008).

A national Forest Service workshop ‘Genetic Conservation of U.S. Forest Trees Threatened by Invasive Insects and Pathogens’ was held in October 2007, and the five-needle pines were one of the four species groups of focus (species threatened by non-native insects or pathogens). The threat of blister rust is compounded by impacts of mountain pine beetle (*Dendroctonus ponderosae*) in the western states (Gibson *et al.* 2008), climate change, and fire (both fire exclusion, and catastrophic fires). Concerted action will be needed to ensure that the genetic resources of these species are available for the future. The Forest Service is well-placed to undertake the conservation of these species, but success will depend on key factors such as partnerships, funding, and innovative strategies to rehabilitate or restore damaged ecosystems. Short conservation and genetic overview documents are currently being prepared for all nine species to provide summaries of past research and possible genetic conservation needs.

National Parks and National Forests currently provide *in situ* conservation areas for many of the white pine species. However, with the current and looming impacts of blister rust and other factors, more effort is needed to retain these resources *in situ* (Schoettle and Sniezko 2007). In addition, *ex situ* genetic conservation, principally via seed collections, will be needed.

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Prior to 2008, relatively few seed collections were available for the high elevation white pine species. Funding was available in 2008 for some additional individual tree cone collections from Colorado (*P. aristata* and *P. flexilis*) and New Mexico (*P. strobiformis*). A good cone crop is forecasted for *P. albicaulis* for 2009, and groups in several regions are hoping to greatly increase their seed collections.

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Developmental, Ultrastructural and Molecular Studies of Male and Female Sterility in Western White Pine (*Pinus monticola*)

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Male sterility has been reported in many flowering plants (Johri 1984, Jordan1993) but in only three conifer species: *Picea abies* (Anderson, 1947); *Pseudotsuga menziesii* (Orr-Ewing, 1978); and, *Cryptomeria japonica* (Taira, 2000). There have been no reports of molecular studies of male or female sterility in any conifer. More recently both male and female sterility have been reported in *Pinus monticola* (Wilson and Owens 2003) and now a combined developmental, ultrastructural and molecular study of the phenology and causes of male and female sterility and the possible proteins related to male sterility are reported based on developmental, ultrastructural and molecular studies over several years of half-sib 10-15 year-old sterile and fertile trees in the B.C. Forest Service, Saanich Seed Orchard located about 15 km north of Victoria, British Columbia. The sterile tree was first noticed by a seed orchard technician who had observed over several years that the sterile tree produced pollen cones which aborted just before pollen shedding and it produced no viable pollen, whereas the fertile half-sib tree produced normal viable pollen. We then observed that the sterile tree produced seed cone buds but almost all seed cones aborted soon after the time of pollination.

In both male and female cones pollen mother cells and megaspore mother cells, respectively, undergo meiosis forming tetrads of microspores in the microsporangium and a linear file of four megaspores in the megasporangium. However, these similar stages of development occur at quite different times in pollen cones and in seed cones. In sterile and fertile western white pine, meiosis in the pollen cones occurs in April, just after winter dormancy, whereas, in seed cones meiosis occurs in ovules in the summer, 3 to 4 month after meiosis in the pollen cones.

Meiosis in sterile and fertile pollen cones and seed cones appears normal in sterile and fertile trees. In each microsporangium of the pollen cones, hundreds of microspore mother cells undergo normal meiosis and each forms four microspores in a tetrad survive. Microspores in the tetrad appear normal and separate from one another then start to develop into pollen grains. In the ovules, the outer three megaspores resulting from meiosis of the megaspore mother cell abort and only the inner functional megaspore survives then undergoes free nuclear division to form the multicellular megagametophyte. In the sterile tree, early development of the four microspores in a tetrad is normal but as a result of tapetal malfunction, all of the one-cell microspores abort and do not form mature pollen. The pollen grains began development in April but pollen wall formation was abnormal and pollen did not undergo cell divisions. At the time of normal pollen dehiscence in June, pollen had not developed beyond the microspore stage, it did not dry and remained in the watery thecal fluid. The aborted pollen was never shed and pollen cones aborted before pollination in June. In the sterile tree, the functional megaspore aborted before free nuclear division in July or August causing the ovule to abort and the seed cones usually aborted late (about August) in the first year of development.

Developmentally and ultrastructurally tapetal malfunction in western white pine pollen cones and seed cones is very similar to that described for male sterility in flowering plants. Tapetal tissue lines the microsporangia in pollen cones and megasproangia in seed cones and is thought to aid in nourishment and development of the microspores and pollen and the deveoping

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megagametophytes, respectively. As in flowering plants, we do not know if sterility in conifers is controlled by the nuclear or cytoplasmic genome, but the fact that in conifers it can occur in both pollen cones and seed cones of the same individual may have future significance in breeding programs and possible genetic engineering of conifers. This may help answer the question, “can genetically modified, wind pollinated conifers be safely released into the environment if they are sterile?”

A proteomic approach was used to identify differentially expressed proteins in sterile pollen cones of *P. montilcola* and an initial list of proteins is given. To characterize male sterility at the protein level, the protein profiles of pollen cones from the sterile tree and a half-sib fertile tree within the same half-sib Saanich Seed Orchard were compared at three stages from meiosis to the free microspore stages. Proteins were separated through two-dimensional gel electrophoreses. A total of 40 protein spots were processed by in-gel trypsin digestion and MALDI-TOF MS analysis. The spectra generated were searched against the non-redundant NCBInr and Swiss-Prot databases. The taxonomic category searched was restricted to the green plants (*Viridiplantae*). Analysis of pollen cones from the sterile tree at meiosis in April revealed that 30 and 19 protein spots increased and decreased in intensity, respectively, when compared to pollen cones at the same stage and date from the fertile tree. At this stage, 7 spots were found in sterile pollen cones but not in fertile pollen cones. No protein spot was found to occur only in fertile cones when compared to sterile cones at meiosis. A total of 641 and 648 protein spots were resolved in fertile and sterile cones, respectively. The differentially expressed proteins in sterile pollen cones at meiosis represent 8.6% of the total proteins detected. Analysis of sterile pollen cones at the microspore stage revealed that 13 and 34 protein spots increased, respectively, when compared to fertile cones at the same stage. At this stage, 6 protein spots were found in sterile cones but not in fertile cones. No protein spot was found to occur only in fertile pollen cones when compared to sterile pollen cones at the microspore stage. A total of 646 and 652 protein spots were resolved in fertile and sterile pollen cones, respectively. The proportion of differentially expressed proteins in sterile pollen cones at the microspore stage was 8.1% of the total proteins detected. This study has identified 34 of the 49 differentially expressed protein spots in sterile pollen cone of *P. monticola*. The expression patterns of these proteins need to be verified using western blot analysis and/or real-time PCR. Forty-nine protein spots have yet to be analyzed by MALDI-TOF-MS and MS/MS analysis. Information obtained from this study will provide a clear picture of the protein complement associated with pollen-cone sterility in *P. monticola* and perhaps other conifers and point towards interesting proteins that may help unravel the mechanism of male and female sterility in conifers at the protein level.

It should be noted that this is the only conifer in which both male and female sterility have been studied using developmental, ultratstuctural and molecular techniques and unfortunately that the sterile *P.monticola* tree has since succumbed to white pine blister rust but other sterile conifers, including *Pseudotsuga* (Orr-Ewing, 1977, 1978), Picea (Anderson, 1947) and *Cryptomeria* (Taira, 2000), have been reported and are likely to have very similar development and protein patterns. Seed orchards are a good site to locate sterile trees because they are usually carefully monitored every year.

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Genotypic and Phenotypic Diversity in Siberian Stone Pine: Associations with Soil Traits and Altitude

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Identification of issues involved in distribution of plant species and their limiting factors are among the major topics in forest ecology. Ability of species to establish new populations in unoccupied regions is critical in the preservation of biological diversity. An understanding of the processes affecting distribution is necessary for the prediction of future species responses to the climate change. At present, the evolutionary perspectives of a species is mainly defined by sustainability of populations occupied range boundaries because ecotones are more sensitive and vulnerable to the impacts of climate fluctuations and human activities. Climatic variables especially temperature and precipitation are the main factors in distribution of plant species along with day length, light intensity, humidity, and wind speed. The non-climatic factors are competition, herbivory, pests and pathogens, dispersal ability, genetic variation, antropogenic factors (harvesting, pollution, land use etc.), edaphic factors (soil and nutrients). The listed factors mostly act in various combinations. The resulting outcome may affected by the biological characteristics of the species.

Among Palearctic conifer tree species, Siberian stone pine, *Pinus sibirica* (Du Tour) is distinguished by its great ecological plasticity. It is one of the main forest-forming conifer species in the boreal zone of Eurasia. Territory occupied by forests with *P. sibirica* domination is nearly 36 million hectares (Iroshnikov & Politov, 2004). The northern limit of the species distribution reaches the Arctic circle. As in other stone pines (subsect. *Cembrae*), Pinus sibirica seeds are dispersed by corvid birds, nutcrackers, namely *Nucifraga caryocatactes* L. The northward distribution of *P. sibirica* is supposed to be limited not only by unfavourable climate factors but also by restriction of nutcrackers' activity due to low and unstable cone yield and seeds quality in the north. In the west. *P. sibirica* range is limited by distribution of spruce (*Picea abies/P. obovata* complex), which is a more competitive species in compare to *P. sibirica*, in the Eastern European climate. At the southern boundary isolated populations can be observed. They mostly grow in the Mongolian Altai, the South-Eastern Khangai and the South-Western Khentei (46°40'-47°00'N). The most important limiting factor in those regions is precipitation. The eastward distribution of *P. sibirica* is restricted by the continental climate and permafrost (Semechkin *et al.*, 1985).

Despite the continuous distribution, there are a number of ecotopes within the species range where its growth and reproduction is limited. In the mountains, environmental conditions and vegetation change rapidly over short distances. The altitudinal gradient is generally characterized by decreasing effective temperatures, increasing humidity and solar radiation in higher elevation (Körner, 2003). For example Siberian stone pine is widely distributed in Southern Siberian Mountains were it forms a tree line. Another example of the climatic factors' effects in very small regions is a 'wet line' near a bog, where the ground gets too wet for tree survival and the edge of a species range is clearly visible. Under process of 'waterlogging' Siberian stone pine represents in forest-bog ecotones. In the taiga zone water-logged territory is near 163 million hectares. It is under constant tension because of sensitivity to the changes in local hydrology. It expands and contracts in response to wetter or drier climatic periods, respectively (Vomperskii, 1994). So, the two distribution limits, caused by great variation of environmental factors at a short distance, are the important elements of Siberian stone pine biodiversity, and its investigation is significant for genetic resource conservation.

We studied physiological adaptation, growth, reproduction

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Table 1. Growth characteristics, reproduction and genetic structure of Siberian Stone pine subpopulations along forest-bog and altitudinal transects.

