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# Proceedings of a Symposium on White Pine Provenances and Breeding

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Experiment Station

General Technical  
Report NE-155



International Union of Forest Research  
Organizations Working Party S2.02-15



XIX World Congress  
Montreal, Quebec, Canada  
August 5-11, 1990

## PREFACE

On August 5-11, 1990 the 19th World Congress of the International Union of Forestry Research Organizations was held at Montreal, Quebec, Canada. As part of that Congress, Working Party S2.02-15, White Pine Provenances and Breeding, met on August 7 to present a series of technical papers. The first portion of this session stressed the Mexican white pines which have been neglected in previous symposiums.

Time allocated for presentation of each paper at the Congress was severely limited to allow for the maximum number of papers. They are published in full in these proceedings and will now reach the wider audience they deserve. The editor of these proceedings did as his position gave him license to do and edited each paper. The editor takes full responsibility for any editorial errors they may contain.

The Officers of this Working Party who took the responsibility for organizing this meeting were:

Dr. Peter W. Garrett, Chairman  
U.S. Forest Service  
Durham, New Hampshire

Dr. Kyung J. Lee, Co-Chairman  
Seoul National University  
Suwon, Kyonggido  
Republic of Korea

Dr. Kurt Holzer, Co-Chairman  
Institut F. Forestpflanzung  
Wein-Mariabrunn  
Austria

During the business meeting, following the technical sessions, the membership of this Working Party in attendance voted to return Dr. Garrett and Dr. Lee to the same offices for the period 1991-1995. Dr. Holzer was not able to serve a second term and his position was filled by Dr. Ion Blada, Forest Research Institute, Bucharest, Romania. The Working Party expressed its thanks for Dr. Holzer's contributions to the success of the organization and his many contributions to the field of forest genetics.

Peter W. Garrett, Editor  
Chairman, Working Party S2.02-15

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WHITE PINE PROVENANCES AND BREEDING

XIX WORLD CONGRESS  
MONTREAL, QUEBEC, CANADA  
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BIOSYSTEMATICS OF THE MEXICAN  
AND CENTRAL AMERICAN WHITE PINES

Dr. Teobaldo Equiluz Piedra

SUMMARY

A short review of taxonomy, variation and distribution of white pines in Mexico, Guatemala, Honduras and El Salvador is reported here. Confusion about the taxonomic treatment of the P. ayacahuite var. brachyptera, P. strobiformis, P. reflexa complex in the northeastern States is still present. The author suggests using var. brachyptera until better information is available. It is concluded that more research is needed in these taxa before many populations of P. chiapensis are eliminated by farmers and other illegal cutting. The other taxa are not in immediate danger of extinction.

Taxonomic Review

The Mexican and Central American white pines belong to the Subgenus Strobus, Section Strobus and Subsection Strobi according to the classification developed by Little and Critchfield (1969) and Equiluz (1985, 1985a).

The pines of the group ayacahuite have been treated with different rank by several authors, due to the common intermediate forms developed in the northern range of its distribution. For over fifty years the northern populations in Mexico were known as Pinus strobiformis Engelm., until the publication by Shaw (1909), where he proposed two new varieties for P. ayacahuite. In fact, P. strobiformis was first discovered in the mountains of Cosihuiriachi, Chihuahua, Mexico by Dr. F.A. Wislizenus in October 1846. In 1874 Dr. J.T. Rothrock found this taxon on the Santa Rita Mountains of Arizona (Sargent, 1897).

P. flexilis James and P. reflexa Engelm. have been incorrectly used, particularly in the northeastern populations. In order to avoid confusion in the future it is recommended that these names not be used until more research is done to unravel the entire complex of P. reflexa, P. flexilis, P. strobiformis, and P. ayacahuite var. brachyptera.

Recently, a new species was described by Carvajal (1986), without Latin designation. This taxon named P. nova-galiciana is illegitimate and questionable because of the absence of supporting data to distinguish it from the var. veitchii.

On the other hand P. lambertiana has a very restricted distribution in the southern limits of its range in Sierra de San Pedro Martir, Baja, California.

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Finally, for many years P. chiapensis Andresen was considered to be a distant variety of P. strobus until 1964 when J.W. Andresen decided to raise this variety to specific rank (Andresen, 1964; 1966, Equiluz, 1985). Gausson (1960) was the first to treat this taxon with specific rank.

At present the white pines in Mexico and Central America are represented by five taxa: P. lambertiana, P. ayacahuite var. ayacahuite, P. ayacahuite var. brachyptera, P. ayacahuite var. veitchii, and P. chiapensis. The taxonomic records and synonyms for each taxon are as follows:

- \* Pinus ayacahuite var. ayacahuite Ehrenberg (Linnaea XII, 492; 1938).
  - 1848. Pinus strobiformis Engelm., in: Wislizenus' Tour Mexico. p. 102.
  - 1848. Pinus brachyptera Engelm., in: Wislizenus' Tour Mexico. p. 89.
  - 1857. Pinus veitchii Roezl., Pinus popocatepetli Roezl., Pinus Don Pedri Roezl., Catal. Graines Conif. Mexico. p. 32.
  - 1858. Pinus buonapartea Roezl., in: Gard. Chronicle. p. 358.
  - 1858. Pinus loudoniana Gordon, Pinetum. p. 230.
- \* Pinus ayacahuite var. brachyptera Shaw (The Pines of Mexico, p. 11, 1909).
  - 1848. Pinus strobiformis Engelm., P. brachyptera Engelm., in: Wislizenus' Tour Northern Mexico. p. 89 - 102. (USA Senate Misc. Pub. 26).
  - 1878. Pinus flexilis var. reflexa Engelm., Rothrock Weeler's Report VI. p. 258.
  - 1882. Pinus reflexa Engelm., Bot. Gaz. VII. p. 4.
  - 1892. Pinus ayacahuite var. strobiformis Lemmon. Cone Bearing Trees Pacific Slope. p. 4.
  - 1897. Pinus strobiformis Sudworth, no Engelm., USDA Bull. 14. p. 17.
- \* Pinus ayacahuite var. veitchii Shaw (The Pines of Mexico. p. 10. 1909).
  - 1857. Pinus veitchii Roezl. Catal. Graines Conif. Mexico. p. 32.
  - 1858. Pinus buonapartea Roezl. in: Gard. Chronicle. p. 358, and Gordon, Pinetum. p. 218.
  - 1858. Pinus loudoniana Gordon, Pinetum. p. 230.
  - 1986. Pinus novo-galiciana Carvajal, Illeg. (Phytologia 59(2): 131-132).
- \* Pinus chiapensis Andresen (Phytologia X(6): 417-421, 1964).
  - 1940. Pinus strobus var. chiapensis Martinez (Anales Inst. Biologia, Mexico. 11(1):81.

1960. Pinus chiapensis (Mart.) Gaussen, Trav. Lab. For. Toul. t. Sect. 1, Vol. I, Part 2, Cap. 11, 91. p. 198.

\* Pinus lambertiana Douglas. (in: Trans. Linn. Soc. Botany XV. p.497 and 500, 1827).

#### Distribution

The P. ayacahuite complex has the widest distribution among the Mexican white pines. It is found on good sites along streams and other protected areas, forming large pure or mixed stands. These pine taxa require deep soils, good drainage and cold weather. In southern Mexico and Central America they reach the mountain tops where the low latitude is offset by the cold and humid climates of the cloud forest above 2000 meters.

Table 1 summarizes the distribution and range for each taxon. At least one species, P. lambertiana, has a restricted range in Sierra de San Pedro Martir, State of Baja California Norte (Fig. 1).

Pinus chiapensis is well represented along the Sierra Madre del Sur and the east front of the Gulf of Mexico. It grows in tropical and subtropical climates and in association with hardwoods and many tropical crops at its lowest elevations. At higher elevations P. chiapensis is associated with P. ayacahuite and with some deciduous hardwood species on the Sierra Madre Oriental. In Guatemala, P. chiapensis is severely threatened and only one small disturbed stand remains, while in Mexico several populations were cut to make way for coffee plantings, fruit trees and pasture.

Pinus lambertiana in Mexico is located in a National park and any harvesting is prohibited. These populations have few trees scattered throughout the park.

In general, the P. ayacahuite complex is represented in three large areas. The variety brachyptera in the north, variety veitchii in central Mexico, and the tropical variety in the southern Sierras through Guatemala and Honduras. P. ayacahuite may be extinct in El Salvador.

Both P. ayacahuite and P. chiapensis produce magnificent trees up to 50 meters tall and 1 meter in diameter. The wood is easily worked for crafts and is in demand for boards and other solid wood products. Variety veitchii also is of great economic importance for Christmas trees and solid products but the variety brachyptera is an undesirable species due to its brittle wood.

The white pines are much less important than the hard pines in Mexico and Central America. Large forests are very rare since these species are associated with Abies spp., Quercus spp. and other pine species. However, both temperate and tropical taxa are becoming more important in commercial plantations as exotics.

Table 1. Geographic distribution of the white pines in Mexico and Central America. Martinez, 1948; Critchfield and Little, 1966; Andresen, 1964, Aguilar, 1976; Equiluz, 1982, 1985, 1988; Styles and Hughes, 1983.

Taxon	Elevation (m)	Latitude N	Longitude W	Distribution
<u>P. ayacahuite</u>	1800-3400	14°15'-18°05'	87°55'-100°50'	Mexico (Guerrero, Oaxaca, Chiapas) Guatemala (Zacapa, Quiché, Totonicapán, Sololá. Quetzaltenango. San Marcos, Huehuetenango) Honduras (Sierra Santa Bárbara, Celacque and Pacayal, La Paz) El Salvador (Cerro de Montecristo)
<u>P.a. var. veitchii</u>	2500-3700	18°30'-21°20'	97°15'-103°50'	Mexico (Puebla, Hidalgo, México, Morelos, Distrito Federal, Tlaxcala, Veracruz, Michoacán, Jalisco)
<u>P.a. var. brachyptera</u>	2000-3200	22°15'-31°20'	101°00'-110°00'	Mexico (San Luis Potosi, Durango, Jalisco, Chihuahua, Sinaloa, Sonora, Nuevo León, Coahuila, Tamaulipas, Zacatecas)
<u>P. chiapensis</u>	500-2200	15°45'-20°35'	91°50'-100°55'	Mexico (Guerrero, Oaxaca, Chiapas, Puebla, Veracruz). Guatemala (Huehuetenango)
<u>P. Lambertiana</u>	2100-2500	30°58'-31°10'	115°25'-115°32'	Mexico (Baja California Norte)

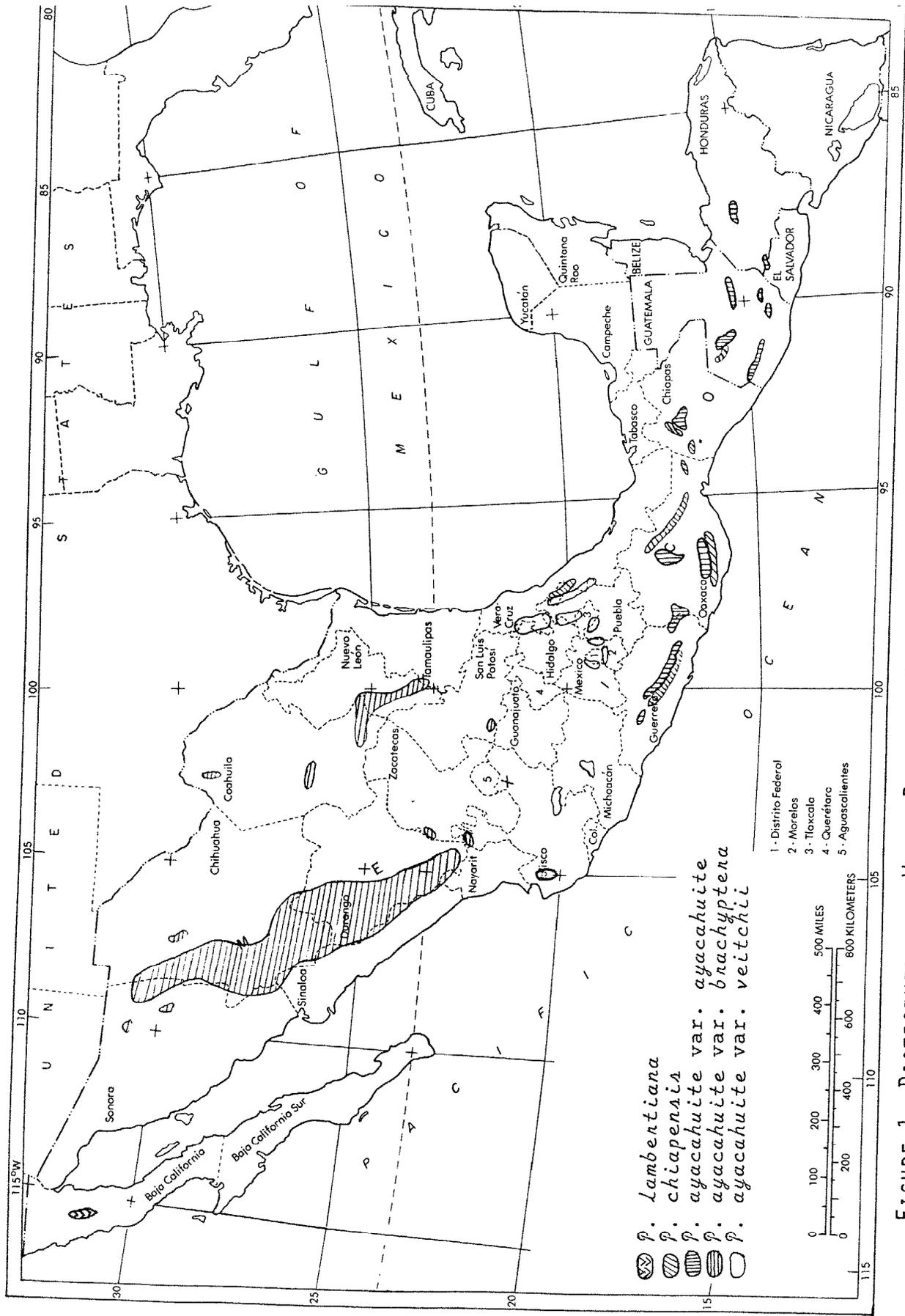


FIGURE 1. DISTRIBUTION OF WHITE PINES IN MEXICO AND CENTRAL AMERICA.

## Variation

As might be expected, P. ayacahuite and its varieties are quite variable in their morphology. The most conspicuous traits are seed and wing dimension. Wing length and cotyledon number were the most variable traits studied by Niembro and Moro (1987) in P. ayacahuite (var. veitchii) from Veracruz.

A pattern of clinal variation in seed wing length is found from north to south (Equiluz, 1984). The var. brachyptera shows only a rudimentary wing, while the var. veitchii has a well developed wing, and the var. ayacahuite has the largest wing in this group.

Cone length is another variable trait with var. veitchii having the largest cone - up to 35 cm. The var. ayacahuite has cone lengths up to 30 cm and var. brachyptera cones have a maximum length of 25 cm. (Equiluz, 1985a).

A recent isozyme variation study (Hernandez and Equiluz, 1990) based on eight populations from the range of P. ayacahuite, two of the var. veitchii, and two of the var. brachyptera, showed a discrete hierarchical clustering, confirming their present taxonomic status (Hernandez, Equiluz and Conkle, 1990).

Pinus chiapensis is also very variable throughout its range, particularly when comparing the high and low elevation populations. A morphological variation study presented by Hernandez (1986) on six provenances (116 trees) from Oaxaca and Chiapas showed statistical differences in 21 of 25 traits. Another variation study reported by Yanez (1981) with three populations of P. chiapensis, based on 15 morphologic traits, showed tremendous variation in more than half of the traits. However, P. chiapensis is a true species as stated by the work of Andresen (1964, 1966).

In spite of the studies on provenances, and tree-to-tree variation reported so far, the white pines in these countries require more extensive variation studies using the latest techniques such as isozyme, DNA, and terpene analysis, in order to better define their relationships.

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## WHITE PINE PLANTINGS IN THE HIGHLAND OF COLOMBIA

Dr. Clements C. Lambeth, William S. Dvorak, Masatoshi Endo

### SUMMARY

Smurfit Carton de Colombia experience with the Mexican white pines began with a single row planting of Pinus chiapensis in 1971. Because of good growth and form, the species has been planted in 15 trials and pilot plantations at elevations between 1600 and 2600 m, at 3°N latitude and 1900 to 2700 mm annual precipitation. Results and general observations are presented.

Whereas most tropical pines require only five to six months in the nursery, P. chiapensis needs eight months. Early field survival can be poor. However, if good seedlings are planted and soil moisture is adequate, survival is good. Seed germination is poor for seed stored longer than two years.

Early stand development is generally slower than that of other Pinus species such as patula, tecunumanii, kesiya, and oocarpa. Trees exhibit a yellow color until about three years of age. After that point a rapid growth phase begins and by age five years P. chiapensis will pass other pines in height growth. Notable exceptions occur in areas of high rainfall where growth can be rapid even in the first two years. Individual trees can reach 15 m by age five years. Rapid diameter growth lags behind that of height but there is no doubt that P. chiapensis is a promising species and one of the best volume producers.