Characters	Forest-bog transect								Altitudinal transect				
	1*	2*	3*	4*	5*	6*	7*	8*	1*	2*	3*	4*	5*
Site altitude, m ASL	164	150	142	135	128	124	127	124	470	660	1280	1830	2100
Humus**, tn/ha	105	185	72	413	145	-	152	-	168	103	124	139	45
Soil moisture**,%	155	233	76	328	96	-	211	-	21	31	76	99	79
Sample size	30	30	30	24	30	31	41	15	22	23	25	26	30
Age	89	155	156	292	187	214	308	118	189	160	240	219	83
Stem height, m	20	21	22	25	17	12	13	5	40	32	25	22	5
Stem diameter, cm	33	41	42	50	23	24	23	15	80	50	60	40	10
Tree ring width, mkm	190	188	134	102	67	50	41	48	200	168	135	151	82
CSRCh	130	136	123	177	134	147	143	253	134	120	079	114	214
Chlorophyll, mg/g dry weight	3.43	4.48	3.52	3.14	3.38	1.88	2.11	0.79	2.49	4.84	2.06	1.91	1.97
Carotenoides, mg/g dry weight	0.62	0.80	0.64	0.59	0.66	0.36	0.42	0.26	0.46	0.83	0.38	0.44	0.51
No of female cone per shoot	2.0	2.38	2.45	2.32	1.88	1.52	2.0	1.82	2.36	2.54	2.3	2.07	1.59
No of male cone per shoot	9.47	11.2	12.1	11.7	9.8	4.3	7.6	2.3	12.4	10.9	7.1	6.3	3.7
Developed seeds per cone	71	-	67	71	59	51	59	66	78	77	83	84	58
Sound seeds,%	62	-	61	61	70	58	79	79	80	66	75	68	42
P, %	52.4	52.4	52.4	47.6	52.4	47.6	42.4	33.3	42.9	47.6	52.4	42.9	47.6
H_o	0.111	0.112	0.110	0.091	0.119	0.100	0.115	0.092	0.079	0.088	0.109	0.095	0.125
H_e	0.109	0.126	0.123	0.135	0.120	0.118	0.111	0.089	0.071	0.098	0.102	0.088	0.122

*-site number according to description given in the text above; **-reserves in the 50-cm upper soil layer; CSRCh-coefficient of sensitivity of residual chronology, P,% - proportion of polymorphic loci,%; H_O - average observed heterozygosity; H_E - average expected heterozygosity.

and genetic diversity of *P. sibirica* populations growing in ecotones at altitudinal (forest – mountain tundra) and humidity (forest–bog) limits. To provide detailed evaluation of the processes we used a complex approach, combining methods of dendrochronology, plant physiology, morphology, soil science and population genetics. Two Siberian stone pine transects comprised of a series of consecutive sites along a limiting factors from normal to unfavorable microenvironments were studied. The forest-bog ecological transect is located between the Ob' and Tom' rivers (the southern subzone of *taiga* in the West Siberia, 56°11'N, 84°25' Å). The transect is cf. 2 km long, and the difference between the lowest and the highest altitudes was 40 m. The transect consists of 8 sites, differed by soil humidity, from herbaceous forest types, through green mossy forest types, to bogged forest, with Siberian stone pine domination: (1) with low herbs; (2) with ferns; (3) with green mosses; (4) tussock bog (5) waterside ridge with dwarf shrubs; (6) sedge bog; (7) rising ground with dwarf shrubs, herbs and sphagnum, within the bog; (8) sphagnum bog (Table).

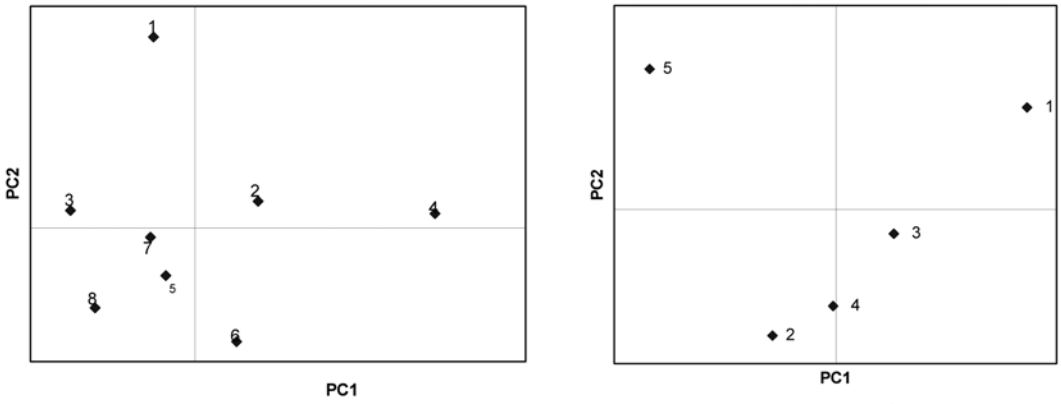
The *altitudinal transect* is located in the Northeastern Altai near Lake Teletskoe in the floodplane of the Kyga river (51°19' N, 87°50' Å). It was 14 km long with the difference between the lowest and the highest altitudes 1640 m. This transect included five sites: Siberian stone pine forest (1) with high herbs and ferns located on a floodplain terrace (470 m); (2) with high herbs, ferns and shrubs (660 m); (3) with high herbs, ferns, shrubs and green mosses (1280 m); (4) subalpine forest (1830 m); (5) open forest (forest-tundra ecotone, 2100 m) - the upper tree-line, were prostrate growth form of *Pinus sibirica*, including sterile plants, occurs (Table 1).

Tree ring width is an integrated characteristic of tree vegetative development and so we used dendrochronological methods to estimate the xylem increment. Across the forest-bog transect mean tree ring width was approximately 3 times lower at waterlogged sites (5-8) as compared with drier conditions (1-4). Tree ring growth dynamics were similar among sites within the groups, but ring width curve shape was specific in the hillock bog forest type. Similarly, tree ring width reduces with increasing of site elevation across altitudinal transect. A tree ring growth dynamic is similar among sites of lower part of transect (1, 2), higher part (4, 5) and in the middle part of transect (3). Trees from the upper limit had higher index of sensitivity marking significant reaction to climatic changes.

Other indicators of environmental stress are needle structure and pigment complex. Contents of the photosynthetic pigments were determined by spectrophotometer at wave lengths 665 nm (chlorophyll *a*), 649 nm (chlorophyll *b*) and 440.5 nm (carotenoides), respectively. Adaptation of *P.sibirica* needles for high-altitude and waterlogged environments resulting in needle xeromorphism related with decreasing of stomatal density, diminution of external assimilated surface and extension of internal one, as well as with prevailing of bound cell water fraction relative to free cell water fraction. Contents of photosynthetic pigments in Siberian Stone pine needles decreased from favorable conditions towards boundaries of both populations that could be explained by increased insolation resulting from low density of the stands. The specific reason for the physiological processes in the forest-bog ecotone is hypoxia (oxygen starvation) of root system, while in the forest-tundra ecotone. However, the most important factor in the higher elevations is higher UV radiation.

Growth and reproduction are two interdependent processes of tree development. Height and diameter of trees are decreased towards a boundary of a site across both transects. Traits of vegetative development of female shoots such as number of shoot organs (indicates rate of organ initiation) increased along the altitudinal transect but internodes length (relation of shoot length to number of shoot organs, indicates shoot elongation) is decreased. In forest-bog transect the number of shoot organs decreased and internodes length varied greatly. The cone initiation and maturation described generative development decreased across the both transect (Table). Analysis of male shoot structure shows that at high altitude environments generative morphogenesis is depressed: the shoots are relatively long, but the rate of organ initiation is low. On marginal sites, the proportion of microstrobiles in a number of shoot organs is the lowest. The same tendency we observed at the forest-bog transect. The seed yield (number and average weight of seeds) and quality (amount and proportion of empty, undeveloped and full seeds) decreased abruptly at the tree line that is putatively explained by spring frost damage and short growth period. Trees in bogged sites have high quality cones and seeds. To explain this phenomenon we used information on the periodicity of cone yields in Siberian stone pine. In optimal environments trees give cone yield once in 3 years, in the middle part of transect once in 4-5 years, while in the bogged site once in 9-10 years. Thus, such intense concentration of resources in time may allow trees to generate the high quality cones and seeds. On the whole, generative development decreased across both transects along with a limiting factors. Probably shoot growth (structure and elongation) is the simpler process which can be steady in wide range of environments in comparison to generative organs development.

Genetic structure of the studied Siberian stone pine populations was determined by 21 loci controlling 14 isozyme systems using starch gel electrophoresis. In the forest-bog, the highest genetic diversity (estimated as H_E) and the proportion of polymorphic loci were observed at the tussock bog site (4), the middle part of transect, and observed and expected heterozygosity were lower at most moisturized site (sphagnum bog forest). To explain the distribution of genetic diversity we take into account two factors affecting population genetic structure, gene flow and natural selection. We assume that nutcrackers providing gene flow by seed dissemination have no preferences in which site of transect to cache seeds. So this mode of dissemination maintains an initial homogeneity of genetic diversity in the embryo gene pool. Consequently, the variation of H_E can be explained by the differences in natural selection strength. Microenvironments at hillock bog site are favorable for the plant growth, soils are rich in humus and available nutrition elements and well moisturized because of near-surface subground waters. Selection in favor of heterozygous genotypes is common in conifer populations (Krutovskii,



a b

Figure 1. Distribution of forest-bog (a) and altitudinal (b) transects sites by ordination of genetic distance matrix by allozyme loci frequencies using Principal Coordinate Analysis.

Politov and Altukhov, 1995, Politov and Krutovskii, 2004) and the absence of heterozygote excess indicates low level of selection on the site. Genetic subdivision of population was estimated by F_{ST} statistics. Approximately 2.1% of the total genetic diversity was accounted for inter-site variation.

Multidimensional analysis of genotypic data performed by using Principal Component Analysis showed that humid sites are grouped together in the *PC* dimension plot. Significant correlation of humus content in soil and *PC1* scores was found ($r=0.83$, $p \leq 0.05$). The results attest for significant association of soil traits and internal population substructure of the forest-bog population (Figure 1a).

Along the altitudinal transect, the observed and expected heterozygosities increased with site elevation (Table). We assumed that in altitudinal transect the main factor forming gene flow is a transfer of seeds by nutcrackers from lower and middle elevation forest belts to the top of the mountain. It means that natural selection and gene flow act in opposite direction across the transect. Hard selection acting at the tree line not reduced higher level of genetic diversity maintained by gene flow. The high observed heterozygosity values in trees at the upper tree limit could be explained by selective advantage of highly heterozygous trees. About 2.2% of the total genetic variation was due to variation between the sites. Distribution of the studied sites in dimension of PCs approximately corresponds to their position on a transect, except for site 2 (Figure 1b).

Thus, our study showed that at forest-bog transect, the dynamics of tree ring indexed chronologies related positively with precipitation during growth season in dried conditions and had negative correlation at waterlogged sites, whereas at altitudinal transect the chronologies correlated with air temperature during growth season positively. In the boundary sites trees had maximal sensitivity coefficient of xylem increment, which is evidence of a great sensitivity of the trees to climatic fluctuation. Decreasing of reproductive function, as well as changes in physiological features, show sensibility of Siberian Stone pine for environmental stress at ecological limits within the species range. Internal genetic substructure of studied populations associates with combination of main environmental factors such as soil moisture and fertility at forest-bog transect and air temperature at altitudinal transect. The findings attest that allozyme polymorphism can be partly involved in adaptation of Siberian stone pine to survival at marginal environments. Marginal populations could be used for ecosystem monitoring of global and local cyclic climate changes.

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Genetic Variation of *Pinus cembra* L. in the Ukrainian Carpathians by Microsatellite Loci

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Swiss stone pine, *Pinus cembra* L., is one a key coniferous species forming the timberline forest stands in parts of the European Alps and the Carpathians. The “island” characteristics of the alpine zone in the Carpathian Mountains and chronic deforestation foster range fragmentation of *P. cembra*, especially in Ukraine at the northeastern border of the species' range. Thus, restricted gene flow may affect the genetic structure of *P. cembra* populations through increased inbreeding. Our previous studies based on allozyme markers showed that *P. cembra* samples from the Ukrainian Carpathians and the Alps are characterized by lower levels of genetic variation and higher levels of inbreeding than the samples of the related stone pine species *P. sibirica, P. koraiensis* and *P. pumila*. The Carpathian populations of *P. cembra* revealed higher levels of expected heterozygosity and differentiation in comparison with Alpine samples, associated to range fragmentation of the species in this region (Belokon *et al.* 2005, Politov and Krutovskii 2004, Politov *et al.* 1992).