CAMCORE trials show differences among provenances and families within provenances for growth and quality with the best provenances coming from La Trinidad and Pueblo Nuevo in the Chiapas Department of Mexico. Four seedlots from Zimbabwe were strikingly inferior to those from the native range. In general, forking is the only serious stem deformity. Results to date show great promise for developing a local land race for Colombia through seed production areas or clonal seed orchards. Studies of seed production potential will be needed.

Low specific gravity (.35) may result in unacceptably low cellulose yield for chemical pulps. On the other hand, it has a very uniform and white wood which may result in more even cooking and easier bleaching. The low density and light color may make it highly desirable for mechanical pulps. Studies indicate it will be suitable for light construction and finished products.

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## Introduction

Although P. chiapensis has been studied botanically for several decades, it wasn't until the late 1940's and 1950's that the first collections were made with the intent of testing the species as an exotic in plantations. A broader effort involving several countries, including Colombia, began in the late 1960's and early 1970's. Dvorak and Brouard (1987) reviewed the resultant literature on studies established from these collections. They concluded that P. chiapensis has good productivity and may be competitive with other pines in tropical and subtropical regions. Plantings in the warmer temperate regions of the United States and New Zealand were failures. Colombia was mentioned among the countries with the highest productivity potential.

In the first planting (1971) by Smurfit Carton de Colombia, P. chiapensis had greater height and diameter than five other pine species at 6.3 years, including P. patula and P. oocarpa which were being planted commercially at that time (Ladrach, 1978), (Table 1). These results were based on only one plot but interest in the species was generated and other arboretum plantings ensued in the late 1970's and early 1980's. South African trials also show promise for P. chiapensis as a potentially commercial species (Darrow and Coetzee, 1983).

Through its membership in the CAMCORE Cooperative (Central America and Mexican Coniferous Resources - based at North Carolina State University) the company began detail testing of provenances and mother trees from seed collected in the natural range of P. chiapensis in 1981, an ongoing effort. There are 15 research plantings and a number of pilot, commercial plantations of several hectares in size. Most of these plantings are too young to report here but they reflect a strong interest in the species and will provide valuable information on adaptability limits in the Andes Mountains. Here we report results of those trials three years of age or older and general observations on the management of P. chiapensis.

## Nursery

Nursery management of P. chiapensis is essentially the same as that used for other tropical pines with the exception of duration. Most pines require only five to six months in the nursery whereas P. chiapensis requires eight months to reach a plantable size. Although lower than that of other pines, the germination rate of P. chiapensis has been acceptable for seed stored two years or less, beyond which time germination can be unacceptably low. A yellow appearance of foliage and a somewhat poor and coarse root system are observed at the nursery stage, symptomatic of the slow growth.

Seed are sown in black plastic bags with 125 cm<sup>3</sup> of topsoil mixed with 25% cinder from the Company's coal burning energy plant. Borax (68%) and NPK (15:38:10) are the only fertilizers used. A shift to plastic tubes with root-directing ridges to prevent circular root systems is underway. The nursery is located at 1500 m elevation and has a relatively even year round temperature which averages 20°C. Nursery fungi are manageable and no more serious than those of other pines. Fungicides are applied regularly. Mycorrhizal

inoculation is carried out by sprinkling the top of nursery bags with soil from a mycorrhizae bank where pines are permanently maintained. It is likely that a different nursery management regime, perhaps with more frequent root pruning, could improve the seedling quality of P. chiapensis.

#### Field Performance

##### Survival:

Survival in the field may occasionally be poorer than that of other species currently being planted such as P. patula and P. tecunumanii (Table 2). This may be due to poor root systems and a perceived sensitivity to dry conditions. Occasional poor survival can also be seen in operational plantations.

Operations foresters have the impression that P. chiapensis is sensitive to grass and broadleaf competition in the early years. Although no formal studies have confirmed this belief, it is congruent with observations of poor root systems which may make P. chiapensis a poor competitor and one which will need more intense competition control in the early years. On the other hand, once it is established and rapid growth starts, its dense crown has good shading ability and soon dominates the site.

##### Growth:

Pinus chiapensis typically has a period of slow early growth, the duration of which depends greatly on precipitation, especially in the early months after planting, and early competition control. At the Samaria Arboretum (Table 1) there was little delay in reaching the rapid growth phase where it averaged an impressive 8.4 m in height after only three years. Annual precipitation at this farm (2700 mm) is much greater than that at the other studies. The slow growth phase lasts for up to three years and is accompanied by a yellowish foliage and great variability in size. The latter is probably due to sensitivity to microsite variation in soil moisture and/or competition. After establishment, growth is very rapid, the foliage takes on the more light bluish color typical of the white pines and the stand becomes more uniform.

##### Species Comparisons:

Pinus chiapensis typically lags behind the species that have been or are currently being planted by Smurfit Carton de Colombia (P. patula, P. kesiya, P. oocarpa and P. tecunumanii) in the first few years but by age five (and sometimes by age three) was always the winner in terms of height (Urrego, 1989), (Tables 1 and 2). It surpasses other species in height before it surpasses them in DBH. Many of the trials are still too young to determine whether P. chiapensis will surpass the most promising species in volume when the latter are growing where they are best adapted. For example, Smurfit Carton de Colombia recently replaced other species with P. tecunumanii which exhibits excellent yield under a wide variety of conditions around the world in tropical and subtropical regions. In the one trial where P. chiapensis occurs with P. tecunumanii at age five, the latter is shorter than the best P. chiapensis provenances (although it was much taller at age 1) but considerably

larger in diameter (Table 2). In La Arcadia (Table 2) P. chiapensis was about the same height as P. oocarpa at age three years but much taller at age five at which time the latter was still larger in DBH. However, the La Arcadia result is misleading since there was a high percentage of forking in P. chiapensis, in which case only the largest stem was measured. Recent observation indicates that P. chiapensis has considerably more volume per hectare than P. oocarpa in this trial.

Company land ranges between 1000 and 3000 m elevation. The limits of P. chiapensis in this range are poorly understood since it has been tested on a narrow range of elevations in the older trials. A series of species-site interaction trials have been and are being planted to better define the adaptive limits of all promising species including P. chiapensis. It is too young to tell yet how well it will do at high elevation but the third year performance of P. chiapensis at 2500 m at Penas Negras (Table 1) is surprisingly good. The high elevation species (P. patula and P. maximinoi) are the best but if the typical P. chiapensis growth pattern holds at this elevation it could eventually outgrow them. The moderately high precipitation at Penas Negras (2100 mm) could account for the good showing of P. chiapensis at such a young age.

There has been insufficient testing in Colombia to determine the best growing conditions for P. chiapensis but it is generally believed to require fertile soils with good water holding capacity that characterize the volcanic soils (Andisols) in the region. Unfortunately, P. chiapensis will have to compete with Eucalyptus grandis on these volcanic soils - a formidable task. Pinus chiapensis has failed in Brazil and Venezuela when planted on Oxisols and Ultisols, soils of poor water holding capacity and infertility, respectively. Only further experimentation will determine the soil requirements for P. chiapensis in Colombia.

#### Provenance Comparisons:

Given the performance of P. chiapensis in Colombia to date, it is paramount that the best provenances be found for commercial plantations to develop a locally adapted land race and to assure a broad genetic base for the future genetic improvement of the species. CAMCORE seed collections have been invaluable in this respect. Furthermore, since seed are collected and planted by mother trees, the beginning foundation for a tree improvement program is put in place when the tests are established. However, the most immediate task is to identify the best provenances so that seed can be purchased and perhaps more mother tree collections made from the natural range in order to broaden the genetic base.

Only one provenance trial is of sufficient age (five years) for even preliminary choice of provenances. Pine rotations are 10-15 years depending on whether the wood will be used for thermomechanical or chemical pulp. In CAMCORE trial 12-02-01D (Table 2) La Trinidad was the best provenance but Pueblo Nuevo and La Libertad are also acceptable in growth and will certainly yield individual trees suitable for a seed orchard but a high degree of forking (40%) in the latter will limit its utility. All three are from the Chiapas state of Mexico (Table 3). Pueblo Nuevo and La Libertad also performed well in study 12-02-07C (Table 2) though caution must be exercised since these two

provenances were represented by only one family and the study is only three years old. The four families from Zimbabwe did very poorly in study 12-02-01D for reasons not yet clearly understood.

Analyses of variance indicate strong provenance and family within provenance differences statistically significant at the 99% probability level, suggesting that the selection of provenances will be effective and that selection of individuals from the best families will result in genetic gains over nonselect populations.

All provenances tested to date are from 800 to 2000 m elevation in Mexico and Guatemala. Seed from low elevation (500 - 600 m) will soon be tested alongside high elevation types. In general, lower elevation provenances from Central America and Mexico perform best in Colombia and it is hoped that these trials will yield better provenances than those currently under test.

#### WOOD QUALITY

Wood samples have been taken in four areas (Isaza, 1988), (Table 1) at ages five to fifteen years of age. Although the age span was considerable, specific gravity only varied from .33 to .36 leading to the conclusion that there is no abrupt change from low, juvenile wood specific gravity to high, mature wood specific gravity as occurs in most pine species. Juvenile wood specific gravity of P. chiapensis compares favorably with that of other tropical pines (Table 1). However, its low mature wood specific gravity suggests limited use for chemical pulps because of low cellulose yield unless its volume yield is far superior to other pine species. One study also indicated that lignin content is high but the authors concluded that mixtures of P. chiapensis and Eucalyptus urophylla pulp was suitable for kraft pulp (Chaves, et al., 1979). Thermomechanical pulp requires low specific gravity wood and from that stand point P. chiapensis may be desirable if lignin content is not too high. Too much lignin will result in yellowing of paper exposed to light.

A study on the workability of P. chiapensis concluded that it will be suitable for light construction and especially good for finished products such as molding and furniture (Isaza, 1987).

Stem straightness of P. chiapensis is generally good. The only serious quality problem is a high percentage of forking in some provenances with many of them forking at ground level (Table 2). The La Libertad and San Juan Cotzal provenances are particularly bad.

#### CONCLUSION AND RECOMMENDATIONS

Pinus chiapensis shows great promise as a high volume producer in the Andes Mountains of Colombia. Even with a slow start in its first three years it is still able to surpass some of the other promising species. It is highly recommended that the best provenances based on five year results be purchased for pilot plantations on a range of soils and elevations.

Many sources should be tested, especially the low elevation provenances which have not yet been tried. So far, CAMCORE has only found two provenances of P. chiapensis to occur below 800 m elevation in Mexico and Guatemala: Tutla, Oaxaca (300-500 m elevation) and Nopoala, Veracruz (700-800 m elevation).

Studies on the production of seed in older stands in Colombia will provide valuable information necessary for local production of commercial seed from existing stands or through the establishment of seed orchards. Cones should be collected and analyzed as soon as possible.

Very low mature wood specific gravity (.35) may limit the use of P. chiapensis for chemical pulps. It will be necessary to run laboratory and mill trials to determine its suitability. However, its overall low specific gravity which is uniform throughout the stem could be of benefit for thermomechanical pulp and for more even cooking in chemical pulps.

A study of the workability of P. chiapensis shows that it will be good for furniture and fine wood finishes and should be considered as a faster growing alternative to Cupressus lusitanica which is frequently cultivated for high value products.

#### Acknowledgements

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Table 1. Growth of pine (*Pinus*) and cedar (*Cupressus*) species in four arboretums in the Andes Mountains of Colombia at 3°N latitude. (Number after trait indicates age of measurement)

FARM: San Jose PLANTING YEAR: 1971 PLOTS: Three 4X4 trees  
 ELEV.: 1,750 m PRECIP.: 1,950 mm

SPECIES	HT.-6.3 m	DBH-6.3 cm	SP. GRAV.-6.3
<i>P. chiapensis</i>	11.5	18.5	.35
<i>P. patula</i>	9.8	15.8	.32
<i>P. douglasiana</i>	7.2	12.4	.33
<i>P. oocarpa</i>	5.5	11.8	.44
<i>P. maximinoi</i>	5.7	9.5	.33
<i>P. pseudostrobus</i>	4.6	11.7	.35
<i>P. michoacana</i>	4.4	9.0	.31

Note: *P. michoacana* had only 33% survival while the other species had good survival.

FARM: San Jose PLANTING YEAR: 1981 PLOTS: 6X6 trees

	HT.-5 m	HT.-8 m	DBH-5 cm	DBH-8 cm	SP. GRAV.-6.3
<i>P. chiapensis</i>	13	18.7	17	25.5	.36
<i>P. patula</i>	10	15.4	17	20.8	.33
<i>P. kesiya</i>	10	12.9	15	22.1	.34
<i>C. lusitanica</i>	9	12.7	14	20.9	.45
<i>P. caribaea</i> (hond.)	7	9.5	15	21.2	.39

FARM: Samaria PLANTING YEAR: 1986 PLOTS: 6X6 trees  
 ELEV.: 2,000 m PRECIP.: 2,700 mm

	HT.-3 m	DBH-3 cm
<i>P. chiapensis</i>	8.4	10.1
<i>P. maximinoi</i>	7.1	10.5
<i>P. patula</i>	7.0	10.6
<i>P. kesiya</i> (Zimbabwe)	6.6	11.2
<i>P. kesiya</i> (Colombia)	5.9	11.5
<i>C. lusitanica</i>	6.5	8.9
<i>P. oocarpa</i>	6.4	9.8
<i>P. tecunumanii</i> (2.5 years)	6.0	8.5

FARM: Penas Negras PLANTING YEAR: 1986 PLOTS: 6X6 trees  
 ELEV.: 2,550 m PRECIP.: 2,100 mm

	HT.-3 m	DBH-3 cm
<i>P. patula</i>	4.7	7.3
<i>P. maximinoi</i>	4.7	7.1
<i>C. lusitanica</i>	4.6	6.5
<i>P. chiapensis</i>	4.3	4.4
<i>P. kesiya</i> (Colombia)	3.8	7.0
<i>P. kesiya</i> (Zimbabwe)	2.6	3.5
<i>P. oocarpa</i>	3.3	5.5
<i>P. caribaea</i> (hondurensis)	2.4	2.3

Table 2. Growth results of CAMCORE provenance/progeny trials of Pinus chiapensis in the Andes Mountains of Colombia. The number of families is in parenthesis. The other species are bulk check lots. The field design is a Randomized Complete Blocks with nine blocks, 6-tree row plots for families within blocks and families are nested within provenances.

FARM: La Arcadia		PLANTING YEAR: 1981		ELEV.: 1,700 m			
PRECIP.: 1,950 mm		STUDY: 01-02-01A					
	HT. -3 m	HT. -5 m	DBH-3 cm	DBH-5 cm	SURV.%-5	FORK.%-5	
P. oocarpa	3.8	9.1	5.2	14.5	91	18	
P. chiapensis	3.9	10.5	3.1	12.6	63	37	
S.Juan Gotzal (7)							

FARM: SAN Jose		PLANTING YEAR: 1984		ELEV.: 1,750 m			
PRECIP.: 1,950 mm		STUDY: 12-02-01D					
	HT. -1 m	HT. -5 m	DBH-5 cm	SURV.%-1	FORK.%-5		
P. tecunumanii	1.6	9.9	18.9	98	4		
P. chiapensis							
La Trinidad (13)	1.0	11.2	14.0	86	23		
Pueblo Nuevo (13)	1.0	10.6	13.8	91	29		
La Libertad (22)	0.8	10.7	12.8	81	40		
Zimbabwe (4)	0.6	8.2	9.1	76	23		

FARM: Los Guadales		PLANTING YEAR: 1986		ELEV.: 1,750 m			
PRECIP.: 1,950 mm		STUDY: 12-02-07C					
	HT. -3 m	DBH-3 cm	SURV.%-3	FORK.%-3			
P. chiapensis							
Pueblo Nuevo (1)	4.7	4.6	96	15			
La Libertad (1)	4.5	4.5	89	11			
Larrainzar (8)	4.2	4.0	92	6			
Guevea (3)	4.1	4.0	84	9			
Pohlo (5)	3.9	3.6	89	7			
Barrillas (34)	3.6	3.1	87	8			
P. Patula	4.4	6.2	93	2			
P. oocarpa	3.6	5.6	83	11			

Table 3. Provenance information for Pinus chiapensis trials.

PROVENANCE	LAT./LONG.	ELEVATION m	PRECIP. mm
Pueblo Nuevo, Chiapas, Mex.	17°N 93°W	1740-1780	1500-2050
La Trinidad, Chiapas, Mex.	17°N 93°W	1160-1530	1600-2000
La Libertad, Chiapas, Mex.	17°N 93°W	1800-2020	1700
Pohlo, Chiapas, Mex.	17°N 93°W	1340-1380	2200-2300
Larrainzar, Chiapas, Mex.	17°N 93°W	1720-1860	1800-2000
Guevea, Oaxaca, Mex.	17°N 95°W	820-1010	1500
S.Juan Gotzal, El Quiche, Mex.	15°N 91°W	980-1430	2220
Barillas, Huehuetenago, Guat.	16°N 91°W	1400-1700	1800

GENETIC VARIATION IN ROOT MORPHOLOGY OF  
PINUS AYACAHUITE AND PINUS CHIAPENSIS

Dr. William S. Dvorak

SUMMARY

Seedlings from 7 provenances and 61 half-sib families of Pinus ayacahuite and Pinus chiapensis were raised in 1 liter containers in a greenhouse and assessed at 25 cm shoot height for lateral root surface area, number of lateral roots, lateral root length and root/shoot ratios. Seedlings of subtropical hard pines, Pinus maximinoi and Pinus tecunumanii were included as controls. Lateral root surface area as a percent of total root surface area of hard and soft pines was approximately the same, 91.5% vs. 89.5%, but Pinus ayacahuite and P. chiapensis had 15% less fibrous root surface area than did P. maximinoi and P. tecunumanii. Slow growing provenances of Pinus chiapensis from elevations above 1500 m had significantly greater lateral root surface area/unit of shoot height than did fast growing provenances from elevations below 800 m. Significant family differences for both Pinus ayacahuite and P. chiapensis were found for lateral root surface area, number of lateral roots, and lateral root length but not for root/shoot ratios. Genetic gains in lateral root development are possible through family and within family selection. Breeding efforts need to be combined with improved nursery techniques to produce more hardy trees.

Key Words: lateral roots, fibrous roots

Introduction

Pinus ayacahuite Ehrh. and P. chiapensis (Mart). Andresen are white pines endemic to Mexico and Central America. Both species have been established in pilot plantings throughout the tropics and subtropics and appear to have good potential on sites with well drained soils that receive abundant precipitation (Dvorak and Brouard, 1987; Poynton, 1976). However, on sites that are slightly less than ideal, the initial growth of both species is very slow and first year field survival has been poor. Mortality is often attributed to a combination of drought stress and poor root development. If trees survive the first several years, they capture the site and grow rapidly thereafter.

The objective of this study was to quantify the magnitude of genetic variation that exists in the root morphology of Pinus ayacahuite and Pinus chiapensis. If a large genetic component is present, tree breeders can manipulate it to develop white pine trees that are better adapted to marginal sites in the tropics.

Dr. William S. Dvorak, Director: CAMCORE Cooperative, College of Forest Resources, North Carolina State University, Raleigh, North Carolina, USA.

Experimental Methods

Seeds of Pinus ayacahuite and Pinus chiapensis were collected from 61 trees and 7 provenances in Mexico, Guatemala and Honduras by the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University. The provenances sampled were those that best represented the climatic and edaphic extremes in the species range (Table 1). Seeds were germinated in the greenhouse and 24 plants/family were pricked into 1 liter (7.5 cm x 7.5 cm x 18 cm) containers and arranged in a randomized complete block design with 3 replications. Seedlots of the hard pines Pinus maximinoi H. E. Moore and P. tecunumanii (Mart.) Equiluz and Perry were also included in the study as controls. Seedlings were destructively sampled when provenance shoot height averaged 25 cm, the normal size at which seedlings are outplanted to the field in the tropics. Shoots were oven dried at 70°C for 72 hours and weighed to the nearest .001 gm. Root systems were gently washed and the length of the 5 longest primary lateral roots measured to the nearest mm. The total number of primary lateral roots were counted. The surface area of the lateral and tap roots was measured using an automated system (Delta-T, Dacagon®). The surface area of fibrous roots was also determined for 50% of the samples from 2 populations of each species. A fibrous root was defined as being 40 mm or less in length and less than 1 mm in diameter. Root dry weights were obtained using standard procedures.

Analyses of variance were conducted using the General Linear Model of the Statistical Analysis System® for the following traits: lateral root area/shoot height, length of the 5 longest lateral/shoot height, number of laterals, and root/shoot ratio.

Table 1. Location of the P. ayacahuite and P. chiapensis collection sites in Mexico and Central America.

Species	Provenance	Country	Latitude	Elevation (m)
<u>P. ayacahuite</u>	Ixtlan	Mexico	17° 24' N	2600
<u>P. ayacahuite</u>	Las Trancas	Honduras	14° 07' N	2300
<u>P. ayacahuite</u>	Ocotopeque	Honduras	14° 25' N	2150
<u>P. chiapensis</u>	Barillas	Guatemala	15° 48' N	1600
<u>P. chiapensis</u>	Nopoala	Mexico	19° 54' N	800
<u>P. chiapensis</u>	San Gabriel	Mexico	16° 11' N	1600
<u>P. chiapensis</u>	Tutla	Mexico	17° 25' N	500

Results indicate that the total root surface area of the white pines is comprised of 89.5% lateral roots and 10.5% tap roots when grown in the 1 liter containers used in this study. The hard pines had slightly more lateral root surface area (91.5%). There were significant differences among provenances of P. chiapensis in lateral root area/shoot height. The high elevation sources, Barillas and San Gabriel, grew more slowly than the low elevation populations of Tutla and Nopoala but possessed greater lateral root area when assessed at 25 cm shoot height. No significant provenance differences were found in

lateral root area/shoot height for P. ayacahuite (Table 2). Significant family differences were found for both P. chiapensis and P. ayacahuite for lateral root area/shoot height, number of lateral roots and lateral root length/shoot height but not for root/shoot ratios (Table 3).

Table 2. Provenance means of lateral root area ( $\text{mm}^2$ )/shoot height (mm) and their standard errors for Pinus ayacahuite and P. chiapensis.<sup>1</sup>

<u>Pinus ayacahuite</u>			<u>Pinus chiapensis</u>		
Provenance	Mean	Std. Error	Provenance	Mean	Std. Error
Ixtlan	21.04	.90	Barillas	22.43	1.02
Las Trancas	20.81	.73	San Gabriel	22.32	.96
Ocotopeque	17.87	.83	Nopoala	14.48	.58
			Tutla	15.53	.70

<sup>1</sup> The values in the table are read as follows: Ixtlan, (for example) required 21.04  $\text{mm}^2$  of lateral root surface to support 1 mm of shoot height.

Table 3. Summary of analyses of variance for the traits of lateral root area/shoot height (LRA/SH), length of the 5 longest laterals/shoot height (LL/SH), number of laterals (NLAT), and root/shoot ratios (RSR).

<u>Pinus ayacahuite</u>	LRA/SH	LL/SH	NLAT	RSR
Provenance	NS	NS	**	NS
Families	*	**	**	NS

<u>Pinus chiapensis</u>	LRA/SH	LL/SH	NLAT	RSR
Provenance	**	NS	*	NS
Families	***	**	***	NS

NS = not significant; \*, \*\* and \*\*\* probability > F of .05, .01 and .001, respectively.

Pinus chiapensis and P. ayacahuite fibrous roots only accounted for 8% and 15% of the total root surface area, respectively, versus 23% and 32% for P. tecunumanii and P. maximinoi. Family variation in fibrous root percent is shown in Table 4.

Table 4. Family variation in fibrous root percent by species. The number of families (N) in each category are expressed as percents.

Species	N	Fibrous Root Percent				
		0-9%	10-19%	20-29%	30-39%	> 40%
<u>P. maximinoi</u>	12	0	0	50	33	17
<u>P. tecunumanii</u>	14	7	21	36	36	0
<u>P. ayacahuite</u>	13	30	54	8	8	0
<u>P. chiapensis</u>	15	73	27	0	0	0

There appears to be sufficient genetic variation in the root morphology of P. chiapensis and P. ayacahuite to warrant selection and improvement by tree breeders. However, it is not known what degree of improvement will be necessary to obtain trees that will survive moderate droughts of several months. Based on the results of this study, it is apparent that shoot growth rate in the nursery is not necessarily a good indicator of lateral root development.

Development of better nursery techniques in association with tree improvement activities will be needed if more hardy P. chiapensis and P. ayacahuite seedlings are to be produced. Alternative nursery techniques may include the establishment of bareroot rather than containerized nurseries, root pruning, and extending the period of time white pine seedlings are grown in the nursery prior to field planting.

Even though some provenance variation in lateral root development was observed for P. chiapensis, it appears that the greatest genetic gains will come from family and within family selection. Breeding priorities should emphasize improvement in the quantity of fibrous roots.

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COEVOLUTION OF MEXICAN WHITE PINES AND THEIR  
DWARF MISTLETOE PARASITES

Dr. Frank G. Hawksworth

FDC: 165.1: 442.1: 174.7 Pinus ayacahuite Ehren.: 176.1 Arceuthobium Bieb.

SUMMARY

The dwarf mistletoes (Arceuthobium, Viscaceae) are common parasites of the Pinaceae in North America. The genus has been present in North America for more than 20 million years (mid-Miocene), so the dwarf mistletoes have long coevolved with their coniferous hosts. Since they are relatively host specific, the dwarf mistletoes can serve as "genetic markers" to provide clues to the evolution and taxonomy of their host trees. Here I discuss the six dwarf mistletoes that parasitize subgenus Strobus in Mexico, 3 on section Parrya (pinyons) and 3 on section Strobus (white pines). The dwarf mistletoes are very common on pinyons in the United States but surprisingly rare on these pines in Mexico. However, Arceuthobium is quite common on Mexican white pines. Arceuthobium guatemalense occurs on Pinus ayacahuite var. ayacahuite in Guatemala and southern Mexico (Chiapas and Oaxaca). It has not been found on P. chiapensis, although this tree sometimes grows near infested P. ayacahuite var. ayacahuite. It is not known within the range of A. ayacahuite var. veitchii in central Mexico. Arceuthobium apachecum is a common parasite of P. strobiformis in southern Arizona and southern New Mexico and it is known from one locality in northern Coahuila, Mexico. The dwarf mistletoe evidence (parasitism by a distinct species, A. blumeri) suggests that the common white pine in the Sierra Madre Occidental in Chihuahua and Durango (which has been commonly called P. strobiformis) should be classified as P. ayacahuite var. brachyptera. The same dwarf mistletoe occurs in the Sierra Madre Oriental in Nuevo Leon and Coahuila on a tree that has been recently named P. strobiformis var. potosiensis.

Keywords: Arceuthobium, Evolution, Parasitism, Mexico, Taxonomy.

Introduction

The dwarf mistletoes (genus Arceuthobium, family Viscaceae) are widespread parasites of the Pinaceae in the New World (Hawksworth and Wiens, 1972). As presently understood, there are 39 taxa in North America and 22 in Mexico. In Mexico, most species occur in Pinus, but Abies and Pseudotsuga are also

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parasitized. In Mexico 6 species parasitize subgenus Strobus: 3 on Section Parrya (pinyons) and 3 on Section Strobus (white pines). The genus has been in North America since at least mid-Miocene so the dwarf mistletoes have undergone a long period of co-evolution with their hosts (Hawksworth, 1987). We don't know how long they have been in Mexico as there are no pre-Pleistocene pollen records from there, but it is likely that they have been in Mexico essentially as long as the pines. Some of the apparently most primitive North American species occur in Mexico (Hawksworth and Wiens, 1972).

Because of their long period of co-development with their hosts and their high host specificity (each dwarf mistletoe is usually restricted to a single host species or a group of closely related species) these parasites can be "genetic markers" that provide clues to the taxonomy and evolution of their host trees. Here I discuss the taxonomy of the Mexican white pines in relation to their differential parasitism by the dwarf mistletoes.

The dwarf mistletoes are by far the most serious diseases of white pines in Mexico. They cause markedly reduced height and diameter growth, increase mortality rates, and adversely affect cone and seed production. Although they cause serious damage in natural stands, because they are dispersed primarily by explosive seed discharge and not by birds, they are of little consequence in plantations, unless susceptible trees are planted near infected overstory trees.

Dwarf mistletoe-caused mortality is particularly severe on Pinus ayacahuite var. ayacahuite in southern Mexico (Chiapas and Oaxaca) and in adjacent parts of Guatemala (by Arceuthobium guatemalense) and on Pinus ayacahuite var. brachyptera in the Sierra Madre Occidental in Durango and Chihuahua (by A. blumeri).

White pine blister rust (Cronartium ribicola) is not known from Mexico but it has been recently discovered on Pinus strobiformis near Cloudcroft in the Lincoln National Forest in southern New Mexico (Hawksworth, 1990), so it is now within 300 km of the closest white pine populations in northern Chihuahua. This is the only known occurrence of the rust within the natural range of P. strobiformis, and it represents a range extension of about 1000 km from the closest known populations in southern Wyoming on Pinus flexilis.

#### The Subgenus Strobus in Mexico

The subgenus Strobus is well represented in Mexico with about 19 taxa; 12 in Section Parrya (pinyons) and 6 in Section Strobus (white pines).

- I. Section Parrya: The 12 known Mexican pinyons are Pinus californiarum, P. cembroides (with 3 subspecies: cembroides, orizabensis, and lagunae), P. culminicola, P. discolor, P. johannis, P. maximartenezii, P. nelsonii, P. pinceana, P. quadrifolia, and P. remota. Two other pinyons listed by Martínez (1948) in Mexico are not now thought to occur in that country. Pinus edulis occurs in the Big Hatchet Mountains in southwestern New Mexico within a few km. of Chihuahua but it has not yet been found across the border in Mexico. Pinus monophylla was reported in Baja California but the single-needled pinyon there

is now considered to be a distinct taxon Pinus californiarum (Bailey, 1987). Pinus monophylla is a valid species but it occurs in the Great Basin area well north of the Mexican border.

II. Section Strobus: The number of white pines in Mexico is uncertain, primarily because of confusion with the Pinus strobiformis group (Equiluz Piedra, 1990). However there seem to be at least 7 taxa: Pinus ayacahuite (with 3 varieties: ayacahuite, brachyptera, and veitchii), P. chiapensis, P. rzedowskii, and P. strobiformis (with possibly 2 varieties: strobiformis and potosensis).

#### Dwarf Mistletoes on Pinyons in Mexico

The dwarf mistletoes are widespread on the pinyons in the southwestern United States, but surprisingly rare on these trees in Mexico (Bailey and Hawksworth, 1988, Table 1). Arceuthobium divaricatum is common almost throughout the ranges of Pinus edulis and P. monophylla. In Mexico, however, this dwarf mistletoe is known only from northern Baja California where it is locally common on Pinus quadrifolia. The vast Pinus cembroides forests of northern Mexico are not affected, but this tree is parasitized by A. divaricatum at its northern limits in the Davis Mountains in West Texas, which is the only known locality where the parasite and the tree occur together.

In Central Mexico there is a distinctive endemic species that is confined to pinyons (Hawksworth and Wiens, 1980). Arceuthobium pendens is currently known from only two localities: (1) It is rare in the Sierra Miguelito in San Luis Potosi on Pinus discolor (where adjacent P. cembroides subsp. cembroides was not infected), and (2), it is locally common in the Perote area of Veracruz and adjacent Puebla on P. cembroides subsp. orizabensis.

The rare, high-elevation, 5-needled Pinus culminicola of northeastern Mexico is rarely parasitized by Arceuthobium vaginatum subsp. vaginatum on Cerro Potosi, Nuevo Leon. However, there are merely cross-over infections of a dwarf mistletoe that primarily parasitizes hard pines, in this case Pinus rudis.

Table 1. Dwarf mistletoes on pinyons in Mexico.

<u>Arceuthobium</u>	Pinyon	Distribution	Abundance
<u>A. divaricatum</u>	<u>Pinus quadrifolia</u>	Baja Calif. N.	Locally common
<u>A. pendens</u>	<u>Pinus cembroides</u> subsp. <u>orizabensis</u> <u>Pinus discolor</u>	Puebla, Veracruz San Luis Potosi	Locally common Rare
<u>A. vaginatum</u>	<u>Pinus culminicola</u>	Nuevo Leon	Rare

Dwarf Mistletoes on the White Pines in Mexico

Unlike the case for the pinyons, the dwarf mistletoes are quite common on Mexican white pines (Hawksworth and Wiens, 1972; Mathiasen, 1982). The only widely distributed Mexican white pine not known to be parasitized is Pinus ayacahuite var. vetchii of central Mexico. Also, the relatively rare Pinus chiapensis and P. rzedowskii are not known to be affected.

Arceuthobium apachecum is widely distributed in the mountains of southern Arizona and southern New Mexico and is known from only one locality in Mexico (the Sierra del Carmen in northern Coahuila). Its only host is the tree commonly known as Pinus strobiformis.

Arceuthobium blumeri is widespread in the Sierra Madre Occidental from northern Jalisco through Durango to Chihuahua. Isolated populations occur in northern Sonora (Ajo Mountains) and extreme southern Arizona (Huachuca Mountains). The host of this dwarf mistletoe in these areas has been variously treated as Pinus strobiformis or P. ayacahuite var. brachyptera. An extreme disjunct population occurs in the northern Sierra Madre Occidental in northern Nuevo Leon and southern Coahuila. The host in this area has been variously treated as Pinus strobiformis, P. reflexa, or P. flexilis (Equiluz Piedra, 1990) and has recently been designated as a new variety P. strobiformis var. potosiensis (Silba, 1990).

Arceuthobium guatemalense is restricted to Pinus ayacahuite var. ayacahuite in the high mountains of southern Mexico (Chiapas and Oaxaca) and Guatemala. In the mountains north of Ixtlan, Oaxaca at 2700-2800 m., we found mixed stands of Pinus ayacahuite var. ayacahuite (at its lower limits) and P. chiapensis (at its upper limits), but only the former was infected.

Table 2. Dwarf mistletoes on white pines in Mexico.