Recently, the analysis of chloroplast microsatellites (or single sequence repeats, SSRs) in *P. cembra* of the Northern and Southern Carpathians and the Tatra Mountains have revealed high haplotypic variation and large among-population variation (Höhn *et al.* 2005, Höhn *et al.,* in press). The present paper reports first results on the variation of nuclear SSRs of *P. cembra* in the Ukrainian Carpathians. We used three samples (Gadzhina, Yayko and Gorgany) presented by 27, 42, 43 individuals correspondently and 23 trees of *P. sibirica* in a provenance trial from Western Siberia (Napas). First, we tested 10 primer pairs initially developed for the related species, *Pinus strobus* (Echt *et al.* 1996). Due to inconsistent PCR product amplification or low polymorphism these primers were turned out to be hardly useful for *P. cembra* analysis. Among eight primers designed for *P. cembra* (Salzer *et al*., in press), three loci were selected for further work. We combined the amplification of two loci, *Pc 18* and *Pc 23*, in one multiplex PCRn. Loci *Pc 1b* and *Pc 18* were multiallelic and highly polymorphic (Table 1). The studied sample of *P. sibirica* was characterized by lower allele number than *P. cembra* by *Pc 1b* and *Pc 23* loci. We observed the differences in the allele frequencies as well as the specific allele composition between populations and species.

The level of microsatellite variation in the studied populations was higher than it was earlier estimated by allozyme loci (Belokon *et al.* 2005, Politov and Krutovskii 2004). The values of mean observed (H_O) and expected (H_E) heterozygosities of *P. cembra* were H_O=0.435±0.104, H_E=0.684±0.159. The respective values for *P. sibirica* were higher (H_O=0.558±0.072, H_E= 0.749±0.063) than in *P. cembra,* as it was also observed in allozyme studies. The samples of *P. cembra* and *P. sibirica* were characterized by high levels of inbreeding (F_{IS}=0.368 and 0.238, respectively).

The analysis of molecular variance (AMOVA) showed that 10% of the total genetic variance was distributed among species, 5 % among populations and 85% within populations. The level of among-population differentiation (F_{ST}) and Nei’s genetic distances (D_N) were lowest within the Carpathian samples of *P. cembra* (F_{ST}=0.046, D_N=0.069–0.195) and largest among all samples of *P. cembra* and *P. sibirica* (F_{ST}=0.078, D_N=0.386–0.393). The Principal Coordinates analysis showed

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Table 1. Nuclear microsatellite loci, primer sequences, allele size ranges and numbers in Carpathian samples of *Pinus cembra* and in samples of a *P. sibirica* provenance trial

Locus	Primer sequences (5'- 3')	Motif	*P. cembra**		*P. sibirica**	
			Allele size range	N_A	Allele size range	N_A
Pc 1b	F: CCACCATCTTGTTTTTGTGTTC R: TTCTCTCCACCCAGCCTAAA	$(GT)_{19}$	166–208 (166–202)	12 (10)	178–210 (174–212)	9 (9)
Pc 23	F: GGGCATCATTATTTTCTTACAA R: CTTGATATACCATGCCACAACC	$(TG)_6$ $CG(TG)_2$	206–260 (221–257)	10 (12)	205–235 (200–238)	8 (4)
Pc 18	F: TTCCCAAAGACCATAGAACCA R: TCATGAAATATTACGTCCCTTATCC	$(TG)_{12}$	154–162 (152–156)	4 (3)	154–162 (152–158)	4 (4)

Note: N_A – number of alleles, * - data from Salzer *et al.* (in press) are given in parentheses.

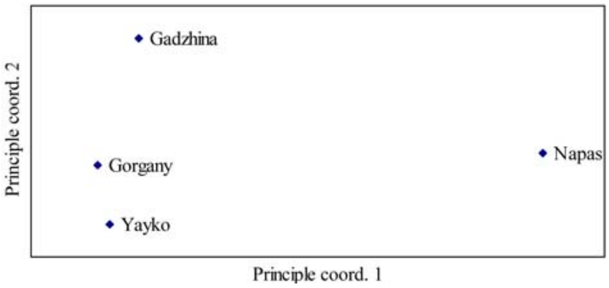

Figure 1. Principle coordinate analysis of *P. cembra and P. sibirica* samples based on three SSR loci.

a good correspondence between genetic differentiation and geographic origin of the studied samples of *P. cembra* and *P. sibirica* (Figure 1).

The data retrieved from nuclear SSR loci complement the earlier allozyme data of *P. cembra* genetic variation. The among-population differences in allele composition and frequencies could be applied for the identification of populations of *P. cembra* and for elaborating gene resources conservation strategy for this species vulnerable in the Carpathians.

We are cordially grateful to K. Salzer for providing us the primer information before publication. The study was supported by Fundamental Research Programs by the Presidium of Russian Academy of Sciences "Gene Pools and Genetic Diversity" and "The Origin of Biosphere and the Evolution of Geo-Biological Systems" and by the Division of Biological Sciences of Russian Academy of Sciences "Fundamental Bases of Biological Resources Management".

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Proceeding
Breeding and Genetic Resources of Five-Needle Pines:
Ecophysiology, Disease Resistance and Developmental Biology
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In vitro and *in vivo* Investigation into the Characterization of Resistance Against White Pine Blister Rust

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White pine blister rust (WPBR) caused by the rust fungus *Coronartium ribicola* J.C. Fischer is one of the most devastating disease of white pine (*Pinus monticola* Dougl.) trees of the Canadian forests (Kinloch, 1999; Hoff, 1980). Different types of resistance with a very low frequency have been observed in a 4 yr field screen program (Figure 1-a & b). The resistant plants have been categorized in 4 major groups: difficult to infect (DI), bark reaction (BR), slow canker growth (SCG) and needle shed (NS). We developed a disease assessment index (DI), based on both *in vitro* and *in vivo* techniques, to evaluate specific reactions to the pathogen (Kinloch, 2003; Hoff, 1980).

The *in vitro* method provides a new approach to study inoculation in an axenic environment under controlled condition. The first step in developing our *in vitro* screening program was to establish an *in vitro* culture protocol for the pathogen, ribes (alternative host) and white pine explants (Figures 2 and 3). Then we developed a disease assessment index, based on both *in vitro* and *ex vitro* techniques, to evaluate specific reactions to the pathogen of the resistant plants.

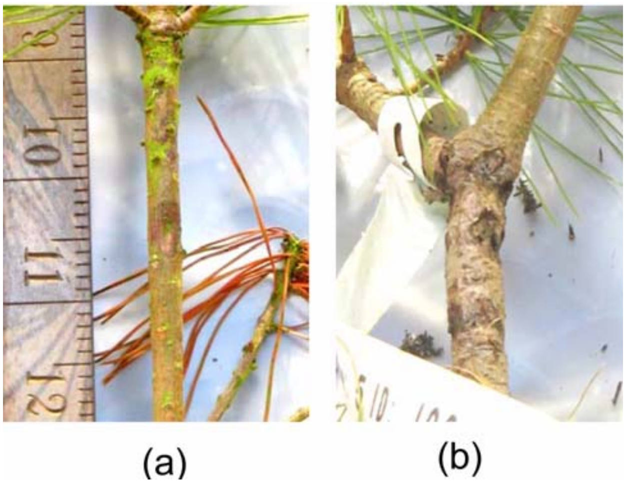

Figure 1. (a) Bark reaction resistance; (b) Slow canker growth resistance/tolerance.

Figure 2. *In vitro* rooted ribes leave after 4 wk in culture.

Figure 3. *In vitro* western white pine-new microshoots have been observed after 5 wk in culture.

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The preliminary results indicate: 1) white pine needle and seedling explants can grow successfully on both WPM (McCown and Lloyd, 1983) and GD based media and produce callus and roots; 2) Ribes leaves can grow independently in culture and produce roots on MS (Murashige and Skoog, 1962) based media; 3) the pathogen can infect and grow successfully on the *in vitro* cultured leaves.

By using molecular techniques e.g. SNPs we are trying to further characterize the resistance and understand the molecular mechanisms behind them. The results of this research could potentially help us to answer to some of the fundamental questions about the WPBR resistance systems.

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Ecophysiology, Disease Resistance and Developmental Biology
Yangyang, Korea 2008

Breeding Eastern White Pine for Blister Rust Resistance Through Interspecific Hybridization and Backcrossing: A Review of Progress in Ontario

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Eastern white pine (*Pinus strobus* L.) is highly valued for its magnificent tree form, fast growth rate, and premium lumber prices. The introduction of white pine blister rust (caused by *Cronartium ribicola* J.C. Fisch.) to North America in the early 1900s has, however, contributed to the dramatic decline of eastern white pine across its range. The Ontario Ministry of Natural Resources' (OMNR) most recent forest inventory indicates that eastern white pine now accounts for only about 2% of provincial growing stock. Although the species occurs in about 3.4 million hectares of forest land, it is dominant on only about 787,000 hectares (OMNR 2006).

The regeneration of eastern white pine in Ontario is hindered by frequent failures. White pine blister rust and white pine weevil (*Pissodes strobi* Peck) are primary causes of poor survival and growth of white pine saplings. Although improving white pine silvicultural regimes helps to reduce economic losses from regeneration failures, the challenge of how best to restore white pine in areas with high blister rust hazard ratings remains. Unfortunately, Ontario's climatic conditions and the pervasiveness of Ribes, the alternate host of the rust, favour the persistence of white pine blister rust. Much of the forest area has been classified as having an intermediate to severe hazard rating (Gross 1985). It is not uncommon to observe white pine plantations with high rates (60%-90%) of blister rust infection (Pitt *et al.* 2006). While white pine weevil decreases tree growth and wood quality, white pine blister rust threatens the species' existence on the landscape.

Ontario began to breed eastern white pine for blister rust resistance in 1946. Forest geneticist, Dr. Carl Heimburger, was among the pioneers in North America who initiated a breeding program in Ontario to explore genetic resistance to the introduced pathogen. Dr. Louis Zsuffa and other OMNR scientists continued this breeding effort until the mid-1990s. After a brief hiatus, renewed interest revived this research and development effort in 2001.

A major effort in Ontario was to breed among eastern white pine trees to enhance blister rust resistance. This effort achieved limited success despite a large number of controlled crosses created among putatively resistant selections. Although differences were observed among progeny in their response to blister rust infection, the overall strength of resistance across the genotypes was weak in protecting saplings from blister rust attack. Facing these realities, researchers in Ontario adopted a strategy that uses resistance screening with open-pollinated (OP) seedlings to identify individuals with meaningful and heritable resistance from *P. strobus* natural populations. Until such individuals are identified, controlled crosses between *P. strobus* selections are to be minimized. Vegetative propagation using rooted cuttings and somatic embryogenesis is being pursued for potential resistant individuals resulting from non-additive genetic effects.

In parallel with the breeding of *P. strobus* trees, Ontario started acquiring five-needle pine germplasms from around the world, in an attempt to introduce major resistance genes that are possibly missing in *P. strobus* (Heimburger 1972). Seeds or scions from up to 18 five-needle pine species were acquired. Among these introduced species, *Pinus wallichiana*, *P. peuce*, *P. monticola* and *P. parviflora* are of major interest.

The goal of Ontario's breeding with interspecific hybrids of *P. strobus* is to gain satisfactory blister rust resistance while minimizing the non-*P. strobus* genome (e.g., 6.25% or less) in the progeny. One aspect of this breeding goal is to reduce concerns over the use of exotic species on Crown land; and the other is to recover desirable characteristics of *P. strobus*, such as good adaptation, fast growth rate and superior wood quality,

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as well as magnificent morphological appearance.

A feature of Ontario's breeding strategy is the combination of backcrossing and intercrossing. Backcrossing the interspecific hybrids to *P. strobus* theoretically reduces the proportion of non-*P. strobus* genome by half in the offspring, but consecutive cycles of backcrossing result in the possible loss of resistance alleles across gene loci if blister rust resistance is conferred by more than one major resistance gene. Intercrossing between hybrids provides an opportunity to recover lost alleles and to increase the homozygosity of resistance gene loci if parents involved in the mating are complementary.