<u>Arceuthobium</u>	White Pine	Distribution	Abundance
<u>A. apachecum</u>	<u>Pinus strobiformis</u> var. <u>strobiformis</u>	Nor. Coah.	Very rare
<u>A. blumeri</u>	<u>Pinus ayacahuite</u> var. <u>brachyptera</u> <u>Pinus strobiformis</u> var. <u>potosiansis</u>	Chih., Son., Dgo. Nuevo Leon	Very common Locally common
<u>A. guatemalense</u>	<u>Pinus ayacahuite</u> var. <u>ayacahuite</u>	Chis., Oax.	Locally common

## Conclusions

The scarcity of dwarf mistletoe infection on the pinyons in Mexico precludes their providing much information on the taxonomy of their hosts. However, the apparent selective parasitism of Pinus discolor, and not of associated P. cembroides subsp. cembroides in the Sierra San Miguelito in San Luis Potosi, is further evidence of the distinctness of these two pinyons.

The parasitism of white pine on the other hand, provides several suggestions of taxonomic affinities of their host trees. The apparent selective parasitism of P. ayacahuite, and not of associated P. chiapensis in Oaxaca, is additional evidence of the distinctness of these two white pines.

The host of Arceuthobium apachecum in southern Arizona, southern New Mexico, and northern Coahuila is best referred to as Pinus strobiformis. However, the white pine in the Sierra Madre Occidental from Jalisco to southern Arizona has also been classed as Pinus strobiformis (Critchfield and Little, 1966), but this is parasitized by a different dwarf mistletoe (A. blumeri). Thus, the dwarf mistletoe evidence corroborates the morphological findings (Equiluz Piedra, 1990) that this tree should be referred to as P. ayacahuite var. brachyptera.

The disjunct white pine in the Sierra Madre Oriental, which is characterized by stubby cones with mostly non-reflexed scales, is also parasitized by A. blumeri. Its unique cone morphology suggests that it is a distinct taxon and the recently assigned name Pinus strobiformis var. potosienses (Silba, 1990) is available for it until detailed studies of this and adjacent populations can be made. It would be of particular interest to determine the taxonomic status of the white pine in the Sierra Madera in central Coahuila to determine whether it is most like the northern Coahuila populations (var. strobiformis parasitized by A. apachecum) or the southern populations (var. potosiensis parasitized by A. blumeri). No dwarf mistletoe parasitism of the white pines in this mountain range is known.

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SURVIVAL AND GROWTH OF PINUS GRIFFITHII  
GENETIC TESTS IN TENNESSEE

Dr. S.E. Schlarbaum and Mr. R.A. Cox

SUMMARY

Genetic test plantations of blue pine, Pinus griffithii, were established in 1988 at three Tennessee locations. The studies were analyzed for survival and growth. Significant differences occurred among families in survival and growth at each location. Differences due to block effects were significant at only one location. Survival and height growth were probably affected by a severe drought, rather than cold winter temperatures. Families that had a high percentage of survival and exhibited good growth at each plantation came from a wide geographic spectrum.

Keywords: Pinus griffithii, blue pine, genetics, variability

Introduction

The Asiatic blue pine, Pinus griffithii McClell. (syn. P. wallichiana A.B. Jacks.) and the North American eastern white pine, P. strobus L., are two Haploxyton pine species that are sexually compatible, despite geographic isolation. The rapid growth of hybrids between these species (Genys, 1979; Kriebel and Dogra, 1986) is of particular interest in North American regions where P. strobus is an important commercial species. Kriebel and Dogra (1986) reported that P. strobus X griffithii hybrids grew comparably with a majority of elite P. strobus families in a North American test planting, although the paternal blue pines were described as being below average trees. Hybridization of elite blue pines with elite eastern white pines may produce trees that would outgrow either parent. Blue pine also has other desirable characteristics: resistance to blister rust (Genys, 1979) and ozone (O<sub>3</sub>) pollution (Genys and Heggstad, 1978).

In Tennessee, tree improvement research has been conducted on eastern white pine for more than two decades. Superior genotypes for growth and SO<sub>2</sub> resistance have been selected (Thor and Gall, 1978). The performance of blue pine hybrids merits investigation on the performance of different seed sources of Pinus griffithii in Tennessee to eventually determine genetically superior blue pine genotypes. Blue pine selections would then be integrated into the

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existing white pine breeding program. In addition to potential for growth improvement, hybridization between blue pine selections with Tennessee selections may produce trees that exhibit hybrid vigor and resistance to blister rust and SO<sub>2</sub> and O<sub>3</sub> air pollution.

#### Experimental Materials and Methods

Seedlots from a IUFRO seed collection of *Pinus griffithii* (Kriebel, 1976a) were obtained from Dr. Howard B. Kriebel in 1986 (Table 1). Following stratification, the seedlots were germinated in 1987, and the resulting seedlings transplanted into Cone-tainers<sup>®</sup> (115 cm) for growing until field planting.

Table 1. Origin of blue pine seedlots used in Tennessee plantings.

IUFRO No. (s)	Location code	Seed Origin			Seedlot bulked (B) or kept by individual tree (I)
		Country,	district,	locality	
6001	1.0	Afghanistan, (nonspecific)			B
6008	2.0	India, (nonspecific)			B
6009-6011	3.0	Nepal, Tistung, Makawanpur			I
6012	4.1	India, Kashmir, Kangan			I
6115-6125*	4.2	India, Kashmir, Liddar Valley			I
6019	5.1	India, Himachal Pradesh, Manali			I
6032	5.2	India, Himachal Pradesh, Chamba			I
6033	5.3	India, Himachal Pradesh, Sanjoal			I
6034	5.4	India, Himachal Pradesh, Simla			I
6036-6045*	6.1	Pakistan, Hazara, Panjoul			I
6046-6055*	6.2	Pakistan, Hazara, Malkandi			I
6114	6.3	Pakistan, Hazara (Murree) (nonspecific)			B
6178-6186	6.4	Pakistan, Hazara, Kund			I
6178-6196*	6.5	Pakistan, Hazara, Kuzagzli			I
6197-6206	6.6	Pakistan, Hazara, Sharan			I
6207-6216	6.7	Pakistan, Hazara, Shogran			I
6217-6226*	6.8	Pakistan, Hazara (Murree), Thandhiani			I
6126-6135	7.0	Pakistan, Gilgit, Naltar			I
6137-6146	8.0	Pakistan, Chitral, Bamburet			I
6147-6156	9.1	Pakistan, Dir, Kalkot			I
6157-6166	9.2	Pakistan, Dir, Kumrat			I
6169,6172	10.0	Pakistan, Swat, Ushu			I
6227-6234	11.1	Pakistan, Azad Kashmir, Dawaryan			I
6235-6244	11.2	Pakistan, Azad Kashmir, Dungian			I
6245-6254	11.3	Pakistan, Azad Kashmir, Kern			I
6256-6265	11.4	Pakistan, Azad Kashmir, Sharda			I
6266-6274*	11.5	Pakistan, Azad Kashmir, Tararkhal			I

\* Not all seedlots were included in tests

Three experimental plantations were established in the Spring of 1988 at various locations in Tennessee using 1-0 container stock. The planting conditions varied among locations. The Bledsoe State Forest (BSF) planting in central Tennessee was on a sandy loam soil of variable depth (parent material - sandstone). The second planting was at Standing Stone State Forest (SSF) in north-central Tennessee on a clay loam soil of variable depth (parent material - limestone) with exposed bedrock. A site on the Ames Plantation (AP) in western Tennessee was selected for the third study. The seedlings were planted in silt loam soil approximately 1.5 m deep over sandy coastal plains sediment. The SSF and AP plantings were on well drained sites, while the BSF planting was on a moderate to poorly drained site. A fourth plantation in eastern Tennessee was terminated in 1988 due to poor survival.

The large number of families, coupled with low seedling numbers in some families, influenced experimental design selection. An incomplete block design was chosen to allow for representation of the maximum number of families in each planting, while relatively reducing the total number of seedlings planted per family. For location BSF and SSF, 132 and 96 families, respectively, were arranged in incomplete blocks with plot size = 12 and 3 replicate plots per family containing 10 seedlings/plot. Seedlings were spaced 1.8 m apart within rows, with 3 m between rows. The field design for the AP location differed only in plot size, containing 5 seedlings/plot. This design was generated via computer software TENNDES<sup>B</sup> developed by John F. Schneider and William L. Sanders, Agricultural Experiment Station, The University of Tennessee.

The survival data utilized GLMM<sup>C</sup>, developed by the Department of Statistics, Louisiana State University, to complete a recovery of interblock information analysis using mixed model methods (Henderson, 1975). Prior to analysis, the percentage data were transformed to angle to enhance the assumptions appropriate to analysis of variance. A least squares analysis of variance was completed via PROC GLM<sup>B</sup> in SAS<sup>B</sup> for height growth. Second year survival data from the BSF and SSF plantations and first year survival data from the AP plantation were analyzed. Analyses of second year height growth measurements were made for each plantation.

## Results and Discussion

The analyses for survival and second year height growth revealed significant differences among families at each plantation. Block effects were significant only at SSF, possibly reflecting the variation in soil depth at this site. Overall survival was greatest at BSF (47%), followed by SSF (45%) and AP (30%). High mortality in North American blue pine genetic tests is usually ascribed to lack of cold-hardiness (Kriebel, 1976b; Genys, 1979). In these plantations, however, the relatively low survival at all locations was probably due to severe drought conditions that existed in 1988 and the 3 preceding growing seasons. Winter conditions in 1988-89, however, were relatively mild in Tennessee and probably did not adversely affect plantation survival. The average maximum height attained by any family was similar at all plantations: BSF = 20.7 cm, SSF = 22.7 cm, and AP = 21.3 cm. Some families showed little growth since planting, despite favorable environmental conditions in 1989. It is unknown why seedlings from some of these families have not begun to grow, as they appeared to be reasonably healthy.

Survival means (not shown) for families from different geographic areas were inspected for general trends over all plantations. The Afghanistan (location code = 1.0) seedlings were planted only at BSF, where they exhibited good survival (83%) and moderate growth. In contrast, seedlings from the bulked Indian seedlot (2.0) showed poor survival at BSF but better growth. Seedlings from Nepal (3.0) showed poor survival at all 3 locations. Survival of seedlings from other provenances were too variable among locations to clearly discern general trends. Similar geographic trends in height growth cannot be detected among locations. This variability was reflected by height and survival means of some families common to all locations. Survival means of 4 families from Pakistan, Hazara, Shogran (6.7) illustrate the variability that can be encountered. Seedlings from 6214 showed poor survival at all locations. Relatively high survival of seedlings from 6207 and 6211 occurred at BSF and AP, but was reduced at SSF. Alternately, survival of seedlings from 6280 was good at BSF, moderate at SSF, and poor at AP. Although general trends in survival and height were often difficult to detect, some individual families performed similarly at different locations. Families 6009 and 6141 had low survival percentages, while families 6142 and 6261 had better survival regardless of location. Height growth of families 6011, 6032, and 6233 was approximately the same at all locations.

Inspection of family survival means (not shown) at each respective plantation showed no geographic trends, other than those mentioned above, and high variability within families from the same geographic area, e.g., Pakistan, Azad Kashmir, Kern (11.2). Families from a number of geographic areas had high survival, ca. 90%, in addition to families with survival under 20%. Inspection of height means of families with good survival at each plantation revealed similar variability within geographic areas.

Although there were differences among families in height growth at age 2, it probably does not reflect the potential for growth in the future. Genys (1979) correlated second year growth with ten year growth of different blue pine seed sources and found no relationship. An additional consideration in this study is the effects of the 1988 drought on growth. Height differences among families probably reflect differences in drought tolerance and subsequent recovery rather than true growth potential.

#### Conclusions

Significant differences were detected among families in survival and height growth at each plantation. These differences are probably due to drought response rather than different levels of cold-hardiness. With few exceptions, geographic trends over all plantations and within plantations were not detected. Inspection of data on families common in each plantation shows that some families survive and grow similarly regardless of location, while others do not. Families that exhibit good growth and survival, at each respective plantation, are represented from a wide spectrum of geographic origins. Geographic patterns may emerge in the future, as normal winter conditions in Tennessee will screen families for cold-hardiness.

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THE GENETIC RESOURCES OF  
PINUS PEUCE GRSB. IN BULGARIA

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SUMMARY

The eastern end of the natural range of Pinus peuce Grsb. on the Balkan Peninsula, is situated in Bulgaria, and it is represented by large populations that occur in the Pirin and Rila Mountains. In the Central Balkan Range, the West Rhodopes, Vitosha, and Slavyanka it comprises smaller stands or occurs as individual trees. This tertiary relict occupies 14,000 ha within the range from 1400 up to 2200 m altitude where it forms either pure, or mixed stands, most often with Pinus sylvestris L., Picea abies (L.) Karst., Pinus heldreichii Christ. and Pinus mugo Turra.

The variation in main phenotypic features such as branch pattern, habitus, bark cracking, strobile coloration, etc. has been studied. The variation in crown shape, branch angle, and plate-line bark patterns seem to be the most useful for purposes of tree breeding.

The genetic resources of this species are conserved mainly in situ. There are currently 5028 ha set aside in National Parks, 584 ha in reserves, and 626 ha in seed production stands. One hundred and fiftytwo candidate "elite" trees of outstanding growth and resistance have been selected and their progenies have been tested under three ecological conditions.

Experimental Methods and Results

Pinus peuce Grsb. forms its most productive stands in the upper part of the mountain belt and in the alpine zone, at altitudes of 1600 to 1900 m. This species is very resistant to unfavorable climatic conditions and it is most suitable for stabilizing and extending the upper forest limits. This property, as well as its valuable timber, make it extremely suitable for an improvement program, under the above mentioned conditions. The high resistance of this species to pests and diseases, particularly Cronartium ribicola and Armillaria mellea, recommends it for interspecific hybridization with other five-needle pines which would bring fast growth rates to the hybrid.

During the period 1971-1976, on 19 plots at different sites, all near the upper forest limits in the Balkan Range - the Rila Mountains, Osogovo, and the Pirin Mountains - 27 ha of provenance plantings of Pinus peuce were established.

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In recent years, studies on the state of the genetic resources of Pinus peuce have been carried out for the purpose of determining its usefulness in breeding programs. This work has been conducted in all of the major mountain ranges of Bulgaria, including the Pirin and Rila Mountains, the Balkan Range, the West Rhodopes, and Slavyanka, where this species occurs naturally. All of the natural range (within Bulgaria) of Pinus peuce was investigated with separate collections from individual mountains, populations from various altitudes, and isolated populations. Each sample plot is comprised of at least 200 trees with 15 representative individuals providing seed in any given seed year. In this study, various methods such as biometrical, phenological, early testing, progeny trials, etc. were used to characterize the genetic variation present and to determine the possibilities for breeding purposes.

The contemporary flora of the Balkan Peninsula was differentiated at the end of the Pleocene. In the beginning of the Myocene, Pinus peuce had almost thoroughly occupied, together with the other conifers, the mountainous parts of the Peninsula (Stefanoff, 1943). Tectonic processes resulted in the formation of mountain ranges, yet the factors of genetic dynamics determined the rate of spread and differentiation of corresponding phytogeographical centers. As climate progressively turned continental in the Pleocene period, this determined the vertical movement of P. peuce in the higher parts of the mountains where air humidity had been higher. The Balkan Peninsula was only slightly influenced by freezing in the Pleistocene, so it appeared to be a refuge for a great number of forest tree species. Favorable conditions for the development of coniferous vegetation including P. peuce had existed in the Rilo-Rhodopean Massif due to the moist and chilly climate that existed there. The paleobotanical and palinological of this species (Stefanoff and Yordanoff, 1935; Bozhilova, 1986), as well as its present condition, indicate that from the Tertiary Period to the present time, it has constantly been subjected to a reduction in its natural range, and to fragmentation within the range, because the climate has become more continental over time. In the course of history, the natural range of Pinus peuce has also been reduced due to anthropogenic activities. The habitual cutting and burning of Pinus peuce forests for enlarging alpine pastures and for harvesting top-quality building timber has significantly accelerated this process over the past 100-150 years.

In Bulgaria, Roumelian pine (Pinus peuce), in the eastern part of its range, occurs in fairly large stands in the Pirin Mountains. In the Central Balkan Range and the West Rhodopes it occurs in isolated populations, while in Vitosha and Slavyanka it is found in small groups or as single individuals. Similar stands in some mountains of the Dinary System in Yugoslavia and Albania, as well as isolated formations in the northern mountains of Greece belong to its western locality.

The morphological features that determine the Pinus peuce in Bulgaria as var. vermiculata J. are its shorter and thinner needles and its shorter strobiles (Chernyavski et. al., 1959).

The Roumelian pine forests in Bulgaria occupy 14,000 ha between 1,400 and 2,200 m elevation. The most intact grouping of this species is in the Pirin Mountains where it occupies 7,175 ha. This complex consists of two parts: the northeastern unit of 3,775 ha that occurs between 1,600 and 2,200 m and the southwestern unit of 3,400 ha occurring between 1,700 and 2,100 m.

In the Rila Mountains Pinus peuce occupies an area of approximately 6,230 ha and consists of three major complexes: The northern unit covers 3,684 ha where this pine has formed mixed stands with Pinus sylvestris L., Picea abies (L.) Karst., and Pinus mugo Turra. up to 2,100 m elevation. The southern unit consists of 1,635 ha and ranges from 1,700 to 2,100 m elevation. The central unit, with an area of 911 ha in the Rilska River valley also occurs as mixed forests and reaches an elevation of 2,000 m. This occurrence indicates that the upper forest limit in the interior portions of the Rila Mountains reaches lower, and the mixture of species in this location, is expressed better than it is at the margin. So, in spite of the anthropogenic activities that have effected the present state of the Pinus peuce forests in the Rila Mountains, their occurrence agrees with the general regularities of autochthonous development.