The second feature of the breeding strategy is the use of an open-breeding population. An open-breeding population means that selected hybrid progeny are backcrossed with a theoretically unlimited number of *P. strobus* trees. Because it is the small proportion of non- *P. strobus* genome from an exotic species that provides the major resistance genes, the *P. strobus* trees used in the backcross breeding have limited influence on the overall blister rust resistance in the progeny. Therefore, to increase genetic diversity, during each cycle of backcrossing, it is desirable to backcross the interspecific hybrids with as many genetically independent *P. strobus* trees as possible.

The third feature of the breeding strategy is the integration of major gene resistance from hybrid progeny and minor gene resistance from *P. strobus* trees that demonstrate relatively higher level of "partial resistance". This is achievable by controlled crossing between hybrid progeny showing major gene resistance and the *P. strobus* trees showing good partial resistance.

Screening progeny for blister rust resistance is a necessity after each cycle of breeding. An artificial blister rust inoculation protocol is used in Ontario (Lu *et al.* 2005) that inoculates white pine seedlings after the first growing season at high inoculum density and cultures the post-inoculation seedlings under optimal growing conditions in the greenhouse. The major advantages of this approach are that it: (1) assures that every seeding is heavily infected with blister rust, (2) requires little space, increasing the number of seedlings that can be screened, and (3) substantially shortens the time needed to carry out a resistance screening experiment, from over 5 years to 2 years.

The most notable advancement to date is the confirmation of satisfactory blister rust resistance in the second-generation backcrosses (e.g., B_2) of *P. strobus* with *P. wallichiana*, which have on average an 87.5% *P. strobus* parentage (Lu *et al.* 2005; Lu, unpubl. data). Segregation data of post-inoculation seedling survival and pedigree information have indicated the roles and transmitting pathway of major genes from *P. wallichiana* in conferring blister rust resistance in the hybrid progeny, which may have involved at least three gene loci. Morphologically, trees from the second-generation backcrosses appear very similar to *P. strobus* with good cold hardiness, fast growth rates, straight stem form, as well as satisfactory blister rust resistance. Progressive improvement in adaptation and growth has been shown by the hybrid backcrosses in laboratory experiments and field trials (Lu *et al*. 2006; 2007).

Future research and development priorities in Ontario are to: 1) breed for more advanced-generation backcrosses of *P. strobus* with *P. wallichiana* to increase genetic diversity; 2) test the resultant progeny on more northerly sites for growth and adaptation; 3) develop efficient vegetative propagation techniques using rooted cuttings and somatic embryogenesis; 4) plan for seed production using seed orchard; and 5) study resistance mechanisms and 6) study the inheritance of interesting morphological, physiological and growth traits relating to hybrid heterosis.

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SHORT ABSTRACTS

Ecological and Genetic Considerations for Conservation of an Endangered Mexican White Pine

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We present an overview of recent studies carried out on *Pinus chiapensis* (Mart.) Andresen (=*Pinus strobus* var. *chiapensis* Martínez) and provide conservation recommendations. Because of its wood quality and being commonly used by 12 ethnic groups, this pine is an outstanding forest resource at mid-altitude humid mountains of southern Mexico and Guatemala. *Pinus chiapensis* is the most abundant species in secondary stands originally occupied by tropical montane cloud forest playing a key role in ecosystem regeneration, particularly in areas managed under slash-and-burn practices. However, many natural stands of this pine are severely reduced. Molecular studies based on isozymes and DNA markers reveal low genetic diversity and the lowest compared with its closest relatives (*P. ayacahuite, P. monticola* and *P. strobus*). Heterozygosity and seed viability increase significantly with population size, and inbreeding depression decreases significantly seed viability, suggesting the involvement of genetic factors on population decline. Low population size is associated with both lack of perturbation in well-preserved habitats and high deforestation rates in severely disturbed habitats. Conservation and management practices require preserving and restoring connections between suitable habitats to enhance gene flow between populations, and on careful programs that monitor and control slash-and-burn practices. Restoration practices should use seeds from as many local trees as possible to decrease inbreeding risks. Spline climate models predict significant increases in temperature and aridity along the range of *P. chiapensis*. Thus, assisted migration would be needed to match forecasted climate changes.

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Thirty Years of Breeding in Five Needle Pines in Romania: An Overview

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The Romanian breeding program in five needle pines has two phases. The first phase was launched in the 1977 year and its main aim was to combine, in the hybrid genotype, the *Pinus strobus* fast growing with the *P. peuce* and *P. wallichiana* genetic resistance to blister rust (*Cronartium ribicola*). The second phase has focused on the *P. cembra* species and it has started 10 years later, i.e. in the 1987 year. Its objective was to test provenances and half- and full-sib families and to estimate the genetic and non genetic parameters and to improve its growth and branching traits. An overview of genetic research on *Pinus strobus* × *P. peuce* and *P. strobus* × *P. wallichiana* F1 hybrids and on *P. cembra* species is presented.

Key words: breeding program, F_1 hybrids, *Pinus strobus* × *P. peuce, P. strobus* × *P. wallichiana, Cronartium ribicola, P. cembra,* half- and full-sib families, genetic parameters

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Taxonomy and Phylogeny of Soft Pines: A Review of Traditional and Molecular Approaches

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Traditional subdivision of soft pines (*Pinus*, subgenus Strobus) into sections Parrya and Strobus and further subdivision into subsections within these sections has been extensively criticized since the start of application of molecular methods to the taxonomy and phylogeny of the group. In almost all existed molecular phylogenies the whole section of Strobus pines seems to be compact, and relationships evident from phylogenies based on one marker set are being disproved by next marker set applied and so on. While particular studies based on limited marker sets reveal some phylogenetic signal, in general, the relationships among the species comprising the group are unclear when all molecular data are summarized. Now it is becoming evident that within section Strobus there are no strict taxonomic borders between species with winged and wingless seeds and with dehiscent and indehiscent female cones. This situation is promoted by easy crossability of almost all soft pine species at least within sections. Reticulate evolution (periodical or, for sympatric species, even constant gene exchange through incompletely isolated lineages after their first separation) seems likely to be responsible for such complicated picture. Among putative explanations of this patterns the following plausible reasons can be considered: 1) winglessness and indehiscent cones could have been arisen more than once in the evolution of pines of the section Strobus alongside with co-evolution of these species to bird dispersal, 2) these traits arose in one species and than transferred through species borders by interspecific hybridization being immediately supported by selection as an evident adaptation to bird dispersal or 3) a combination the multiple origin of winglessness+indehiscence and wide dispersal of this trait through hybridization.

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Mating System and Allozyme Heterozygosity Dynamics in Siberian Dwarf Pine *Pinus pumila* (Pall.) Regel Populations

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The mating system and the temporal dynamics of heterozygosity in five *P. pumila* natural populations from the Pacific region, optimal for the species, were studied using isozyme analysis. Seed samples were collected in northern Koryakia, southern Kamchatka (three samples), and the Kunashir Island (Kurils). The highest level of outcrossing was observed in population from Koryakia (t_m=0.939). It may be due to the absence of dense stands which put obstacles in the way of pollen dispersal. In the case of old and continuous thickets (populations from Kamchatka and Kunashir) the outcrossing level is lower (t_m=0.919−0.683). It may be caused by considerable pollen sedimentation at maternal trees. Wright's fixation index was used to estimate the level of inbreeding in embryos from dormant seeds resulting from open pollination and in maternal plants. A substantial level of inbreeding was observed in the embryo samples but not in four out of the five adult samples. The inbreeding level at the reproductive age was higher only in the sample from Koryakia, which can be explained by a relatively young age of plants in the population frequently affected by fires. A general increase in heterozygosity in the course of ontogeny, characteristic of other conifers as well, was attributed to elimination of inbred progenies and by balancing selection in favor of heterozygotes, which is a key factor maintaining allozyme polymorphism in populations.

Key words: *Pinus pumila*, allozymes, heterozygosity, mating system

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Patterns of Genetic Structure and Diversity in Western White Pine (*Pinus monticola*)

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Western white pine (*Pinus monticola*) ranges across a large region of western North America. Over the past several decades, this species has declined precipitously in abundance mainly due to the introduction of blister rust and reduced opportunities for regeneration. Genetic diversity and structure among populations at 15 sites across the native range of western white pine were assessed using Amplified Fragment Length Polymorphism (AFLP) markers. Based on 66 polymorphic AFLP loci, genetic diversity varied among the 15 collection sites. Sites below 45°N latitude tended to exhibit higher levels of genetic diversity than sites at higher latitudes. Genetic distance analysis revealed two major clades comprising northern and southern populations, but other well-supported relationships are also evident within each of the two major clades. Complex genetic relationships among populations are perhaps attributable to multiple factors, such as migration, adaptation, and multiple glacial refugia, especially in higher latitudes. Genetic diversity and structure demonstrated in this study can provide a basis for the recognition and selection of western white pine populations for species management and conservation programs, especially in consideration of current and future climate.

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Biogeographic, Ecologic and Genetic Impacts of Global Warming on *Pinus monticola* and Other Five-Needled Pines of Western North America

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Predicting the impact of climate change to distributions of five-needled pine populations requires an understanding of several components: 1) accurate location information and climate data to determine the environmental drivers and climatic envelope of a species, 2) genecology and identification of climatic ecotypes obtained from both quantitative and molecular genetics, and 3) model development requiring regression/classification approaches immune to overfitting. We demonstrate the capability of this approach with five-needled pine populations of western white pine (*Pinus monticola*). General circulation models are used to predict the current and future suitable climate spaces for the species and ecotypes. Furthermore, we demonstrate the capabilities provided by molecular data, and show similarities between analyses of molecular data and adaptive traits related to growth potential. Finally, these techniques are demonstrated on the other five-needled pines in western North America.

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Exploring Western White Pine Structural Defense Mechanisms for Resistance Against White Pine Blister Rust

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Western white pine is one of the most significant conifers in North America for its economic, social and ecological value. *Cronartium ribicola* J.C. Fischer is a destructive rust pathogen that has devastated western white pine forests in this region over the last century. We collected samples from different white pine populations across Canada and USA to develop an efficient *in vivo* and *in vitro* screening method for phenotype expression of WPBR infected plants. The results from our field screening program demonstrated four different kinds of presumptive partial resistance against WPBR; difficult to infect (DI), bark reaction (BR), slow canker growth (SCG) and needle shed (NS). To better understand the disease, plants from each category investigated in details using scanning and transmission electron microscopy. The results from most of our DI families indicated a significant difference in the needles structure with of those from control/susceptible families. Basically, the amount of epicuticular wax was significantly more than the control families and the majority of the DI stomata were occluded. Evaluation of epicuticular wax using chloroform method confirmed the electron microscopic results. In general, the amounts of epicuticular wax extracted from DI families were consid-

erably higher than control plants. These structural defense characteristics could potentially help white pine breeding programs in characterization of resistant trees.

Key words: Quinquefoliae, *Pinus monticola*, five-needle pines, plant pathology, tree biotechnology, plant breeding, electron microscopy.

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Pinus Maximartinezii Rzedowski (White Pine) a Mexican Genetic Resource that is Endemic and Endangered Species

"Advances Achieved by Biotechnology Tools to be Preserved"

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Mexico has spread more than 60 pine species; which are under high pressure, mainly for low culture on harvesting all kind of woods, although several Mexican pine species are endangered, mainly those producers of pinion seeds as *Pinus lagunae* and *P. maximartinezii.* Under this sight, Biotechnology tools could maintain and improve all of these resources by appliance of regeneration protocols and genetic breeding. This study involved the initiation or induction of somatic embryogenesis as well as the multiplication and maintenance of embryogenic tissue, and testing maturation of somatic embryos of pinion pine (*Pinus maximartinezii* Rzedowski). A new medium were applied utilizing a nitrogen ratio (0.1) and carbon sources (sucrose and/or maltose) all of them were evaluated a long of the regeneration period. The explants used in these experiments were the whole gametophyte with the immature zygotic embryo and excised immature zygotic embryos. The basic concentration of plant growth regulators were 2 mg/l 2,4-D and 1 mg/l BA. Proliferation of embryogenic tissue was obtained in both explants. In order to maintain the proliferation, each cell line or genotype were transferred to three media with different nitrogen ratios (0.1, 0.4, and 0.8) plus carbon source (sucrose and/ or maltose), exchanging embryogenic masses among media to establish all proliferating genotypes. As maintenance of embryogenic tissue it has been utilized the storage of immature embryos at 4^{o}C as suspension, with the same success as was achieved by crypreservation in liquid nitrogen in previous studies, where the immature embryos started to proliferate from 15 days to 6 months, depending on the genotype. Suspension cultures was also achieved, utilizing only ¼ of the PGR basic concentration, subculturing every 8-15 days, initializing with 20-40 embryos/ml, and arising form 500 to 1500 embryos/ml. These previous results were the basis to start the maturation protocol, where the aim is to stop proliferation of immature embryos in order to initiate the formal maturation protocol.

Key words: regeneration, somatic embryogenesis, genetic variation.

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Sampling Strategy for Genetic Conservation of *Pinus koraiensis* in Northeast Asian Region-based on Genetic Variation Parameters

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Sampling Strategies based on genetic data from a number of isozyme loci and I-SSR markers were established for the conservation of *Pinus koraiensis* in Northeast Asian regions. The allelic multiplicity and the expected and observed genotypic multiplicities were defined as the main measures to be maximized in sampling. Increase patterns in genetic diversity associated with the sequential random sampling of populations and individuals were depicted by using a simulation program, SGD (Sampling Genetic Diversity). By iterated samplings of intended number of populations and individuals, SGD enabled to estimate the average amount of secured genetic variation in each sample. By analyzing capturing curves, named for the increase patterns in genetic variation associated with the sequential random sampling, target amounts of genetic variation for conservation were determined and represented as the number of populations and individuals to be retained. And a partially preferential sampling of the populations with the alleles of putative conservational value was also suggested. Besides the in situ conservation focused first, sampling strategies were proposed for the ex situ conservation in the form of field genebanks. Conclusively, required activities related to the sampling strategies were proposed to some extent.

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Perspectives of Ecological and Silvicultural Aspects for the *Pinus koraiensis* Forest

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The Korean pine (*Pinus koraiensis*) has been widely planted for several decades in South Korea, having contributed to afforestation with other coniferous species, such as Japanese larch (*Larix kaempferi*). The species has been favorably evaluated as to produce timber and pine nuts. Even though some of Korean pine artificial forests reached the age class, natural stand of the species is seldom found below 600 m, and its upper limit is about 1,200 m. Southernmost range of the species in S. Korea is in Mt. Chiri, where it shows up between 1,100 and 1,500 m above sea level, growing extremely slow. Similar to other plantations in S. Korea, most of Korean pine forests are pure even-aged stands, economical and convenient in the management, but considered to be ecologically unstable. In the region of Northeast China, however, the Korean pine is naturally mixed with more than 10 deciduous tree species on the gentle slopes and in the valley, including *Tilia amurensis*, *Fraxinus mandshurica*, *Juglans mandshurica*, *Acer mono*, *Ulmus laciniata*, *Phellodendron amurense*, *Betula*

costata, and *Quercus mongolica*, forming the mixed broadleaved-Korean pine forest type. The relict broadleaved-Korean pine forest community occurs in a favorable environment in which a variety of plants are found, Its structure and function are more complex than any other forest types in the region. All the species are organized in an irregular uneven-aged mixed forest, and the pure Korean pine forest is hardly seen. Under mixed forest canopy, there are well-developed understory, herbaceous layer, moss ground cover, and occasionally lianas. The establishment of Korean pine forest has commonly and effectively been done with monoculture. The reliance upon single-species stands still predominates in much of S. Korean forestry, mostly for the benefits of simplicity of management and predictability of yield. It is time to change the conception. Scientific knowledge is needed to translate good intentions into realized goals. Any actions in the treatment of Korean pine forests need to be based on an understanding of how the Korean pine stands would grow naturally and how they could be manipulated effectively. It is necessary for us to make it a lesson from the dynamics and processes of natural Korean pine forests in Northeast China, where the mixed broadleaved-Korean pine forest type remains. Ecologically sound silvicultural systems should be developed for present Korean pine forests as well as other coniferous plantations, which have occupied more than 30% of total forest land. Advisable succeeding generation could be multi-species and uneven-aged or two-storied stand, taking advantage of forest successional process. Given the current situation and a knowledge of stand development patterns, appropriate silvicultural practices should be used to ensure adequate regeneration containing a mixture of desired species so as to achieve the sustainable forest management in the future.

Selection Breeding of Korean Pine

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Korean pine is a native white pine species in Korea and was extensively planted during the period of 70's and 90's for timber and nuts. However, the planting of the species has decreased due to government policy towards to diversification of planting species. Nevertheless, it still occupies 13% of the total afforestation area for the past 5 years in the nation. Therefore, we launched a Korean pine breeding program in 1959. Until 1986, we selected 300 superior trees, propagated them by grafting, used them as stock materials for seed orchards and maintained in clone banks in 4 different locations. Attempts have been made to apply data from progeny trials to the seed orchards. Two hundred and forty four families were ranked by estimating GCA from their standardized volume growth after incorporating age and the number of established sites. To improve the genetic gain in the seed orchards, many parameters including heritability and genetic correlation of those progenies derived from plus trees were estimated and applied. For the past 30 years, the seed orchards have produced about 141 tons of seeds from those clones which is enough to meet the demand for afforestation of the species in the nation. The quality of seed orchard is affected by several factors including genetic value of clones, genetic relatedness among clones, the quantity of flowering, pollen contamination from surrounding forests. Therefore, genetic thinning is being carried out in consideration of both genetic gain and genetic diversity. All ramets in the seed orchard are also systematically pruned to make the height lower so that making harvesting job easier and to stimulate flowering in the seed orchard.

Effective Population Size and Genetic Value Under Various Genetic Thinning Intensities in a Clonal Seed Orchard of *Pinus koraiensis*

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The main purpose of the present study was to find out a proper thinning intensity for keeping high genetic gain and maintaining genetic diversity in a clonal seed orchard of *Pinus koraiensis*. A clonal seed orchard of *P. koraiensis* was composed of 167 clones (4,194 ramets) and established with the size of 14 hectare in 1982. Genetic values for volume growth were evaluated on the basis of the general combining ability from open-pollinated progeny tests. Effective population size was estimated by the ramet variation among clones in the seed orchard as $n^2_{\text{total}}/\Sigma n_i^2$ where n_{total} is the total number of ramet in the seed orchard and n_i is the ramet number of the i^{th} clone. Genetic thinning applied was the linear thinning model, which was the mixture of family and within family selection. Given GCV values of -1.0, -1.5 and -2.0, we applied various thinning intensities ranged from 10% to 90% with 10% interval. Before thinning, average genetic value was 0.119 and effective population size was 90.32. Under all selection intensity, the effective population sizes increased as the thinning intensity increased. The average genetic value was increased when the thinning intensity was increased up to 70%, 60% and 50% and decreased at stronger intensity under the given GCA values of -1.0, -1.5 and -2.0, respectively. At GCA of -1.0 and thinning intensity of 70%, the average genetic value was estimated to be 0.674 and the effective population size was 83.3. At GCA= -1.5 and thinning intensity of 60%, the average genetic value was estimated to be 0.539 and the effective population size was 93.3. At GCA= -2.0 and thinning intensity of 50%, the average genetic value was estimated to be 0.445 and the effective population size was 98.5. We decided the thinning intensity at the condition of the GCA= -1.5 and thinning intensity of 50% because the effective population size was the same as before thinning. At this condition, the average genetic value was estimated to be 0.529 and the effective population size was 90.6.

Key words: *Pinus koraiensis*, genetic thinning, genetic value, effective number

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On the Genetic Diversity of Mature *Pinus cembra* Stands as a Response to Species Competition

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Swiss stone pine (*Pinus cembra* L.) stands from two elevational transects in the Eastern Alpine mountains were studied, in order to analyze genetic diversity as a response to species competition. Altogether five stands were investigated, representing forest communities from the middle montane to the low subalpine zone. Species diversity and structural diversity of the stands were measured according to FUELDNER 1995. Genetic diversity and variation were analyzed by means of isozyme gene markers. The findings showed comparable trends in both transects. It was found, that species mingling is highest in the stands with special site conditions. There, species competitiveness is comparable amongst the dominating tree species (spruce, larch, pine) and human impact is reduced by difficult access, e.g. by the steepness of the slopes. By contrast, the reduced diversity of the stands from high elevations is not only do be explained by the strong limiting effects of harsh site conditions, but also by human impact (cattle crazing). The analysis of the genetic structures revealed trends of declining genetic diversity from the high elevation , where *Pinus cembra* is competitive to the other tree species, to the lower elevation, where spruce is dominating the forest community. The findings are discussed in the light of conservation and management of Cembran pine genetic resources.

Diallel Crossing in *Pinus cembra*: V. Age Trends in Genetic Parameters and Genetic Gain for Total and Annual Height Growth Across 16 Years of Testing

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A full diallel mating design (p=10 parents) carried out in a natural population of Swiss stone pine (*Pinus cembra* L.) from the southern Carpathian Mountains. At age six, after nursery testing, the material was field planted in one site, using a completely randomized block design with 100 families, four replicates and 15 tree row-plots per replication, spaced 2.5×2.5 m. Total and annual height growths were assessed at successive ages across 16 years of testing period i. e. nursery test between ages one to six and field test between ages seven and 16. Plot means of the measured traits were analyzed using the general least-squares method by means of the computer DIALL program prepared by SCHAFFER and USANIS (1969). Across testing period significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) differences occurred in total height growth for general and specific combining ability effects. These results suggest that the two traits were controlled by nuclear additive and non-additive genes. In an ascendant trend, the additive variance, as a percent of the total genetic variance, ranged between 5% at age six to 67% at age 16 for total height growth while that for annual height growth ranged between 33% at age four to 78% at age 16. In a descendent trend, the dominance ratios $\sigma^2_{SCA}/\sigma^2_{GCA}$ for total height growth ranged between 8.2 at age two to 0.3 at age 16, suggesting that the additive variance could be used in a breeding program. Parents with significant general combining effects for the two traits were found. For total height growth, the narrow-sense families mean heritability estimates varied in an ascendant trend between 0.06 at age two and 0.65 at age 16 while the narrow-sense individual tree heritability varied between 0.02 and 0.37. Age-age additive genetic correlations for total height growth rose from 0.85 at age two to 0.95 at age six and then leveled off across the field test indicating that if the goal is to improve 16-year height, early selection can be considered at age six. By selecting the best 20 families and the best 20% of individuals within families, a genetic gain in total height growth of 8.8% and 9.9%, respectively, could be achieved at age 16. The improvement of growth using both family and individual selections could be applied. The very high age-age and trait-trait genetic correlations suggest that both early and indirect selection could be applied effectively.

Key words: *Pinus cembra*, diallel crossing, additive variance, dominance ratio, genetic effect, age-age genetic correlation, heritability, early selection, indirect selection, genetic gain.