In the Rhodopes, Roumelian pine has been localized only in their northwest spur, on an area of 170 ha, mainly as plantations. To the east and to the south it occurs only as single trees.

On Vitosha this species occupies an area of 104 ha, mainly as plantations that have been grown for more than 60 years. Growth of planted trees has been good and their viability has been high. The natural occurrence of Roumelian pine on this mountain complex is as single trees mixed with the spruce stands that form the upper forest limits.

The most northern formation of this species in Bulgaria is also the most northern limits of the species on the Balkan Peninsula. It consists of 193 ha in two parts. The formation in Slavyanka, whose total area is barely 57 ha, is among the smallest ones.

In spite of the complete spatial isolation that exists today between the Rila-Rhodopean Massif, Vitosha, and the Central Balkan Range, the occurrence of a number of plant species including Pinus peuce, provides the availability of phytogeographical links between them. The Rila-Rhodopean Massif being in close proximity to populations on mountains in both Greece and Yugoslavia appears to be the point from which Pinus peuce began its spread in Bulgaria.

Pinus peuce is distinguished by its high tolerance to extremes in climate, to windthrow, windbreakage, and breakage due to snow loading. It is extremely resistant to both insect pests and fungal diseases. Its biological resistance is combined with high productivity throughout its range. It shows the highest rates of growth between the ages of 20 and 40 years. Its growth in diameter is shown to be most intense between the ages of 30 and 80. At those ages, the current increments in height and diameter vary from 20 to 46 cm and from 1.6 to 3.8 mm respectively, and the annual increment in volume ranges from 4.3 to 13.2 m<sup>3</sup>/ha, depending on site conditions (Nedyalkov and Krastanov, 1962).

The high density of stems in mature Pinus peuce stands results in high basal area and volume on a per/ha basis. Data from the Noth Pirin Mountains indicate volumes of as much as 1,671 m<sup>3</sup>/ha, an average height of 40 m, and a diameter of 2 m for 500 year-old trees (Nedyalkov and Nickolov, 1986).

Roumelian pine occurs mainly on silicate soils, and to a lesser extent on carbonate soils, where it forms the corresponding edaphotypes.

While conducting investigations on the variability of this species, the following crown shapes were observed: 1. Spindle-shaped crowns (f. vermiculata). This form occurs at higher elevations, mainly on sunny exposures, on humus and carbonate soils. Individuals whose strobiles are red before maturation predominate. 2. Individuals with cone-shaped crowns (f. conica). This type occurs on lower altitude sites, mainly on shady portions of the mountains, and on silicate soils. These trees generally have green strobiles. 3. Trees with column-shaped crowns (f. columnaris). Crowns of this type are narrow - from 1 to 2 m in width and branches are short and thin. This is a highly productive form because of the many stems that can be crowded onto a given area. Its decorative value is also very high.

Trees are also distinguished by their branching habit and three forms are recognized: horizontalis, pyramidalis, and pendula. Analogous to other pine species, the forms of Pinus peuce separated according to this trait have morphological characteristics that have resulted from continuous adaptation to particular ecological conditions.

The forms separated according to branching habit are closely related with these, that are determined according to crown habit and their overall appearance is submitted to the law of homologous laws for hereditary variation.

Based on bark cracking, the following forms have been determined: 1. smooth-barked, 2. scale-barked, 3. spruce-like cracked bark, and 4. longitudinally plate-like cracked bark.

According to coloration of strobiles, three forms can be separated: 1. var. chlorocarpa, 2. var. erythrocarpa, and 3. var. dichlora. The first one occurs mainly at lower altitudes and on warmer sites. Its growing season and flowering start about 10 days later and its growth is slower than the other types.

The most productive Pinus peuce form is one with spindle-shaped crowns, plate-like cracked bark, and red immature cones. For determining heritabilities is some quantitative features, and for studying the structures of Pinus peuce populations, half-sib progeny trials have been established under 3 types of ecological conditions. These experiments involve progenies of 180 individuals and 13 provenances.

National parks and reserves are among the most reliable means for in-situ conservation of genetic resources. In the Pirin National Park, 4,924 ha of the best stands of P. peuce are conserved. They consist of central populations on sites considered optimum for this species, and of marginal populations that have adapted to extreme conditions. A large area of the Roumelian pine complex in the Pirin Mountains helps the restriction of gene migration and genetic drift, as well as the establishment of climax balance in places where it has been disturbed by anthropogenic activity. The Bayuvi Dupki Biosphere Reserve is included there also. In this Reserve the Roumelian pine is located between 1,800 and 2,200 m on a total of 259 ha.

The Rila formations that are most representative of Pinus peuce include 5 reserves: Skakavitsa, Skakavets, Ibar, Marichini ezera and Parangalitsa. As most of the Roumelian pine stands in the Rila Mountains are parts of erosion-controlling and recreational forests, watersheds, etc., their gene pool has been relatively well conserved.

The greatest part (159 ha) of the unique P. peuce formation in the Central Balkan Range is in the Tsarichina Biosphere Reserve.

The national parks and reserves in Bulgaria involve 5,249 ha of P. peuce stands which conserve the heterozygosis of this species, as well as possibilities for its evolutionary development. Roumelian pine genetic resources are also being preserved in another 108 seed stands with a total of 626 ha. Additionally, 152 select candidate-elite trees of this species, and progenies of these trees growing on three different ecological sites, provide a supplemental reserve stock to aid in the conservation of the gene pool.

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GENETIC CHARACTERIZATION OF FIVE WHITE PINE SPECIES  
AND THEIR INTERSPECIFIC HYBRIDS BY ISOZYMES

Dr. Ebby Chagala

ABSTRACT

The possibility of using isozymes for identifying five white pine species, Pinus strobus, P. monticola, P. griffithii, P. peuce, P. koraiensis, and their interspecific hybrids, was investigated by starch gel electrophoresis of enzymes using mature needle tissue. It was demonstrated that each species and their interspecific hybrids could be uniquely identified based on their allozyme genotypes. The most useful alleles were for loci SDH1, MDH2, 6-PGD1, 6-PGD2, PER1 and PER2.

Key words: White pines, electrophoresis, isozymes, genetic markers

Introduction

Gel electrophoresis of enzymes has proven to be very useful in forest tree species for numerous studies (Feret and bergman, 1976; Falkenhagen, 1985; Cheliak et al., 1987). Such studies have included the use of isozymes as genetic markers for species and interspecific hybrid identification (Adams and Cotinho, 1977; Tobolski and Conkle, 1977; Joly and Adams, 1983; Viquez-Lopez, 1988). The use of isozymes as diagnostic characters provides information for a better utilization of species and their interspecific hybrids. Such knowledge is important for the success of breeding programmes, and is needed in white pines.

White pines are among the most important timber species in the world. There are more than twenty white pine species, and the eastern white pine (Pinus strobus Loud.) is the most widely planted and studied. However, other species such as western white pine (P. monticola Dougl.), Himalayan white pine (P. griffithii McClell.), Balkan pine (P. peuce Gries.) and Korean pine (P. koraiensis Sieb. & Zucc.) constitute an important and broad range of material for the white pine breeding programme. These species show varying resistance to diseases such as white pine blister rust (Cronartium ribicola J.C.), and some are also used for interspecific hybridization. There have been problems of species and interspecific hybrid identification in white pines using morphological characters, but there have been no reports on the use of isozymes as genetic markers for this purpose.

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This paper reports on identification of five white pine species, P. strobus, P. monticola, P. griffithii, P. peuce, P. koraiensis, and their interspecific hybrids by unique allozymes.

#### Materials and Methods

Five white pine species: Pinus strobus, P. monticola, P. griffithii, P. peuce and P. koraiensis, interspecific hybrids P. strobus x griffithii, P. strobus x monticola, P. strobus x peuce and P. monticola x peuce and their reciprocal crosses were studied using needle tissue. Clones and families used were collections established in arboreta of the Ontario Ministry of Natural Resources at Maple, Midhurst and Melancthon. As these collections are from various parts of the species range, samples were selected such that they represented different sources of each species. All clones, families and interspecific hybrids available in these collections were included in the study. A total of 129, 19, 30, 57 and 24 individuals of P. strobus, P. monticola, P. griffithii, P. peuce and P. koraiensis respectively were used. Details of the origins of the material are reported elsewhere (Chagala, 1990).

Needle samples of the previous year's growth were collected in January and September, 1987 and also January, 1988. Immediately after collection, the samples were placed in polythene bags and into a cooler with ice. These were then transported to the laboratory for extraction and storage. Sample extraction was either done the same day or after overnight storage at  $-5^{\circ}\text{C}$ .

Sample preparation was according to Mitton et al. (1979) with some minor modifications. About 100 grams of needle tissue was cut into small pieces and placed in a mortar. Liquid nitrogen was then added and the contents were ground to a fine powder with a pestle. Extraction buffer (Buchert, personal communication) which had been kept cold in an ice bath was added (2.5 ml), and the contents were swirled with a pestle until they became a paste. The paste was then poured into 1.5 ml microcentrifuge tubes and immediately placed in ground ice until all the grinding was completed. The microcentrifuge tubes containing the extract were then stored at  $-70^{\circ}\text{C}$  until needed for electrophoresis.

The extracts were subjected to horizontal starch gel electrophoresis. Electrophoretic equipment was described by Rajora (1986). The amount of starch and composition of gel and electrode buffers for the four buffer systems designated as B, P, I and M were as those described by Cardy et al. (1980), Clayton and Tretiak (1972), Selander et al. (1971) and Shaw and Prasad (1970) respectively. Electrophoresis for all buffer systems was run at a constant current (75 mA for buffer systems B, P and M and 85 mA for I) until the buffer front reached a distance of 7.5 cm (for buffer systems P, M and I) or 11.5 cm (for buffer system B).

The eight enzyme systems assayed and found to give clear banding patterns were: adenylate kinase (AK), glutamate oxaloacetate transaminase (GOT), isocitrate

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dehydrogenase (IDH), malate dehydrogenase (MDH), peroxidase (PER), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SDH). These enzymes were assayed on buffer systems as follows: AK, IDH, MDH and 6-PGD - P; GOT - I; PER - B; PGM and SDH - M. The stains for AK and GOT were modified from Dancik (1986) and Siciliano and Shaw (1976), respectively, while those for IDH, MDH, PER, 6-PGD and PGM were as described by Rajora (1986) and for SDH by Siciliano and Shaw (1976). When multiple loci were encoded in an enzyme, the fastest anodally migrating zone was designated as locus 1 and the most anodal allele at that locus as allele A. The numbering of alleles and additional loci within an enzyme system progressed in the cathodal direction.

## Results

Banding patterns and inheritance of isozymes are reported elsewhere (Chagala, 1990). Figure 1 showed that for the enzyme SDH, *P. monticola* and *P. peuce* could be identified from the other three species by the unique allele E. The other enzyme systems analyzed could also be used for identifying different species except IDH which was monomorphic in all the species. Alleles useful for identification purposes in each species are summarized in Table 2. These alleles could be used for identification purposes to different degrees depending on their frequencies which varied among species from very high to very low or zero.

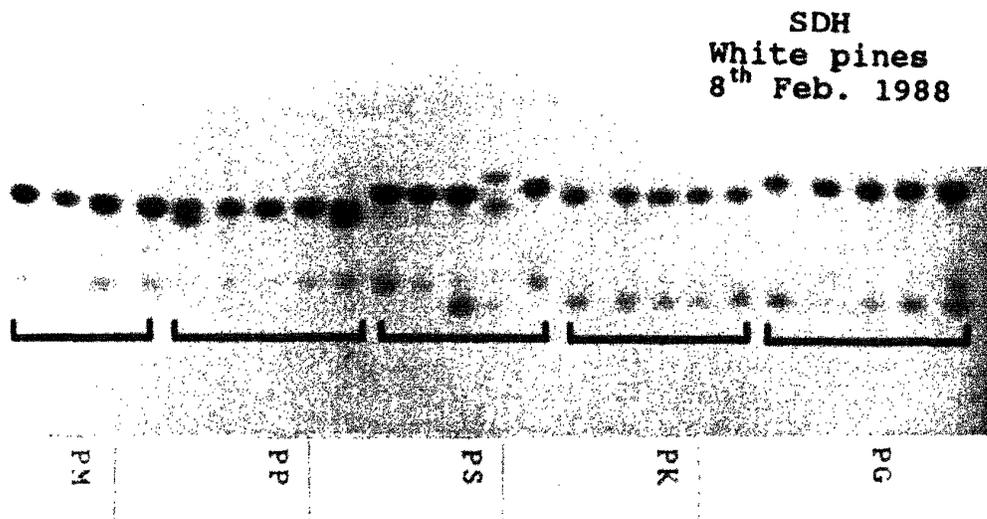


Figure 1. Species identification using SDH for *P. monticola* (PM), *P. peuce* (PP), *P. strobus* (PS), *P. koraiensis* (PK) and *P. griffithii* (PG).

From Table 1, we can conclude that there was no single enzyme which could be used for the identification of all species in this study. However, each enzyme could be used for identifying one or two species. For example, 6-PGD could be used for identifying P. peuce and P. griffithii. On the other hand, P. strobilus could be identified using a single allele PGM1-C, and P. peuce by three different alleles, MDH2-D, 6-PGD2-B and PER1-I. These alleles either occurred at a very low frequency or were absent in the other species.

Table 1. Isozyme loci and sets of alleles which were found most suitable for identifying P. strobilus (PS), P. monticola (PM), P. griffithii (PG), P. peuce (PP), P. koraiensis (PK) and their interspecific hybrids.

Locus	Species				
	PS	PM	PG	PP	PK
PGM1	C	B	A	B	A
SDH1	D	E	D	E	D
SDH2	C	B	C	B	C
AK	B	B	B	C	C
MDH2	A	C	B	D	B
MDH3	C	C	C	C	A
6-PGD1	B	B	B	A	B
6-PGD2	A	A	A	B	A
PER1	D	A	H	I	D
PER2	D	B	D	C	E
PER3	C	C	C	A	A
GOT2	B	B	B	A	B

The results also showed that the enzyme systems analyzed could be used subsequently to identify the different combinations of interspecific hybrids among these species. Figure 2 shows some interspecific hybrid combinations that were used including reciprocal crosses. These results indicated that hybrids could be easily identified using unique alleles observed in the parent species. The results also indicated that hybrids from reciprocal crosses could be similarly identified. Generally, as summarized in Table 1, the alleles most useful for identifying all the species and their interspecific hybrids are for loci PGM1, SDH1, MDH2, 6-PGD1, 6-PGD2, PER1 and PER2.

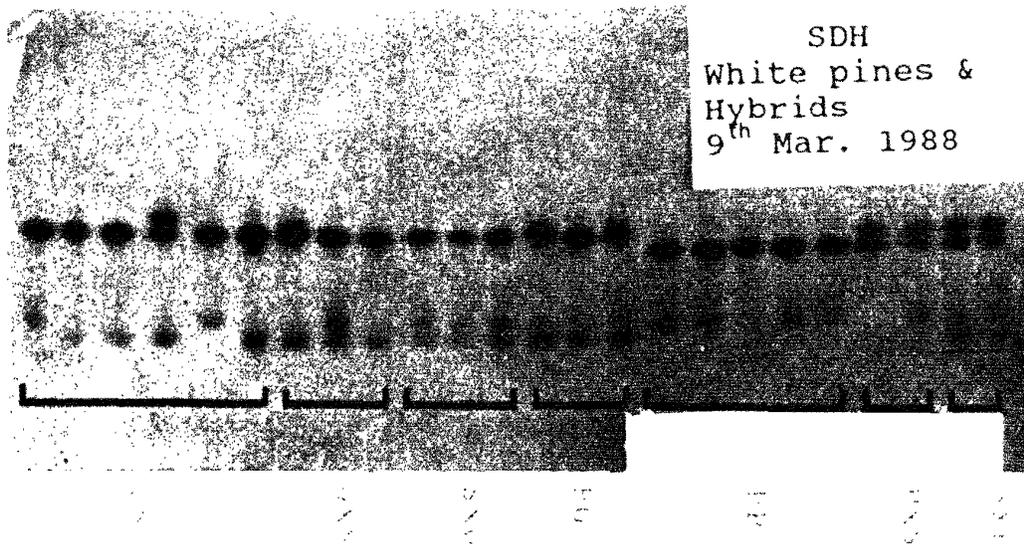


Figure 2. Species and hybrid identification using SDH for P. strobus (PS), P. griffithii X P. strobus (GXS), P. strobus X P. griffithii (SXG), P. griffithii (PG), P. peuce (PP), P. peuce X P. strobus (PXS) and P. strobus X P. peuce (SXP).

#### Discussion

Banding patterns for the five white pine species had the same phenotypic features such as the number of loci, the relative mobilities of the bands and the degree of activity. As such, differences in loci could not be used for identification purposes. Similar observations have also been reported in other conifer species (Adams and Cotinho, 1977; Conkle, 1979; Dancik and Yeh, 1983; Yeh and Arnott, 1986). However, there were substantial differences in allele frequencies at some loci indicating high differentiation of alleles among these species. The differences in allele frequencies were found useful for identification of these species and their interspecific hybrids. Such results confirm the usefulness of isozyme analysis as a diagnostic tool.

Apart from MDH1 and IDH which were monomorphic in all the species, unique alleles at all the other isozyme loci could be used for identifying the species and interspecific hybrids studied. For example, P. strobus could be identified from all the other species using PGM1-C while P. peuce could be identified by either using MDH2-D, 6-PGD2-B or PER1-I. In all cases, hybrids, including those from reciprocal crosses, could be easily identified using the characteristic banding patterns of each species without prior knowledge of the

parental species identity. However, in those cases where the alleles occurred in other white pines, once the parental genotype was known, the hybrid could also be identified. The most useful alleles were for loci PGM1, SDH1, MDH2, 6-PGD1, 6-PGD2, PER1 and PER2.

The establishment of unique gene markers for the different white pine species and their interspecific hybrids will greatly facilitate the certification of control crosses. The demonstration of the use of isozymes to identify such closely related species in the present study also confirms their use as a tool in systematic studies. As the classification of the genus Pinus is still uncertain (Critchfield and Little, 1966; Mirov, 1967), the value of isozymes as a taxonomic tool has therefore to be emphasized.

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USE OF SOMACLONAL VARIATION TECHNIQUES TO PRODUCE  
RUST RESISTANT WHITE PINE

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SUMMARY

Tissue culture techniques are being used in an attempt to recover somatic variation in eastern white pine embryo cultures. Plantlets with increased resistance to Cronartium ribicola as determined by in vitro inoculation with basidiospores will be multiplied clonally in vitro. Some of these plantlets will be rooted, and the seedlings will be screened for resistance in the greenhouse. Resistant seedlings will be clonally propagated for field evaluation.

Key words: Blister rust, biotechnology, forest diseases

Introduction

Eastern white pine (Pinus strobus L.) was once the most valuable forest tree in the Lake States region of the United States. In 1889 more than 16.5 billion m<sup>3</sup> of white pine lumber were cut in Michigan, Wisconsin and Minnesota (Betts, 1954). During the next 20 years production dropped in half, and by 1932 only 467 million m<sup>3</sup> were cut nation-wide. Annual production through the 1950's averaged about 1.7 billion m<sup>3</sup>. Today white pine plays only a minor role in the forest industry because of the lack of available growing stock. One reason this species is not being planted in greater amounts is white pine blister rust caused by Cronartium ribicola A. Fischer (Garrett, 1985). Since it first appeared in the Lake States region around 1913, this fungus has been a major cause of mortality of eastern white pine throughout the region.

Efforts to find eastern white pine with resistance to blister rust began in 1937. Trees with apparent resistance were selected from stands severely affected by rust. Open-pollinated seedlings and grafts from these select trees were exposed to the rust fungus. Although a high percentage of seedlings became infected with stem cankers, only a few of the grafted trees developed cankers (Riker et al., 1943). Work has continued on the production of rust resistant trees since Riker's early work, and seed orchards have been developed from many of the grafts of trees with putative resistance (Patton and Riker, 1966; Ahlgren, 1955; Heimbürger, 1962). Only a few of these trees however, have shown the ability to confer rust resistance to their progeny at rates needed for production of rust resistant forest stands (Patton and Riker, 1966). Thus, after 50 years we still do not have a proven, practical system that will produce large numbers of rust resistant seedlings for field planting. This problem remains one of the most critical obstacles to restoring eastern white pine as a major component of our Lake States forests.

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Our research group is investigating a different approach to solving the blister rust problem by using our tissue culture and pathology experience. The Forest Disease Project at the North Central Forest Experiment Station has a mission to develop more rapid systems for imparting disease resistance in forest trees. This program, which started about five years ago, uses the phenomenon of somaclonal variation to obtain genetic change without going through the long process of traditional sexual breeding. Somaclonal variation is the variation exhibited by plants grown in aseptic culture. Although the causes of somaclonal variation are not completely understood, the phenotypic and genetic variation found in various plant crops indicates that many factors are involved. These include minor point mutations, polyploidy, transposable elements, aneuploidy, and epigenetic changes. Somaclonal variation can be pre-existing genetic variation that is expressed in regenerated plants, or it can be induced by the tissue culture process itself (Scowcroft and Larkin, 1983).

We selected hybrid poplar for our initial research on somaclonal variation in a forest tree. Our objective was to produce resistance in these trees to Septoria canker caused by the fungus Septoria musiva Peck. Callus cultures of susceptible clones were initiated from stem internode explants taken from greenhouse plants. Callus cultures were maintained for 5 to 13 months before shoot proliferation was induced. Elongated shoots were excised and rooted before they were placed in the greenhouse. New, fully expanded leaves were collected from these plants and inoculated with conidia of S. musiva on the abaxial surface. Plants with increased resistance to Septoria were recovered from two of the three previously susceptible clones. Many regenerants were significantly more resistant to Septoria canker than their source plants (Ostry and Skilling, 1988). These putative plants have also been tested for field resistance for four years. Many of these plants continue to show strong resistance to Septoria canker. The best selections are now being asexually propagated for more extensive field evaluation.

Our research group has also used tissue culture of European larch (Larix decidua Mill.) to produce trees with resistance to Scleroderris canker caused by the fungus Gremmeniella abietina (Lagerb.) Morelet. In this model system we use the cotyledon culture technique to produce somatic variants with resistance to G. abietina (Diner et al., 1986). The plantlets are inoculated in vitro with conidia of G. abietina. Those plantlets that survive three inoculations are multiplied both in vitro and ex vitro by means of axillary buds to produce large clonal lines of clones with putative resistance. At this time we have 100 clonal lines that are being multiplied for field testing.

We have recently developed a tissue culture system for screening European larch from different seed sources for resistance to Mycosphaerella laricina Hartig. By inoculating larch plantlets in vitro with the needlecast fungus, we have determined the relative susceptibility of larch seed lots within 6 months of seed germination. The results from these tests correlated very strongly with field evaluations of the same seed lots. The field evaluations, however, required 4 years to complete.

Using the knowledge we have acquired with Populus and Larix, we have recently started working on a system for producing blister rust resistant white pine.

We are using an embryo culture technique with seed from putative resistant trees and from non-tested white pine from seed orchards. Currently we are producing large numbers of white pine plantlets from these seed sources. If our rates of somatic variation are similar to those we found in European larch, we should find 1 to 2 percent of these plantlets with increased resistance to blister rust. To identify these resistant plantlets, we must challenge them with the rust fungus. Standard procedures are to inoculate seedlings with rust basidiospores and to wait several months for symptom development to determine if the plant is resistant to the rust fungus. During this time any putatively resistant plants are losing their capacity to be regenerated asexually. To maintain the plants in a regenerative state, we are inoculating with basidiospores while the plantlets are in tissue culture. Some preliminary work on this type of system has been done by other investigators. Harvey and Grasham (1969) grew cambial explants of western white pine (Pinus monticola Dougl.) infected with C. ribicola. This was callus already infected with the rust and was not intended to serve as a challenge system. Diner et al. (1984) used callus from lines of Pinus lambertiana Dougl. that were both resistant and susceptible to C. ribicola in their challenge system. They observed a hypersensitive reaction on the resistant callus. Diner and Mott (1985) also inoculated plantlets of western white pine in vitro with vegetative hyphae of C. ribicola using intact needles and shoot apex wounds. They observed intercellular haustoria, characteristic of infection by basidiospores. They were not, however, attempting to determine resistance of the individual plantlets.

Our inoculation system, with basidiospores applied directly to the intact plantlets, is closer to a natural system. However, because of the succulent needle epidermis of in vitro plantlets, we may be bypassing a resistance mechanism present in the epidermis. Thus our challenge system is conservative and we may not recognize an existing resistance mechanism in the epidermis. Based on other systems we have studied, we expect the in vitro basidiospore inoculation technique will produce visible infection symptoms in less time than ex vitro systems. Our in vitro Scleroderma inoculation system with European larch produced ratable results in 60 days versus 12 months under field conditions. Our basidiospore inoculations consist of multiple isolates of C. ribicola to avoid the possible problem of the existence of races of the fungus. Those plantlets with putative resistance will be subdivided in culture. A portion of each genotype will be screened under greenhouse conditions to verify their resistance before field evaluation. Subportions of these same genotypes will remain in tissue culture to supply the plants needed to develop clonal lines of the more promising material. Our strategy will be to develop rapid in vitro propagation methods that will retain clonal integrity and to use these techniques to produce large quantities of plants with resistance to C. ribicola.

The potential advantages of this system are both speed and the ability to produce large numbers of resistant genotypes. If the white pine project follows the pattern of the Populus and Larix studies, we should have our first rust resistant white pine plantlets this year. This in itself is not a great advance over existing grafted seed orchard material. However, if we can produce

large quantities of resistant trees from many genotypes through the tissue culture process, we will have made a major advance on the problem that has resisted solution by forest tree breeders for the past 50 years. For the first time we might be able to establish multiclonal plantations of white pine with increased resistance to C. ribicola.

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## IMPROVEMENT OF PINUS MONTICOLA FOR BRITISH COLUMBIA, CANADA

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### SUMMARY

The joint program of Forestry Canada and the British Columbia Ministry of Forests to produce western white pine seedlings resistant to white pine blister rust is described and progress to 1990-01 is reported. 313 parents have been selected for testing for a coastal seed orchard and 167 more are selected for an interior-zone orchard. Screening against rust has begun for 98 of these parents, using open-pollinated seeds collected in situ. Seed orchards should be established between 1995 and 1997 for coastal and interior zones, respectively. Orchards will consist mainly of seedlings selected for specific rust defenses from more-resistant parents. Support research, to refine testing or advance delivery of well-adapted, rust-resistant, planting stock, is described.

### Introduction

Western white pine, Pinus monticola D. Don., is native to British Columbia in two large, disjunct areas: one lying mainly west of the Coast Mountain range summit to latitude 50° 40'N, and the other lying along the western slopes of the Monashee-Cariboo Mountain ranges and east to the Rocky Mountain trench to latitude 53°N. These areas are commonly called the south-coastal, and the interior wet-belt, zones (hereafter called "coastal" and "interior" zones).

Early exploitation of western white pines by the white settlers was heavy; the species grew to 60 m tall and up to one m stump diameter; it could be used for high-quality finish material such as doors, window sashes and furniture. This was reflected in its high stumpage value, which often was some multiples of those of its associated species. A strong price difference still exists in the interior zone.

White pine blister rust (Cronartium ribicola J.C. Fisch.) was introduced to the west on infected seedlings from France about 1910 (Boyce, 1948) and spread rapidly through both regions, causing severe damage or mortality in existing coastal stands by the late 1920s and to interior stands by the mid-1930s. Regeneration of white pine into openings caused by logging or fire usually was prolific, but the rust killed or weakened most of it, so that its future as a commercially valuable species seemed threatened by the mid-1940s. Canada duplicated United States efforts to control the rust silviculturally starting in the 1930s, then began a program of testing promising individuals for genetic

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resistance in the late 1940s (Porter, 1960). This program was abandoned in 1960 when the scientist in charge resigned.

The pressure to intensify forest management on a shrinking land base and the recognition that western white pine seemed more resistant than its coniferous associates to some of the common root diseases, particularly Phellinus weirii (Murr.) Gilb., and the success of the programs to produce genetically-resistant seedlings in the U.S. (Bingham, 1983), have led to increasing attempts to establish such plantations or to manage natural stands for white pine. The estimated annual requirements of rust-resistant seedlings is approximately five million, although the target keeps rising as the area delineated by root rots increases.

In 1983, a Memorandum of Understanding was signed between the Canadian Forest Service (now Forestry Canada) and the British Columbia Ministry of Forests for the development of western white pine with increased resistance to blister rust. This paper describes the aims and goals of our program, progress to date and describes some of the support research, or that being pursued as possible alternatives to conventional, proven methods. Fuller details are presented by Hunt (1988).

#### Experimental Methods

Base Populations -- A goal of 300 parent trees per zone (coastal and interior) meeting our criteria: rust-free in attacked stands 20-60 years old, cone-bearing, of acceptable stem form and growth relative to neighbours, was set on the assumption that one tree in six might provide useful, genetically-based, resistance and that 50 widely separated genotypes would be sufficient to provide broad genetical variability for other adaptive traits.

Rust Sources and Culture -- Aeciospores are collected from several locations each spring, inoculated onto both a cultivated Ribes garden containing clones of several species, and natural groves. The infections are monitored and intensified during the uredial stages by wafting spores from infected to "clean" plants. Once the telial-horn stage is reached, usually in early September, infected leaves are picked from several sources and placed on screens of open mesh approximately 50 cm above the seedling tops in a controlled-environment room for spore casting and inoculation.

Rust Inoculation -- Open-pollinated seeds are collected from the specific trees during good cone crops, randomised at the start of their second year into 9-tree rowplots in 45-cell "container" blocks (5 families per block) and exposed to rust under cool (12-16°C) humid conditions. Fine droplets of distilled water are applied to the seedlings by portable electric misters. When spore cast reaches about 3000 per cm<sup>2</sup>, the stock is moved away from the rust source and kept in similar temperature, humidity and moisture conditions until spore germination occurs. Light, to open stomata, is supplied during spore germination and penetration. Following that, stock is returned to a nursery until examination the following spring.

Selection of Seed-Orchard Stock -- Our process follows that developed in Idaho and Oregon: Seedlings are to be selected for specific defense mechanisms (Table 1) from families displaying reduced rust susceptibility. Stock is examined up to five times after inoculation, at 8, 12 to 18, 24, 36 and 48 months. Spot numbers per seedling are recorded during the first two inspections, whereas rust development, or absence from previously-attacked seedlings, is assessed from the second through fifth inspection. Seedlings selected at the end of the process will be 6 years old; they will have been maintained in transplant beds since the start of their fourth year, so will be easy to move to the seed orchard. These selections may be augmented by scions collected from outstanding individuals containing known resistance mechanisms from the Idaho and Oregon programs. Seed orchards are the responsibility of the B.C. Ministry of Forests. We anticipate that the first seed orchard, for the coastal zone, will be established in 1995 and that for the interior will be planted about 2 years later. Useable seed crops should be obtainable approximately 6 years after establishment of each orchard.

Table 1. Proposed features used to select families and seedlings for a rust-resistant seed orchard of Pinus monticola.

Hypothesized Resistance Type <sup>1</sup>	Selection Unit	Resistance Mechanism	Resistance "Line"
Multi-genic ("Horizontal")	Family	Reduced needle spots/m foliage	All
"	Family	Slow canker growth ("stem tolerance")	A
Single-gene ("Vertical")	Seedling	Early shedding of rust-spotted leaves	B
"	Seedling	Fungicidal short shoot reaction	C
"	Seedling	Rust halted via bark reaction	D

<sup>1</sup> Hoff, R.J, G.I. McDonald, 1980. Improving rust-resistant strains of inland western white pine. USDA, For. Serv., Research Paper INT-245, 13 p.

Support Research -- Studies are in progress to determine genetic variability in white pine at the regional (coastal vs. interior), provenance-in-region and individual levels, and second-year variability, by assessing seed (Meagher and El-Kassaby, this conference) nursery and field performance, as well as protein profiles and DNA variability using restriction-fragment-length polymorphisms (RFLP) (White, 1989a, 1989b). Narrow and wide crossing is underway to test the

inferences of low genetic variability among provenances or zones detected by Steinhoff et al. (1983) and Rehfeldt et al. (1984). Other studies are underway to assess genetic variation in the rust, using both RFLPs and inoculations of separate B.C. rust sources on common seedlots: a "rust races" test (Meagher, 1990). The impact of root diseases on white pine provenances and competing species is under test on nine sites, while six more sites test common seed sources from Canada and the U.S. against the "local" rust and environment.

Other studies are focused on delivering genetically-improved stock. Drs. Stephen Ross and Joe Webber, B.C. Ministry of Forests, Victoria, are working to enhance early and consistent cone production (both seed and pollen) so that seed orchards will meet their targets regularly and economically. Cone and seed insects are being studied by Dr. Gordon Miller, Forestry Canada, Victoria. Dr. Martin Lapp, Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, is developing techniques for the mass propagation of selected seedlings; success would permit delivery of planting stock containing a specified balance of defense mechanisms appropriate to each forest site.

#### PROGRESS TO 1990-01

Base Population, Rust Inoculation and Selection of Seed-Orchard Stock -- Selection of parents is nearly complete for the coast (313 trees), including 15 clones from Washington-Oregon, but is only half complete for the interior (167 trees) including 22 clones from Idaho. Seeds of 135 coastal and 61 interior parents have been sown; 98 families (18,646 seedlings) have been inoculated with rust in three years, starting in 1987, 1705 seedlings have been evaluated at 48 months and 407 of these are potential selections for the coastal seed orchard. 6166 seedlings will be examined for rust in the spring of 1990 and 6750 more were exposed to rust in 1989; they will be examined first in May of this year. Sowing in 1990 is to be reduced because of poor seed crops the last two years, but indications of a good cone crop this year are widespread.

Support Research -- Assessment of plantations of Idaho seed source on coastal lands (e.g. Bower, 1987) support Rehfeldt et al. (1984) contention that western white pine is broadly adapted, but heavier spring frost damage was suffered by Idaho vs. local trees at one site in the southern interior (Meagher, 1988) and a strong latitudinal trend in frost damage with seed source was found at another site (Hunt, unpub. data). A study of the effects of controlled freezing during the autumn on range-wide seed collections is under way at the University of British Columbia, Vancouver, to test for differences in timing and depth of fall frost resistance. Variability in white pine's chloroplast genome is reported by White (1989a, 1989b).