In vitro Culture and Cryopreservation of an Endangered Species *Pinus armandii* var. *amamiana*

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For *in vitro* culture conservation via organ culture, mature embryos were excised from the seeds of *Pinus armand*ii Franch. var. *amamiana* (Koidz.) Hatusima, an endangered species only inhabiting the south west islands of Japan. Adventitious buds were induced on the surface of the embryo on 1/2 DCR medium containing BAP and they grew to shoots after subculturing to medium containing activated charcoal. Shoots were cultured in the different temperature conditions namely 25, 20, 15 and 4°C to assess the effects on cool preservation of the culture. They can survive at least 4 months under each temperature. From the elongated shoots, root primordia and roots were induced in RIM medium containing IBA as an auxine. Regenerated plantlets were grown in the field for three years. For cryopreservation of somatic embryos, embryogenic cell suspensions were induced from a mature and immature seed of *P. armandii* var. *amamiana* on MS liquid medium supplemented with 1 μM 2,4-D and 3 μM BAP. The suspensions were incubated in the dark at 25°C. Induced suspension cells were transferred to ammonium free MS liquid medium supplemented with 1 μM 2,4-D, 3 μM BAP and 30 mM L-glutamine and subcultured every 2 weeks. For long-term storage somatic embryogenic cells were cryopreserved in the liquid nitrogen using vitrification method. After thawing in 40^{o}C water, the embryogenic cells were subcultured for recovery. The cultures were incubated under a 16h light/ 8h dark photoperiod. After 1-2 months of culture, differentiation of embryos progressed and cotyledonary embryos were obtained. These embryos were transferred on ammonium free MS solid medium under 16 h photoperiod. After 2-3 weeks plantlets with roots and green cotyledons were obtained. Plantlets were transplanted to vermiculite containing modified MS liquid medium in 200 ml culture flasks, then out planted after habituation procedure to the field. The growth of the plantlets from organ culture was better than that from somatic embryos in the three years' field performance.

Somatic Embryogenesis in Five-Needle Pines of Canada and Its Application in Multi-Varietal Forestry, Research, and Conservation

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Eastern white pine (*Pinus strobus*), western white pine (*P. monticola*), whitebark pine (*P. albicaulis*), and limber pine (*P. flexilis*) are the four five-needle pines that are native to Canada. Eastern white pine is the most wide ranging and tallest of the conifers growing in eastern Canada, extending from Newfoundland to Manitoba, whereas western white pine, whitebark pine, and limber pine are the western species growing in British Columbia and Alberta. Eastern and western white pines were once economically important trees owing to their desirable wood quality and size; however, due to many decades of over-harvesting and susceptibility to white pine blister rust (*Cronartium ribicola*), they are reduced to only marginal importance in commercial forestry at the present. Whitebark and limber pines are high-elevation trees growing as isolated groups, and are ecologically important keystone species critical for watershed protection and wildlife. All five-needle pines in Canada are susceptible to white pine blister rust, which seriously impacts economic and ecological resource management. Somatic embryogenesis (SE) is a recently developed biotechnology whereby genetically identical trees can be mass produced using tissue-culture techniques. The use of SE technology offers exciting new opportunities in research elucidating genetic response to disease and insects, to provide an additional dimension for species conservation and restoration, and to implement multi-varietal forestry (MVF), defined as the use of genetically tested tree varieties in plantation forestry. Somatic embryogenesis is available for eastern, western, and whitebark pines, and attempts to induce SE in limber pine are in progress. The state of the five-needle pines in Canada and the development of SE in these species are presented. The main focus of this presentation is the use of the SE technology, in conjunction with cryopreservation, in packaging and deploying improved white pine varieties for commercial MVF and in developing strategies for species conservation and restoration.

Key words: blister rust, cryopreservation, eastern white pine, limber pine, multi-varietal forestry, somatic embryogenesis, western white pine, whitebark pine.

Somatic Embryogenesis, a Tool for Accelerating the Selection and Deployment of Hybrids of Eastern White Pine (*Pinus strobus*) and Himalayan White Pine (*Pinus wallichiana*) Resistant to White Pine Blister Rust (*Cronartium ribicola*)

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White pine blister rust (*Cronartium ribicola* Fisch.) is an exotic pathogen that was accidentally introduced into North America in the early 20th century. The devastating effect that this pest has had on both naturally and artificially regenerated stands in eastern Canada is a significant obstacle to the re-establishment of the natural stands that once covered extensive areas. In spite of the significant improvements in growth and adaptation attained under Quebec's white pine (*Pinus strobus*) breeding program, efforts to increase the species' resistance to white pine blister rust were unsuccessful. The transfer of blister rust–resistant genes from other five-needle pine species is not new in Canada and Europe; a number of promising hybrids have already been developed through interspecies crosses. The most promising hybrids for eastern Canada are *P. strobus* L. crossed with *P. wallichiana* A. B. Jacks. In general, these hybrids together with F2 and F3 backcross hybrids exhibit satisfactory blister rust resistance, as shown in studies conducted in both Europe and Canada. However, the first-generation hybrids (F1) are not as well adapted and they remain susceptible to the harsh winter conditions in eastern Canada. Consequently, Quebec's program focuses on the production of F2 backcross hybrids and efforts are being devoted to increasing the native gene component and enhancing adaptability. The technique of somatic embryogenesis has sparked renewed interest in the selection of resistant hybrids since this approach offers high potential for large-scale multiplication and deployment. The embryogenic cell lines will make establishment of clonal tests possible and facilitate the selection of resistant, well-adapted clones. The improvements attained in seed production from F2 hybrids, together with the advances achieved with somatic embryogenesis, will be discussed.

Light-energy Processing and Needle Level Related Adaptive Traits; and Growth of Eastern White Pine (*Pinus strobus* L.) from Large, Central vs. Small, Isolated, Marginal Populations under Three Silvicultural Treatments

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Adaptive traits related to light-energy processing under different light levels have significant ecological implications for plant fitness. Our objective was to examine and compare light-energy processing, chloroplast pigment content, nitrogen economy, and needle level morphological adaptive traits; and growth of white pine (*Pinus strobus* L.) from large, central populations (central Ontario (ON)), in comparison with small, isolated populations (Newfoundland (NL)) under three silvicultural treatments (ST). Light levels were on average 100%, 42.0%, and 20.4% transmittance for the full sun, and intermediate and high shade ST, respectively, using a cleared or thinned 30-year-old red pine overstory to 2 levels. After 8-years, overall height growth was 4.10, 3.25 and 1.70 m for full sun, intermediate, and high shade ST, respectively ($P<0.001$). Overall, ON populations had greater total height, diameter and current height growth than NL populations (all $P<0.001$). At low light levels (10 and 25 mol m^{-2}s^{-1}) high shade ST trees had higher photochemical quenching (*qP*), apparent photosynthesis (*APs*), and lower chlorophyll fluorescence (F_{pc}) compared to intermediate and full sun ST. There was an inflection in rankings at 50 mol m-^{2}s^{-1}. At and beyond 100 mol m^{-2}s^{-1}, full sun ST trees had higher *qP*, *APs*, lower F_{pc}, as compared to intermediate and high shade ST. There were no thermal dissipation efficiency (*qN*) differences among ST across all light levels. Light-energy processing traits had no regional effects. Average total chlorophyll concentration (CHL) and content (CHLC) increased in response to the intermediate shade ST but did not respond further or decreased to the high shade ST. Region was significant for CHL and CHLC with ON greater than NL. Chlorophyll a:b and CHL: carotenoid (CAR) ratios and needle level morphology responded significantly to all three ST. Needle N and C:N showed no regional effect and N showed a modest ST effect probably due to limited N availability in forested stands. Tree height and volume showed a curvilinear and linear relationship to light level, respectively. Tree height showed a positive linear relationship to *qP* (r^2=0.668), *APs* (r^2=0.371), chlorophyll a:b ratio (r^2=0.598), and needle N (r^2=0.728) (all $P<0.001$). Tree height showed a negative linear relationship to F_{pc} (r^2=0.539), CHL:CAR ratio (r^2=0.576), C:N ratio (r^2=0.706), specific needle area (r^2=0.680), and needle area N^{-1} (r^2=0.748) (all $P<0.001$).

Key words: components of growth, fitness, light levels, needle-level adaptations, nitrogen economy, silvicultural treatment.

*Full article, Major *et al*., 2009, Forest Ecology and Management, 257: 168-181.

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Nut Production of Korean Pine Forests in the Russian Far East and Problems of Sustainable Forest Management

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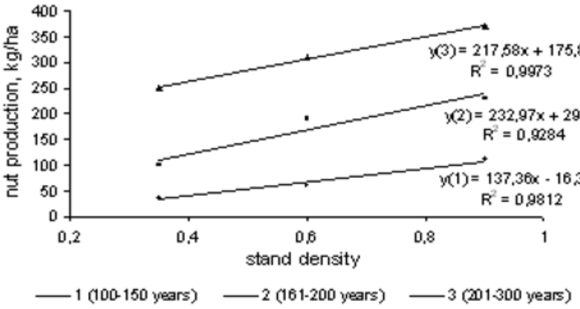
The Far East grounds of Russia, a northeast part of the Euroasian continent - one of few regions of a planet where broad-leaved - Korean pine (*Pinus koraiensis*) forests (*KPF*) were still kept, including intact forest landscapes. Value of these forests as unique natural phenomenon is noted in the International project «Global 200» where forests of South-East of Russia in a complex with the rests of broad-leaved - Korean pine forests of the next provinces of Northeast of China are considered as a natural heritage of the world level. Forest fund of Primorie region, having area of more than 11.5 million ha, is represented by natural forests, considerably changed after long-term use, but still preserving climax nature. Broad-leaved - Korean pine forests, most abundant in tree, shrub and herb species and occupying near 1/5 of the region's area, have maximal resource capacity in gaining of non-timber forest products and the main quantity of useful plants is concentrated in coniferous-broadleaved forests of mountain slopes and in valley multispecies forests. The area of these forest ecosystems is promptly reduced and in Russia at forest inventory to Pine forests already carry forest communities, where a share of a Korean pine as the core of forest forming tree, makes only 20-30%. In the southeast of Russia now one of the major problems of forest management is the problem of preservation forest ecosystems with Pinus koraiensis. Edible plants deserve special attention. The special role, undoubtedly, belongs to a Korean pine, that form the best genetic reservations of the most productive forest ecosystems in region. In schedules are reflected mean parameters of dynamics of Korean pine nut production in the most productive hillsides coniferous forests in a middle and it is plentiful bumper-crop years.



Direct positive connection of a crop of nuts with a share of participation of a Pinus trees in structure of forest stands and is observed by their completeness. Top yields are in Pine forests where the basic generation of a cedar reaches of 210-250 years. Such forest stands at a share of a pine tree of 6-8 units in structure of, produce on the average in a year of nuts of 130-150 kg, and within plentiful crops - up to 350 -450 kg on one hectare. The beginning of fructification of a pine trees in middle density forest stands registers in 60-80 years, and in plantations and silvicultures

- in 18-20 years (Senchukova, 1965; Kozin, 1990). These average parameters on Korean pine fructification are used for an estimation of resource capacity of this non timber forest products. In these forest ecosystems usually for one decade 1-2 plentiful crops of nuts and 2-3 - averages when economically proved craft of nuts is possible observed. In the field of safety of the best part of most diversity *KPF*, theoretical works and practical measures are necessary to allocation and preservation genetic reserves as prime objects of protection, to revision of structure zones with Korean pine forests. The best genetic reservation are *KPF* in southern and average parts of Primorskii Krai (the weight of 1000 seeds of a Korean pine in air dry condition on the average makes 450γ - in northern part of an area and 550γ - in a southern part of Pine forests). There is a set important works declared in the project of the special program “Cedar”, including creation of Korean pine plantations in agricultural landscapes and plantings of a secondary forests specially for obtain a crop of nuts.