Preliminary analysis of early results from the "rust races" test indicate that interior parents, even those screened in Idaho for rust resistance, are more susceptible to the coastal rust sources than are coastal parents (Meagher, 1990). Assessment of rust impact on our field sites will start about 1992, prior to establishment of the first seed orchard. Variability in blister rust in British Columbia also is being studied by White, using RFLP techniques.

Protein chemistry of white pine is being studied by Dr. Ekramoddoullah (this conference) using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques, following modification of protein-extraction procedures to minimise the amount of interfering substances.

Some plantlets produced by Dr. Lapp (Lapp and Malinek, 1988) from single embryos have been planted in a root-diseased demonstration area and others in an area free of root disease. More are expected every year. Grafts have been made from individuals selected for disease resistance by collaborators in Idaho and Oregon. Depending on results from the various tests of white pine and rust variability, these may be incorporated into the seed orchards, or held for other studies.

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#### VARIATION IN THE CHLOROPLAST GENOME OF PINUS MONTICOLA

Dr. E.E. White

#### SUMMARY

The chloroplast genome of Pinus monticola was physically mapped. The genome lacks a large inverted repeat, and dispersed repeated sequences occur. These two structural features might be expected to confer a degree of variability on the genome that would prove useful in population studies. Restriction site variation in the genome of coastal and interior collections was surveyed for evidence of differentiation between geographically disjunct populations. The same variants occurred in both areas; however, the frequency of a variant genome was significantly higher in the interior than in the coastal collections. The results indicate a difference in the pollen cloud of the two parts of the range. Chloroplast DNA is predominantly paternally inherited in P. monticola, though the presence of heteroplasmic individuals indicates occasional bi-parental inheritance (White, 1989a, 1989b).

Dr. E.E. White is a research scientist at the Pacific Forestry Centre, Victoria, British Columbia, Canada.

Current studies on DNA variation in Cronartium ribicola and identification of non-host resistance genes in P. monticola using differential screening of cDNA libraries will be discussed.

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### GENETIC VARIABILITY OF SOME TRAITS IN TWO PINUS CEMBRA NATURAL POPULATIONS

Dr. I. Blada

#### SUMMARY

Twenty six trees randomly selected in each of two genetically isolated Carpathian Pinus cembra L. natural populations were multiplied by grafting. After artificial inoculations with the blister rust Cronartium ribicola Fisch.ex Rabenh. the clones were planted in two parallel trials in one location. Each trial consisted of 26 clones placed in a randomized complete-block design; each clone represented by a 2-graft plot in each of 9 blocks. At age 11, the following results were obtained: (1) Highly significant differences among clones in blister-rust resistance and in growth traits, (2) The Calimani population was the best in growth traits at this lower elevation, (3) The genotypic source of variation, for the most important traits, ranged from 63 to 90% of total phenotypic variation, (4) Highly significant genetic correlations were found between the most important economical traits, and (5) The high heritability estimate could yield an important genotypic gain for both blister-rust resistance and for growth traits.

Key words: Pinus cembra, Cronartium ribicola, genotypic variance, heritability, genotypic gain, growth traits, resistance

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## Introduction

The stone pine (Pinus cembra L.) distribution area is restricted to the colder climates and higher altitudes of the Alps and Carpathian Mountains. Stone pine is especially important for reforestation and afforestation purposes at high elevations to raise the timber line above existing levels to stabilize the soil which would prevent avalanches and reduce torrential flooding (Holzer, 1958). The role of forest genetics is to provide material suitable for these purposes through well designed provenance and race studies (Holzer, 1963).

The within species variability of this tree was not well understood although Rikli (1909) and Holzer (1969, 1970) both mentioned a phenotypical variability in crown shape and stem form. In two trials with clones and half-sib progenies, Holzer (1969, 1975) found a good correlation between height growth and the elevation of the seed source. At low altitudes the provenances from the lowest elevations grew best and the progenies of trees selected near timber line grew better at the higher elevations.

A trial with P. cembra provenances from both the Alps and Carpathians showed that the former produced the best height growth at age 5 (Blada, 1987).

It is well known that P. cembra exhibits a high degree of resistance to blister rust under artificial inoculation (Hoff et al., 1980; Delatour and Byrot, 1980; Stephan, 1986; Blada, 1987). It is probable that interspecific hybridization of P. cembra with other white pines will yield increased resistance to Cronartium ribicola; but there is still some question about how such hybrids can be produced (Holzer, 1975). Recently hybrids were obtained between P. cembra x P. monticola Dougl. and P. cembra x P. wallichiana Jacks. (Blada, 1987). At age 10 these hybrids demonstrated good blister-rust resistance and good height growth (Blada, in preparation).

The objective of this investigation was to estimate the amount of genotypic variability for several traits in two stone pine natural populations.

## Materials and Methods

Parent populations and parent trees -- Two presumably isolated populations of stone pine were selected for this study. These populations, Calimani and Rodnei, are distributed in the northern part of the eastern Carpathians at 1450 and 1650 m altitude, respectively. The 26 trees, randomly selected in each population, were multiplied by grafting on P. strobus L. root stocks in the spring of 1979. The root stocks were in individual polyethylene pots that could be arranged in the desired experimental design during the inoculation period.

Inoculation with Cronartium ribicola -- Artificial inoculation took place between September 1-5, 1979, in a tent constructed according to Bingham's (1972) recommendations. The inoculum was obtained from heavily infected leaves of Ribes nigrum L. harvested from a single plantation. Other inoculation details were similar to those described by Patton and Riker (1966) and Bingham (1972).

Infestation with Pineus cembrae Chd. -- No artificial infestation was necessary because the grafted plants were spontaneously attacked by this woolly aphid that arrived with the parent populations and the grafts. Over the length of the testing period the attacks became heavier and heavier until the plants were suitable for resistance assessment.

Experimental design -- During the inoculation period the 26 clones from Calimani and the 26 from Rodnei were arranged in the tent in a randomized complete-block design; each clone represented by a 2-grafted seedling plot in each of 9 blocks. In order to test the inoculum efficiency, a mix of 4-year-old P. strobus open-pollinated progeny was used as the control. After inoculation, the clones were planted out on a spacing of 2.5 x 2.5 m, in two parallel trials placed in the same location, at about 350 m elevation, and using the same experimental design used in the inoculation tent.

Measurements -- All traits were evaluated at the end of the eleventh growing season, i.e., in October 1989. Cronartium ribicola resistance ( $R_1$ ) was assessed on a 1 to 10 scale where 1 = tree dead or total susceptibility, and 10 = no stem lesions or total resistance. This scale takes into consideration the magnitude of the stem un-open and without aecial stage lesions, but with the fungus present in the stem. In our opinion, this type of resistance could be attributable to two mechanisms such as "bark reactions" and "alive and cankered" (Hoff et al., 1980).

Pineus cembrae resistance ( $R_2$ ) was assessed according to a 1 to 10 scale where 1 = 91-100% and 10 = 0-10% covered stem and branches with woolly wax deposits secreted by the hiemal larva.

Quantative traits such as annual height growth in 1989 (Ha), total height growth (Ht), diameter at 1/2 Ht (D) and crown diameter (CD) were measured in decimeters. By using Ht and D, basal area (BA) and stem volume (V) were calculated as follows:  $(BA) = 3.14 D^2/4$  and  $V = (BA) \times Ht$ .

Quality characteristics were evaluated using subjective scores. Stem straightness (SS) was scored on a 1 (worst) to 3 (best) scale. Stem numbers (SN) scores ranged from 1 to 3, where 1 = three or more stems; 2 = two stems; and 3 = one stem. Branch number/whorl (BW) was established by dividing by 3 the total number of branches counted at 3 whorls. Branch diameter scores ranged from 1 to 3 (thin to thick).

Statistical analysis - - A fix model was used. The formula for this model is from Snedecor and Cochran (1976):

$$X_{ij} = m + C_i + B_j + e_{ij}$$

where: m = the general mean;  $C_i$  = the effect of the i-th clone;  $B_j$  = the effect of the j-th block;  $e_{ij}$  = the random error.

The model for variance analysis and the formula for estimating variance components are given in Table 1.

Table 1. Model for analysis of variance

Source of Variation	Df	MS	E(MS)
Blocks	b-1	MS <sub>B</sub>	$\sigma_e^2 + c\sigma_B^2$
Clones	c-1	MS <sub>C</sub>	$\sigma_e^2 + b\sigma_C^2$
Error	(b-1)(c-1)	MS <sub>E</sub>	$\sigma_e^2$

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$$\sigma_C^2 = MS_C - MS_E/b; \sigma_e^2 = MS_E/b; \sigma_P^2 = \sigma_C^2 + \sigma_e^2; \sigma_P^2 = \sigma_P^2$$

where: b = blocks; c = clones;  $\sigma_C^2 = \sigma_e^2$ ;  $\sigma_P^2 = \sigma_C^2 + \sigma_e^2$ ;  $\sigma_P$  = clones, error and phenotypic variance, respectively;  $\sigma_P$  = phenotypic standard deviation.

The broad-sense heritability ( $H^2$ ) and the genotypic gain ( $\Delta G$ ) were calculated according to the formula:

$$H^2 = \sigma_C^2 / (\sigma_C^2 + \sigma_e^2) \text{ (Nanson, 1970); } \Delta G = iH^2\sigma_P \text{ (Falconer, 1960),}$$

where i = selection differential.

## Results

Genetic variability and variance components -- In both Pinus cembra clonal populations mean squares were highly significant ( $p < 0.001$ ) for all traits except stem forking (Table 2, col. 4 and 6); that is, among the individuals of each base population there were striking differences. Consequently there are large possibilities of selection at the individual tree level.

Duncan's Test shows the ranking of the clones according to their blister-rust resistance and diameter growth as well as the synthetic index of selection. Diameter growth appeared a more heterogeneous trait than blister-rust resistance (Tables 3 and 4).

Clone mean values for all traits/population are given in Table 5. It is apparent that the Calimani clonal population was the best in growth traits such as annual and total growth in height, diameter, basal area at 1/2 height and stem volume growth rate. For example, the stem volume growth is 112% higher in Calimani than in Rodnei populations, but the other traits, i.e., crown diameter, branch number/whorl and branch diameter, indicate a lower qualitative value in the Calimani than in the Rodnei population (Table 5, col. 4). The tree improvement person has to choose between the two populations according to the improvement objectives.

The P. cembra blister-rust resistance was very high (above 9) while resistance in P. strobus control stocks was very small (mean 2.8) (Table 5).

Variance components -- The variance analysis of the data for both clonal populations has yielded the variance components given in Table 6. The genotypic source of variation that contains all genetic effects, estimated by clonal variance, ranged from 70 to 90% and from 63 to 88% of total phenotypic variance for all traits except stem forking in the Calimani and Rodnei populations, respectively (Table 6). These data suggest that genotypic variance is very important for most traits and a breeding strategy based on this variance could be adopted.

Correlations -- For the Calimani populations, the genotypic and phenotypic correlations computed from estimated components of variance and covariance are presented in Table 7. Highly significant ( $p < 0.001$ ) positive genotypic correlations were found between blister-rust resistance and the other tested traits, except Pineus cembrae resistance and stem forking (Table 7, row 1). Growth traits, i.e., annual and total diameter growth, basal area and stem volume, were positively and strongly correlated genetically with some qualitative traits, i.e., crown diameter, branches/whorl, stem straightness and branch diameter. Other details concerning genotypic and phenotypic correlations in both the Calimani and Rodnei populations are displayed in Tables 7 and 8. The highly significant genetic correlations between blister-rust resistance and growth traits, and on the other hand between all growth traits, suggest that an indirect selection could be advantageously adopted. For example, in this case if selected for an easily measurable trait, i.e. diameter, another economically important trait can be improved, i.e. the volume growth rate, which is genetically correlated with the diameter.

Heritability -- Table 6 displays broad-sense heritabilities for all tested traits based on a clonal basis. All traits, except stem forking, indicated high value for heritability estimates; these estimates ranged from 0.695 - 0.896 and from 0.629 - 0.875 for the Calimani and Rodnei populations, respectively. The stem forking trait heritability had a low value for both populations (Table 6, col. 6).

Genotypic gain -- Table 9 displays the genotypic gains that could be achieved for each trait if the best 10 or 15 clones of 26 were selected. A genotypic gain higher than 10% could be achieved in blister-rust resistance, basal area, crown diameter, number of branches per whorl and branch diameter, but the most important genotypic gain could be made in stem volume growth rates.

#### Conclusion

Within the P. cembra natural populations an important genetic variation was found in the most important economical traits. There is sufficient justification to believe that an improvement program with this species would be successful.

With regard to the improvement program, two main decisions could be taken, as follows: (1) If the stem volume growth rate is to be improved, a selection in the Calimani population would have to be performed; (2) If we want to select for narrow crowns, small branch number/whorl or thin branch traits, the selection would have to be made in the Rodnei population.

The strong correlations among traits suggest that indirect selection techniques could be advantageously adopted.

The most important tested traits are under strong genotypic and heritable control; consequently, an important genotypic gain could be achieved.

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Table 2. Analysis of variance of some traits of Pinus cembra clones from Calimani and Rodnei populations.

Source of variation	Df	Calimani		Rodnei	
		MS	F	MS	F
1	2	3	4	5	6
R <sub>1</sub> Resistance to <u>C. ribicola</u>	25	14.624	9.69 <sup>***</sup>	20.150	7.89 <sup>***</sup>
R <sub>2</sub> Resistance to <u>P. cembrae</u>	25	2.298	3.26 <sup>***</sup>	9.805	5.62 <sup>***</sup>
Ha Annual growth in 1989	25	1.025	5.43 <sup>***</sup>	0.802	2.60 <sup>***</sup>
Ht Total height growth	25	46.275	8.99 <sup>***</sup>	32.504	6.46 <sup>***</sup>
D Diameter at 1/2 height	25	0.011	5.85 <sup>***</sup>	0.008	6.14 <sup>***</sup>
BA Basal are at 1/2 height	25	0.003	5.17 <sup>***</sup>	0.001	6.13 <sup>***</sup>
V Stem volume	25	2.265	5.57 <sup>***</sup>	0.691	6.19 <sup>***</sup>
CD Crown diameter	25	13.476	6.33 <sup>***</sup>	14.193	6.95 <sup>***</sup>
B/W Branches per whorl	25	8.494	7.75 <sup>***</sup>	3.136	5.24 <sup>***</sup>
SS Stem straightness	25	0.260	4.92 <sup>***</sup>	0.811	4.98 <sup>***</sup>
SF Stem forking	25	0.019	0.93	0.149	1.51
BD Branch diameter	25	1.289	3.68 <sup>***</sup>	1.228	3.16 <sup>***</sup>

Table 3. Blister rust resistance, diameter growth and synthetic index of selection rankings for Calimani *P. cembra* clones (Duncan's test)

Blister rust resistance ( $R_1$ )			Diameter (D)			Synthetic index of Selection (I)	
Clone	Mean (Score)	Duncan test p <0.05	Clone	Mean (cm)	Duncan test p <0.05	Clone	Value
180	10.0		180	3.8		180	13.8
182	10.0		190	3.8		190	13.8
184	10.0		176	3.7		184	13.6
187	10.0		311	3.6		311	13.6
190	10.0		184	3.6		176	13.4
198	10.0		175	3.6		175	13.2
311	10.0		187	3.5		185	13.2
189	9.9		185	3.4		187	13.2
185	9.8		199	3.4		189	13.2
195	9.8		177	3.3		198	13.2
199	9.8		194	3.3		199	13.2
176	9.7		189	3.3		182	13.1
181	9.7		186	3.3		195	13.0
196	9.7		187	3.2		177	12.9
197	9.7		181	3.2		181	12.9
171	9.6		198	3.2		178	12.7
177	9.6		195	3.2		197	12.7
312	9.3		182	3.1		196	12.6
314	9.3		197	3.0		186	12.4
178	9.2		191	3.0		312	12.2
186	9.1		200	2.9		314	12.2
192	9.0		312	2.9		194	12.0
194	8.7		314	2.9		192	11.9
191	8.1		196	2.9		191	11.1
200	7.2		192	2.9		200	10.1
188	3.9		188	2.2		188	6.1

$$I = R_1 + D$$

Table 4. Blister rust resistance, diameter growth and synthetic index of selection rankings for *Rodnei P. cembra* clones (Duncan's test)

Blister rust resistance ( $R_1$ )			Diameter (D)			Synthetic index of selection (I)	
Clone	Mean (score)	Duncan test p < 0.05	Clone	Mean (cm)	Duncan test p < 0.05	Clone	Value
231	10.0		231	3.2		231	13.2
232	10.0		234	3.1		231	12.8
237	10.0		256	2.8		237	12.6
245	10.0		243	2.8		245	12.6
248	10.0		232	2.8		248	12.6
252	10.0		236	2.7		252	12.6
254	10.0		242	2.7		242	12.5
242	9.8		258	2.7		254	12.5
258	9.8		281	2.7		258	12.5
281	9.8		237	2.6		281	12.5
239	9.6		245	2.6		243	12.4
255	9.6		240	2.6		256	12.4
236	9.6		280	2.6		234	12.3
243	9.6		252	2.6		236	12.3
256	9.6		248	2.6		239	11.9
257	9.5		254	2.5		240	11.9
240	9.3		260	2.5		257	11.9
262	9.3		257	2.4		280	11.9
280	9.3		246	2.3		255	11.7
234	9.2		239	2.3		262	11.6
261	8.3		262	2.3		260	10.7
260	8.2		259	2.2		261	10.4
241	7.8		233	2.1		241	9.8
259	6.4		255	2.1		259	8.6
233	5.4		261	2.1		233	7.5
246	4.2		241	2.0		246	6.5

$$I = R_1 + D$$

Table 5. Superiority in some economically important traits of the Calimani compared to the Rodnei population of P. cembra

Traits	Mean		Superiority <sup>1</sup> (%)
	Rodnei	Calimani	
R <sub>1</sub>	9.01	9.27	3
R <sub>2</sub>	8.11	8.59	6
H <sub>a</sub>	2.22	2.94	32
Ht	15.78	20.54	30
D	.252	.323	28
BA	.051	.085	67
V	.850	1.804	112
CD	6.62	8.88	34
B/W	4.30	5.71	33
SS	2.83	2.93	4
SF	2.91	2.99	3
BD	1.73	2.07	20

<sup>1</sup> The Calimani population superiority.