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Weevil Incidence, Growth, Phenology, and Branch-level Dynamics of *Pinus strobus* from Large and Small Populations Under Three Silvicultural Treatments

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Our goal was to quantify the impact of three silvicultural treatments (STs) on weevil incidence, resulting tree growth effects, branch-level components of growth, and related adaptive trait information of white pine (*Pinus strobus* L.) from large, central populations in central Ontario (ON) versus small, isolated populations in Newfoundland (NL). Light levels were on average 100%, 42.0%, and 20.4% transmittance for the full-sun, intermediate-shade, and high-shade STs. After 8 years, overall incidence of weevil infestation was 42.1%, 23.4%, and 13.7% for the full-sun, intermediate-shade, and high-shade STs, respectively ($P < 0.001$). Weevil damage impact on total height and volume averaged -13.2% and -11.8%, respectively, and was largely consistent across STs and regions. Dominant lateral shoot length (which averaged 68% of leader length, $r^2 = 0.89$) analysis showed that ON populations had 32% longer shoot growth than the NL populations. There was no growth rate difference between NL and ON trees between Julian days 154 (near bud break) to 168 under the three STs. Bud set difference was a primary determinant of shoot-length growth differences between regions: Julian days 171 and 184 for the NL and ON populations, respectively, under all three STs. The primary determining factors related to shoot length were number of needle bundles and region, driven by light levels and day length, respectively, and internode length probably through inbreeding effects. Total height showed a positive curvilinear relationship to light level with a constant difference between regions, and diameter showed a curvilinear relationship to light level with no difference between regions at low light levels and progressively higher diameter for ON than NL populations as light levels increased. Shoot length and number of needle bundles showed a positive curvilinear relationship to light level, and specific needle area showed a negative curvilinear relationship to light level. Each day bud set was delayed resulted in an average of 2.5% or 0.5 cm more growth. To maximize fitness, conservation strategies should also now consider not only preservation but also examining and introducing adaptive trait variation in the context of anticipated environmental change.

Key words: allometry, components of growth, conservation, fitness, light level, *Pissodes strobi,* silvicultural treatments, small population size, weevil damage.
*Full article Major *et al.*, 2009, Can. J. For. Res. 39: 12-25.

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Phylogeography of the White Pine Blister Rust Fungus, *Cronartium ribicola*

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At present, little is known about the worldwide genetic structure, diversity, or evolutionary relationships of the white pine blister rust fungus, *Cronartium ribicola*. A collaborative international effort is underway to determine the phylogeographic relationships among Asian, European, and North American sources of *C. ribicola* and closely related taxa. Here, we present preliminary information on phylogenetic relationships among Eurasian and North American populations of *C. ribicola* using DNA sequences from four nuclear loci. In addition, we are investigating some of the phylogenetic relationships among Asian and North American alternate hosts in the genus *Pedicularis*. Identifying the evolutionary relationships and potential origin(s) of *C. ribicola* that spread through Eurasia and North America, and determining the phylogenetic relationships of its hosts are critical toward understanding this pathogen’s native ecology. Such information will help evaluate risks of cryptic introductions, contribute to the development of biological controls, and develop appropriate regulatory practices.

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Initial Symptom Development of *Pinus koraiensis* Seedlings Artificially Inoculated with Pathogenic Pine Wood Nematodes

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Three-year-old seedlings of *Pinus koraiensis* were inoculated in

a greenhouse with 10,000 pathogenic nematodes, *Bursaphelenchus xylophilus*, to investigate the disease development, stem and leaf water contents and the density of nematodes in stems. The incidence of needle dehydration, xylem drying and pith browning were started about 20 days after inoculation above and below the inoculation sites. Significant differences were found in stem water content, stem and leaf relative water contents 20 days after inoculation between seedlings inoculated with nematodes and those of control. The content of pinewood nematode in inoculated seedlings increased a little from 5 to 10 days after inoculation, but it has dramatically increased from 10 to 20 days after inoculation. The number of *B. xylophilus* recovered from stems of all inoculated seedlings was negatively correlated with the stem water content, stem and leaf relative water contents, and decreased significantly as a seedling becomes diseased.

Key words: *Pinus koraiensis, Bursaphelenchus xylophilus,* water contents, xylem drying, pinewood nematode.

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A Molecular Analysis of *Pinus parviflora* Native to Ulreung Island in Korea: Is *P. armandii* Native to Korea?

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The five needle leaves of *Pinus* on Ulreung Island in Korea, traditionally treated as *P. parviflora* Siebold et Zucc., is recently assumed to be more related to *P. armandii* Franch. in China, S. Japan, and Taiwan because of on non-wing type of seeds. *P. parviflora* and *P. armandii* along with *P. koraiensis* are two major pines, belonging to the white pine group, *Pinus* subgenus *Strobus.* To investigate the possible relationships of individuals on Ulreung Is. with *P. armandii* in China or *P. parviflora* growing in Japan, needle samples of a total of 9 accessions of *P. armandii*, 15 from the taxon on Ulreung and 7 accessions of *P. parviflora* from Japan, were collected, and subjected to the randomly amplified polymorphic DNA (RAPD), sequence analysis of species specific RAPD bands, and also subjected to amplified fragment length polymorphism (AFLP). Sixty Operon random 10mer oligonucleotide primers were screened, and 10 primers were selected showing polymorphism. *P. armandii* specific bands and *P. parviflora* specific bands, and specific RAPD bands were identified and sequenced to understand the genetic diversity and to develop speciesspecific sequencecharacterized amplified region (SCAR) markers. This study showed that profiles of the taxon to Ulreung Is., was somewhat different from those in *P. armandii* in terms of RAPD, sequence analysis, and AFLP data. The molecular analysis of these taxa with morphology will be discussed in detail.

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Current Local Control of White Pine Blister Rust Caused by *Cronartium ribicola* in Kangwon Province, South Korea

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It has been 70 years since white pine blister rust disease, caused by *Cronartium ribicola*, was firstly reported in South Korea. Korean pine (*Pinus koraiensis*) is one of the most resistant 5-needle pines to blister rust in the world. Currently 43.5 ha of Korean pine plantations at elevation over 1,000 m in Kangwon province are damaged by blister rust. The Pyeongchang and Yanggu National Forest Management Stations are in charge of controlling blister rust damaged areas, 18.5 ha and 25 ha, respectively. Current blister rust control efforts involved removing *Pedicularis* plants, the main alternate hosts in a natural ecosystem of South Korea, from 87.7 ha in Pyeongchang from 2001 and 25 ha in Yanggu from 2004. Also, diseased pine host trees are removed annually. White pine blister rust is not a serious problem threatening valuable Korean pine resources now, but it appears that continuous efforts to eradicate the pathogen by destroying alternate host and diseased pines will be able to completely control blister rust in South Korea.

Key words: *cronartium ribicola*, blister rust, *Pedicularis,* alternate host, natural ecosystem.

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A Needle Rust Fungus on the Needles of *Pinus koraiensis, Coleosporium neocacaliae,* New to Korea

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Coleosporium neocacaliae, a needle rust fungus, is recorded for the first time on *Pinus koraiensis* from Korea. Detailed descriptions of the species are verified based on the macroscopic features (symptoms and signs) and light and scanning electron microscopic observations of aecial characteristics, aeciospores and peridial cells, from the collected 13 dried specimens. The present needle rust fungus is apparently different from *C. eupatorii*, a solely recorded *Coleosporium* species in Korea until this time, in having comparatively large sized aeciospores than those of latter species. Verrucose surface structure of aeciospores in light microscopy was revealed to be annulated with two to four annulated rings in scanning electron microscopy. Some additional notes concerning distribution were described.

Key words: *Coleosporium neocacaliae*, needle rust fungus, *Pinus koraiensis*

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Effect of Accelerated Aging on Germinability and Vigor of Korean Pine (*Pinus Koraiensis*) Seeds

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This study was conducted to figure out the effect of artificial aging on seed germinability and vigor of *Pinus koraiensis*, which could be meaningful information to decide the suitable storage time and temperature. Korean pine seeds were artificially aged at different temperatures and times. The seed viability was affected by the accelerated aging temperature and time with changing in germination rate and seed vigor. The germination rates increased up to 15 days of aging treatment at 18°C and 24°C, decreased linearly from 10 days to 35 days at 37°C aging treatment. In addition, seed viability was nearly lost from the beginning of aging treatment at 45°C. The highest aging indices showed after 5 days at 18°C and 24°C, and 35 days at 37, and the lowest values were observed after 25 days, 15 days and 10 days aging treatment at 18°C, 24°C and 37°C, respectively. Seedling growth after 35 days aging treatment decreased with the increase of aging temperature. Accelerated aging test was considered as a suitable method to evaluate the seed viability of tree species. However more studies need to find out the optimum conditions for aging by tree species, because seed characters are much different among tree species.

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Physiological Responses of *Pinus koraiensis* to Potassium Chloride

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Deicing salt has been used to melt snow and ice from the road for traffic safety during winter season in Korea. However, this salt, which is mainly composed of $CaCl_2$, also has been reported that it is accumulated in the roadside vegetation and induces visible injuries such as browning of leaves and needles of roadside trees. Especially on late March through April, when these trees accelerate their physiological activities with new leaves, the damage by deceiving salt sets to be generated. The most significant symptoms by salts on roadside trees are reduction in biomass, chlorosis and necrosis; in extreme case, it would ended up by the entire destruction of a plant. This study was conducted to investigate the cause of plant damage by deicing salt ($CaCl_2$), and different concentration of $CaCl_2$ (0.5%, 1.0%, 3.0%, 5.0%) was treated to *P. koraiensis* (5yr), respectively. The first visible change was chlorosis on the leaves, under the treatment of 3.0 and 5.0% of $CaCl_2$ on 12 and 7 days after treatment, respectively. At the 70 days after treatment, leaf was lastly destructed. Growth rates of new leaves were increased 3.07 and 2.53 cm at each $CaCl_2$ treatment of 0.5 and 1.0%; these individuals showed 48 and 40% decreased growth rates comparing to those of control, respectively. And there were no growth rates of the new leaves at the treatment of 3.0%, 5.0%. Photosynthetic rates drastically reduced all of the individuals except those under the 0.5% $CaCl_2$ treatment: as one case of leaves under 0.5% $CaCl_2$ treatment, the rates were 54 % decreased on the 12th day and 95% reduced on the 73th day after treatment. In addition, total chlorophyll content of leaves were decreased all of the individuals except those under the 0.5% $CaCl_2$ treatment. Leaf water content rate decreased in the treatment of 3.0 and 5.0% $CaCl_2$ under the rate of 50% compare with those of control. The result of leaf chemical properties showed that there was high Ca^{2+} accumulation in the leaves as increasing the concentration of treatment. In consequence, higher concentration of Ca^{2+} in soil causes higher Ca^{2+} accumulation in leaves, and it damages plant physiological activities.

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Relationship Between Cone Abundance and Cone Characteristics of *Pinus koraiensis*

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Pinus koraiensis is a native and an economically important conifer species in Korea, which has high wood quality and disease resistance. The whole quantity of seed for reforestation is supplied from seed orchards. Generally, it was known that the number of flowers and fruits affect the tree growth as well as seed quality. Thus, the relationship between cone abundance and cone/seed characteristics was investigated to improve seed quality and seed orchard management of *P. koraiensis*. According to cone numbers per branch, 12 groups (3, 6, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 cones/branch) with 10 replicates were sampled and examined. The larger the cone numbers per branch, the smaller the cone and seed size. Particularly, the weight of cone and seed per cone were largely decreased. While the number of aborted ovules per cone was largely increased as increasing cone numbers per branch, the number and weight of full seed were decreased. There were no significant differences among 12 groups in number of fertile scale, infertile scale and extracted seeds per cone. Additionally, the implications of the results to the seed orchard management of *P. koraiensis* were discussed.

Key words: Seed orchard, cone analysis, seed quality, aborted ovules

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Provenance Variation and Provenance-Site Interaction in Korean Pine (*Pinus koraiensis* S. et Z.) in Central Korea

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Twenty-one provenances of *Pinus koraiensis* S. et Z were tested on two site in central Korea, and results of the 7th year after plantation were evaluated. There were highly significant differences among the provenances in height, root collar diameter and survival rate in both test sites. All studied characteristics showed significant positive correlation with each other and negative correlation with latitude and altitude of seed source. The fastest growing provenance had up to 27.0% greater height and 32.4% greater diameter than the site means, implying considerable gain can be realized if the best provenances were selected for plantations. Changes in provenance rank at all measured traits suggested that a significant potion of GxE interaction is in existence. Provenances were significantly different for stability variances, indicating the potential to select for stable genotypes across sites or for genotypes that are the most productive at specific sites. It can be concluded that the observed patterns of variation will have implications for genetic resources conservation and tree breeding program for the species.

Key words: *Pinus koraiensis*, Provenance variation, GxE interaction, stability parameters

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Growth Performance of Eastern White Pine (*Pinus strobus* L.) among Provenances at Four Plantations in Korea

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The growth performance of Eastern white pine (*Pinus storbus* L.) was studied with six provenances(originated from three nations) at four plantations in Korea. All growth performances were significantly different among provenances and plantations. The most outstanding provenance in growth(height, diameter and volume) at age 39 was North Carolina in all plantations and this trend had been started from four years old seedlings. In plantations, the growth of volume was best in ChunCheon plantation while that was worst in GunPo plantation. The effect of plantations was thought to be larger than that of provenances growth of *P. strobus*. A pattern of growth and annual growth by ages was different by among plantations. Annual precipitation, foggy days, altitude and sand contents in soil are positively correlated with growth and the correlations between by ages were very highly correlated.

Key words: *Pinus strobus*, growth performance, correlations, growth pattern

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Clonal Variation of Seed Production by Means of Cone Analysis in a Seed Orchard and an Archive of *Pinus koraiensis*

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This study was conducted to estimate the clonal variation of cone characteristic and seed production. A clonal seed orchard was established in Chuncheon in 1981 and a clonal archive was established at the close place by the seed orchard in 1983. Cones were harvested from 166 clones in 2004 and from 168 clones in 2007 and cone characteristics, seed production potential, 1st and 2nd year aborted ovules were investigated. The results from cone analysis were then compared to the total seed production from the seed orchard. Clonal contribution on seed production was also estimated based on the number of clone full-filled seeds divided by the total number of full-filled seeds. Cone fresh weight, dry weight and cone length in 2007 were 30%, 18% and 5% higher than those in 2004, meaning that cones in 2007 are larger and heavier than cones in 2004. Seed production potential was also 14% higher in 2007 than in 2004. Aborted ovule of the 1st year was 1.8 and 22.9 and that of the 2nd year was 4.4 and 9.5 in 2004 and 2007, respectively. The higher rate of aborted ovules in 2007 might be due to the lower flowering (especially male) or poor weather condition in the previous year. The numbers of empty seeds were 47.1 in 2004 and 21.3 in 2007, and the total number of full-filled seed in 2007 was 24% higher compared to 2004. However, the total seed production of the seed orchard in 2007 (4,725 kg) was less than that in 2004 (5,650 kg). This result was caused by less cone production in 2007 (21,655 kg) than cone production in 2004 (29,660 kg). The less cone production, in turn, affected physiologically to the production of larger, heavier cones in 2007. The 50% seed production was contributed by 35% clone in 2004 and 40% clone in 2007.

Key words: *Pinus koraiensis*, cone analysis, seed production, clonal contribution

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Estimation of Combining Ability in Height Growth from a Control Pollinated Progeny Test of *Pinus koraiensis*

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The main aim of this study was to select superior combinations from a control pollinated progeny test of *Pinus koraiensis*. Control pollination was made by disconnected half diallel mating design with 10 female and 9 male parents, and the progeny trial was established with 27 combinations in 1996. At ages of 11 (2004) and 13 (2006), individual tree height was measured, and combining ability and relative height growth rate were estimated. In both 2004 and 2006, KW116 × KW84, KW45 × KW96 and KW84 × GG3 combinations were identified as superior combinations, and KW29 × KW84, KW78 × KW116 and KW96 × KW3 were recognized as inferior combinations. Rank correlation coefficient between two years was 0.963, meaning that there was almost no change in ranking between years. General combining ability showed that KW84 and GG17 were good combiners, but GG7 was lower in specific combining ability in all combinations. Specific combining ability showed that KW78 × KW29 and KW57 × KW3 were good combiners and KW29 × KW84 and GG31 × GG17 were poor combiners in both years. In relative height growth rate, KW78 × KW116 combination, with height rank of 26th in 2006, was the best and

KW45 × KW96, height rank of 4th, was the poorest. Correlation between relative height growth rate and height growth in 2006 was negatively significant (r=-0.476) at 5% probability level.

Key words: *Pinus koraiensis*, progeny test, relative height growth rate, combining ability

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Identification of *Armillaria* Species on Korean Pine Forests in Korea

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Korean pine, *Pinus koraiensis* Siebold et Zuccarini, is one of the mostly planted species to restore deforested landscape and utilize wood for commercial product after the Korean War in Korea. Symptomatic Korean pines have been reported in different regions of Gyeonggi, Chungchung, and Kangwon providences between 2007 and 2008. The symptoms on root and collar of Korean pine included resin exudates on the surface of bark and necrotic dead tissue on trunk when the bark was peeled off. Mycelial fan and black rhizomorph were found on the symptomatic plant tissue. Based on the symptoms and signs, we believed that the disease on the Korean pines was caused by *Armillaria* species. *Armillaria* root disease is commonly present in natural forests in the world. In Korea, *Armillaria mellea, A. ostoyae, A. gallica,* and *A. tabescens* have been found in deciduous and coniferous forests. In Korean pine forests, *A. mellea, A. ostoyae*, and *A. tabescens* were reported as facultative pathogens but they are mostly in saprophytic stage. They have only been investigated for the production of *Gastrodia elata* tuber. In this study, we collected 43 isolates from the mycelia fan and rhizomorph of the diseased Korean pines. *Armillaria* isolates were identified in the species level by means of sequence analysis of rDNA ITS and IGS regions and IGS PCR-based RFLP. The result showed that at least four species of *Armillaria* were involved in Korean pine forests.

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Outcrossing Rates of Korean Pines in Natural Population of Mt. Seorak in Korea Revealed by cpSSR Marker Analysis

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To estimate the outcrossing rates and the number of pollen contributors (i.e., paternal parents) in a natural population of Korean pines in Mt. Seorak, chloroplast DNA simple sequence repeats (cpSSR) markers were analyzed. Multiple cones (3~4 cones per individual tree) were collected from 14 individual trees in 2007. Thirty seeds were randomly selected from each individual tree. After germination, DNA was extracted from embryo and endosperm of the same seed, respectively. To determine chloroplast DNA haplotypes of maternal trees and pollen donors for each seed, 7 cpSSR primers (pt26081, pt36408, pt48210, pt109567, pt15169, pt71936, pt30204) were used for PCR. Amplication products of PCR and DNA size markers (50-350bp) were fractionated by automatic DNA analyzer (LI-COR automatic DNA 4000 sequencer). Chloroplast DNA haplotypes were determined according to the size of amplified DNA. Seeds having been generated via outcrossing were verified by comparing cpDNA haplotypes of embryos with those of mother trees, respectively. In case of an individual tree 3 (KP-3), cpDNA haplotypes of 30 embryos and a mother tree were determined by PCR with 7 cpSSR primers, which resulted in 10 different cpDNA haplotypes. Sixteen embryos of the 30 embryos showed different cpDNA haplotypes (9 different cpDNA haplotypes) compared to that of mother tree, which represented that they should be generated via outcrossing. Estimates of outcrossing rates on the basis of cpDNA haplotypes in natural populations should be regarded as the minimum values on account of the unverified cpDNA haplotypes of the neighbors of paternal candidates. In conclusion, outcrossing rate for the individual tree of KP-3 was turned out to be 0.534 and the minimum number of pollen donors contributed to generation of the distinguished 16 seeds was counted as 9 individuals. Further data on the other mother trees will be presented and discussed on the poster.

Key words: Korean pines, cpDNA haplotypes, cpSSR marker, outcrossing rate, pollen donor

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Analysis of Mating System of *Pinus koriensis* in Natural Population of Mt. Seorak in Korea on Basis of Allozyme Markers

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The mating system of *Pinus koraiensis* in a natural population of Mt. Seorak was investigated by allozyme analysis of progeny arrays using a mixed-mating model. Value of A_e, the effective number of alleles per locus was 1.11 in the filial population and 1.12 in the parental population. The observed heterozygosity (H_o) was 0.078 in the filial population and 0.118 in the parental population. The expected heterozygosity (H_e) was 0.099 in the filial population and 0.104 in the parental population. The multilocus outcrossing rates (t_{m}) were estimated to be 0.986 (SD 0.061) with all 11 polymorphic loci investigated. We found high correlated outcrossed paternity (r_{p} = 0.528) and evidence for biparental inbreeding ($t_{\mathrm{m}} - t_{\mathrm{s}} = 0.019$). While *P. koraiensis* is mostly outcrossed, the observed r_{p} values indicate that outcrossed matings did not occur randomly. 52.8% of the outcrossed progeny pairs within a family were full sibs, indicating neighborhood size ($1/r_{\mathrm{p}}$) of less than two individuals in the paternal mating pool.

Key words: Allozyme, *Pinus koraiensis*, Mating system, Outcrossing rates

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PHOTO GALLERY

▲ Opening by co-organizers

▲ Opening and introduction by IUFRO president (Left) and Working Party Chairs (Right)

▲ Introducing research work on improvement of Korean pine
At the seed orchard (left) and during the presentation session (right)

▲ Oral presentation session at Naksan Beach Hotel

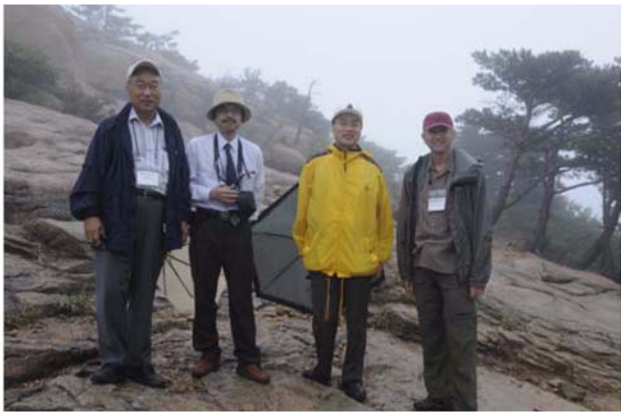
▲ Owens, Richarson, Dyukarev, Daoust. Ishii, Lu and Mudrik

▲ del Castillo, Yi, Park, Daoust, Noshad and Park

▲ Looking at a Korean pine native stand on a misty hill of Sorak Mountain

▲ Waiting for buckwheat noodle

▲ Sniezko, Schoettle, Klopfenstein and Richardson at Kwonkumsung



▲ Roh, Hoshi, Ishii and Daoust at Kwonkumsung

▲ Noshad, Belokon, Dyukarev, Goroshekevich, Zhelev, Klofenstein, Richardson, Sniezko, Owens, King, Kittarat, Song, Kim and del Catillo (from the left)

▲ Lu, Hoshi, Lee, Dmitry, Mudrik, Owens, Schoettle, Sniezko, Dyukarev, Daoust, Belokon, Noshad, Blada, King, La, Park, Klofenstein, Major, Goroshekevich, Ishii, Richardson, Lee and Nghia (from the left)

▲ Belokon trying to use chopsticks for her noodle

▲ Kim, Yi and Han on field trip

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● 국립산림과학원 산림과학기술서비스헌장 ●

우리 국립산림과학원 전 직원은 산림자원의 조성·이용과 환경이 조화된 임업기술을 개발하여 국민의 삶의 질을 높이고 우리의 고객인 국민에게 신뢰와 사랑을 받는 공무원이 되기 위하여 다음과 같이 실천하겠습니다.

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모든 고객께서는 친절하고 공정한 서비스를 받을 권리가 있으며 우리 산림공무원은 고객여러분께 고객만족과 감동의 서비스를 제공하기 위하여 이 헌장을 선포하고 실천해 나가고자 하오니 아낌없는 성원과 적극적인 협조를 부탁드립니다.

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