Note: The blister rust resistance of the P. strobus used as a control in this study was 2.8.

Table 6. Means ( $\bar{x}$ ), variance components ( $\sigma^2$ ) (\* in brackets), phenotypic standard deviation ( $\sigma_P$ ), and broad-sense heritability ( $h^2$ )

Traits	$\sigma_G^2$	$\sigma_E^2$	$\sigma_P^2$	$\sigma_P$	$H^2$
<u>Calimani Population</u>					
R <sub>1</sub>	1.460 (90)	0.170 (10)	1.630 (100)	1.277	0.896
R <sub>2</sub>	0.178 (81)	0.078 (30)	0.256 (100)	0.506	0.695
H <sub>A</sub>	0.092 (81)	0.021 (19)	0.113 (100)	0.336	0.814
Ht <sub>1),2)</sub>	4.569 (89)	0.572 (11)	5.141 (100)	2.267	0.889
D <sub>1),2)</sub>	0.195 (83)	0.022 (17)	0.127 (100)	0.356	0.827
BA	0.024 (80)	0.006 (20)	0.030 (100)	0.173	0.800
V	0.207 (82)	0.045 (18)	0.252 (100)	0.502	0.821
CD	1.261 (84)	0.237 (16)	1.498 (100)	1.224	0.842
B/W <sub>1),2)</sub>	0.821 (87)	0.122 (13)	0.943 (100)	0.971	0.871
SS	0.023 (79)	0.006 (21)	0.029 (100)	0.170	0.793
SF	0.010 (4)	0.220 (96)	0.230 (100)	0.480	0.045
BD	0.104 (73)	0.039 (27)	0.143 (100)	0.378	0.727
<u>Rodnei Population</u>					
R <sub>1</sub>	1.960 (88)	0.280 (12)	2.240 (100)	1.497	0.875
R <sub>2</sub>	0.900 (83)	0.190 (17)	1.090 (100)	1.044	0.826
H <sub>A</sub>	0.056 (63)	0.033 (77)	0.089 (100)	0.298	0.629
Ht <sub>1),2)</sub>	3.052 (85)	0.559 (15)	3.611 (100)	1.900	0.845
D <sub>1),2)</sub>	0.070 (83)	0.014 (17)	0.084 (100)	0.290	0.833
BA	0.011 (85)	0.002 (15)	0.013 (100)	0.114	0.836
V	0.064 (84)	0.012 (16)	0.076 (100)	0.276	0.842
CD	1.350 (86)	0.227 (14)	1.577 (100)	1.256	0.856
B/W <sub>1),2)</sub>	0.282 (81)	0.067 (19)	0.349 (100)	0.591	0.808
SS	0.072 (80)	0.018 (20)	0.090 (100)	0.300	0.800
SF	0.006 (35)	0.011 (65)	0.017 (100)	0.130	0.353
BD	0.093 (68)	0.043 (32)	0.136 (100)	0.369	0.684

1)  $\sigma_G^2$ ,  $\sigma_e^2$ ,  $\sigma_P^2$  estimates multiplied by 100

2)  $\sigma_P$  estimate multiplied by 10



Table 9. Expected genotypic gain ( $\Delta G$ ) for Pinus cembra clones as compared to the general mean of the clonal population

Traits	Calimani		Rodnei	
	$\Delta G(\%)$ when selected		$\Delta G(\%)$ when selected	
	10/26 <sup>1</sup>	15/26 <sup>1</sup>	10/26	15/26
R <sub>1</sub>	11.9	8.1	14.0	9.6
R <sub>2</sub>	3.9	2.7	10.2	7.0
Ha	9.0	6.1	8.1	5.5
Ht	9.4	6.5	9.8	6.7
D	8.8	6.0	9.3	6.3
BA	15.6	10.7	18.1	12.4
V	22.0	15.0	26.3	18.0
CD	11.2	7.6	15.6	10.7
B/W	14.3	9.7	10.7	7.3
SS	4.4	3.0	8.2	5.6
SF	0.1	0.0	1.5	1.0
BD	12.8	8.8	14.0	9.6

<sup>1</sup> 10 or 15 clones selected out of 26 tested

PROVENANCE VARIATION IN EASTERN WHITE PINE (PINUS STROBUS L.):  
28TH-YEAR RESULTS FROM TWO SOUTHERN ONTARIO PLANTATIONS

Drs. Husnia Ibrahim Abubaker and Louis Zsuffa

SUMMARY

Provenance variation in eastern white pine (Pinus strobus L.) was investigated on the basis of 12 range-wide geographic sources, planted at Ganaraska Forest and Turkey Point test locations in Ontario, Canada. Twelve growth and morphological characters were measured and scored on trees at 28 years of age. Statistical analysis consisted of univariate, multivariate and non-parametric methods.

The results showed that eastern white pine was highly variable and displayed well-defined patterns of variation. At both test sites trees from the Atlantic coast had grown fastest, while trees from further inland, especially the Iowa provenance, have been below average performers. Traits such as growth form and resistance to weevil varied more randomly.

Provenance-environment interactions were not significant in height, volume/tree, survival and branch angle, but rank changes at the two plantations were observed in D.B.H., volume/plot, weevil damage, branch diameter and branches/whorl.

Key words: provenance variations, Pinus strobus, provenance x environment interaction

Introduction

Eastern white pine (Pinus strobus L.) ranges across Canada from Manitoba to Newfoundland, throughout United States from Minnesota and northeastern Iowa to the Atlantic Ocean, and southward along the Appalachians to northern Georgia and Alabama. There are several isolated distribution areas in the Central States. This large geographic range of eastern white pine embraces considerable diversity. Thus, white pine is adapted to a wide range of site conditions, and is capable of growing under a wide variety of soil textures and moisture regimes (Stiell, 1978).

Much of the information available on genetic variation in eastern white pine has been derived from a range-wide provenance test initiated by the U.S. Department of Agriculture in 1955. It included 31 provenances from natural stands; thirteen test plantations were established in the U.S. and two in Canada. The studies were designed to determine variation within the range of eastern white pine. The results of these trials are in reports by King and Nienstaedt (1969), Fowler and Heimburger (1969), Wright (1970), Funk (1971), Garrett et al. (1973), Demeritt and Kettlewood (1975), and Zsuffa (1975). In this study, the 28th-year results are reported from two southern Ontario (Canada) plantations, each containing the same 12 geographic sources.

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Stem form, H:D, and forks all showed significant differences among provenances. However, the variance associated with provenances for these characters was low, while the error variance was high. This was probably due to weevil damage which affected the stem form of provenances in this study.

The significant differences among provenances in H:D indicate that, as was hypothesized by Wright (1970, 1976), height and diameter growth may be inherited separately. Thus, height and DBH should be considered separately in selecting provenances.

It was reported that the production of lammas shoots which may cause forks is a genetically controlled feature which probably has a complicated genetic background (Rudolf, 1964; West and Ledig, 1964; Ehrenberg, 1970). On the other hand, Carter et al. (1986) indicated that in Douglas-fir, the number of trees with forks in the main stem increased with spacing.

The analyses of branch angle, branch diameter and branches per whorl show provenance differences. Branch angle is more strongly genetically controlled than branch diameter and branches/whorl. Much of the variation in branch diameter and branches/whorl is confounded with error variance. This was due, at least in part, to the procedures and circumstances in this study. A subjectively graded score was used, and in addition, the trees were too tall and therefore difficult to examine precisely. Moreover, environmental factors, such as differential spacing created by mortality, could have influenced branch diameter and number of branches/whorl.

Resistance to biotic factors: In this study, blister rust at 28 years was minimal at both trial sites, especially in Ganaraska Forest, where it was almost nil. Many smaller dead trees which were attacked by blister rust earlier were observed. This verifies the observation reported by Patton (1961), that resistance apparently increases with age.

Weevil damage had already occurred on several trees by age 7 (Fowler and Heimburger, 1969). During the following 21 years, the insect made its massive attack throughout the experimental areas especially at Ganaraska Forest. The least weeviled sources at Ganaraska Forest were Minnesota, Pennsylvania, Maine and Ontario, while at Turkey Point the least weeviled sources were Pennsylvania, Minnesota, Maine and Tennessee. In general, Nova Scotia, New York and Iowa seed sources were highly susceptible to repeated weevil attacks with very high proportions of heavily weeviled trees at both sites (Table 5). However, all provenances were attacked by weevils. Similar results were reported by Garrett (1972, 1973), who indicated differences in weevil damage among provenances, but that all sources were heavily attacked. He considered it unlikely that provenances with acceptable number of weevil free trees could be located for use in higher risk areas. On the other hand, Wilkinson (1981) pointed out that the selection for reduction of the effects of leader damaging attacks in plantations over a rotation may be a more realistic goal, and evaluation of geographic variation in susceptibility to repeated attack has become a useful information to selection programs. In this respect, the proportion of fast growing trees

that have not been free of weevil attack, but have been attacked infrequently and maintained good form is important. Provenances from Pennsylvania, Maine and Minnesota contain a range of 18.1 to 36.8% of such trees. In addition, Pennsylvania and Maine provenances are fast growing and thus could give good results in breeding for resistance to weevil attack.

#### Provenance-Environment Interaction

GE interactions are more likely to occur when environments differ. Although the trial sites are both in the same region, site type varied. The Ganaraska Forest site is characterized by clay soils and Turkey Point by a well-drained sandy soil. In addition, the spring temperatures and annual rainfall are higher at Turkey Point than at Ganaraska Forest. Further, Turkey Point is near Lake Erie where tornado, winter storm, wind and snow storms are more frequent than at Ganaraska Forest. Some characters, such as height growth, did not show much GE interaction, and the provenances with high or low height growth maintained their relative ranking.

The analysis of variance showed GE interactions for DBH and volume/plot which were close to significance (Table 6). Relative rankings of provenances for these traits indicated that some are superior in growth on both sites, some fluctuate rather widely from site to site, and others are consistently poor.

#### Conclusions

A study of 28-year-old trees from 12 range-wide provenances of eastern white pine in two test plantations in southern Ontario indicated that natural populations of this species are genetically different from one another. The 12 morphological and growth characters studied showed significant differences among provenances. The amount of among provenance variation showed by these characters was unequal and certain traits showed larger variation than others. Provenances with maritime climates were faster growing with fewer forked trees, wider branch angles and finer branch diameters. Southern provenances had fewer branches/whorl than those of the northern provenances.

Provenance-environment interactions were small in height, volume/tree, survival and branch angle, and this suggested that provenances of eastern white pine selected for these characters could be used over a wider range of environments. More significant was the rank change on two test sites in branch diameter, branches/whorl and weevil damage.

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Table 1. Geoclimatic information for the eastern white pine provenances studied <sup>a</sup>

Provenance Number	State or Province	County or District	Latitude ( <sup>o</sup> N)	Longitude ( <sup>o</sup> W)	Elevation (m)	Mean January Temp( <sup>o</sup> F)	Frost-Free Period	Annual Pptn (mm)	Sept thru June (mm)
272 (A)	Georgia	Union	34.8	84.1	745	4.4	190	1316	1089
273 (B)	Tennessee	Greene	36.0	82.8	685	2.2	172	1074	988
274 (C)	Pennsylvania	Monroe	41.1	75.4	550	-4.4	129	1201	974
275 (D)	New York	Franklin	44.4	74.3	490	-8.9	110	947	757
276 (E)	Maine	Penobscot	44.9	68.6	45	-7.8	141	1057	898
277 (F)	Ohio	Ashland	40.8	82.3	305	-2.2	156	914	751
278 (G)	Iowa	Allamakee	43.3	91.5	305	-8.9	152	813	638
279 (H)	Minnesota	Cass	47.4	94.4	395	-14.4	126	589	433
280 (I)	Wisconsin	Forest	45.8	88.9	460	-12.2	90	724	580
281 (J)	Nova Scotia	Lunenburg	44.4	64.6	45	-6.7	137	1110	931
282 (K)	Quebec	Pontiac	47.4	77.0	305	-11.1	80	920	716
283 (L)	Ontario	Algonia	46.4	82.6	200	-12.2	120	899	760

<sup>a</sup> Climatic data for United States and canadian sites are from the U. S. Department of Agriculture (1941) and Environment Canada (1984) respectively.

Table 2. Location, elevation and summary of weather records at the planting sites <sup>a</sup>

Planting site	Latitude ( <sup>o</sup> N)	Longitude ( <sup>o</sup> W)	Elevation (m)	Mean January Temp ( <sup>o</sup> C)	Mean Annual Temp ( <sup>o</sup> C)	Frost-free Period (days)	Annual Pptn (mm)	Sept thru June Pptn (mm)
Ganaraska Forest	44 <sup>o</sup> 57'	78 <sup>o</sup> 44'	262	-8.1	5.9	138	853	706
Turkey Point	42 <sup>o</sup> 42'	80 <sup>o</sup> 27'	213	-5.7	7.9	139	912	837

<sup>a</sup> Climatic data from Environment Canada (1984)

Table 3. Summary of assessment method

Direct measurement	Counting	Scoring (Classes 1-5)
1. Height (to 0.1 m)	1. Survival	1. Stem form
2. DBH (to 0.25 cm)	2. Forked trees	2. Branch diameter
3. Forking height (to 0.1 m)	3. Branches/whorl	3. Branch angle
	4. Secondary flushing	4. Weevil attack
		5. Blister rust damage

Table 6. Mean squares (MS)<sup>a</sup> and percentage of total variance (V%)<sup>b</sup> based upon variance component

Characters	Source of Variation					
	Provenances		Provenance X site		Within Provenances	
	MS	V%	MS	V%	MS	V%
Height	0.410 <sup>***</sup>	51.7	0	0	0.38	48.3
DBH	0.675 <sup>**</sup>	34.1	0.2	10.1	1.10	55.8
H:D	11.80 <sup>***</sup>	38.76	0	0	18.64	61.24
Volume/tree	0.00033 <sup>***</sup>	37.87	0.00002	1.78	0.00051	60.35
Volume/plot	0.2029	20.96	0.122	12.69	0.642	66.35
Forking %	32.41 <sup>**</sup>	14.32	0	0	193.88	85.68
F:H	15.49 <sup>***</sup>	17.07	0	0	75.24	82.93
Survival %	59.29 <sup>***</sup>	57.96	3.64	3.56	39.36	38.48

<sup>a</sup> Significant levels \* 5%, \*\* 1%, \*\*\* 0.1%

<sup>b</sup> If variance component negative, V% listed as 0

Table 4. Provenance means and standard deviation (SD) for growth, forking and survival percent at Ganaraska Forest (1) and Turkey Point (2) at 28 years.

Provenance	Stat.	Height (m)		DBH (cm)		Vol/tree (m <sup>3</sup> )		F:H <sup>a</sup>		Forking %		Survival %		H:D <sup>b</sup>		Vol/plot (m <sup>3</sup> )	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Georgia	Mean	11.8	13.7	15.2	16.9	.111	.163	69.8	84.3	50.0	29.4	72.7	62.5	81.4	80.6	4.31	5.5
	S.D.	1.4	1.6	4.3	4.0	.06	.09	32.2	25.6	11.2	4.8	5.9	3.8	18.6	12.9	.78	1.18
Tennessee	Mean	11.7	13.7	15.7	19.0	.111	.195	72.0	84.5	45.6	27.9	75.5	64.6	78.7	73.9	4.50	6.73
	S.D.	1.1	1.5	3.4	5.0	.05	.10	32.8	26.1	11.1	8.9	5.9	7.0	18.1	13.2	.60	.46
Pennsylvania	Mean	12.0	14.0	16.9	17.7	.132	.183	70.0	82.1	50.0	29.4	80.1	68.1	82.8	80.3	5.70	6.63
	S.D.	.8	2.0	4.2	4.6	.07	.10	31.8	31.0	10.2	12.8	2.3	9.5	19.1	17.2	.45	.49
New York	Mean	12.5	14.7	17.4	20.4	.150	.247	62.0	76.2	61.8	45.6	68.1	44.0	76.2	73.7	5.43	5.7
	S.D.	1.4	1.7	4.9	4.8	.08	.12	32.0	27.7	17.3	11.3	5.9	6.3	19.0	14.7	.31	.87
Maine	Mean	12.6	13.9	16.8	19.0	.137	.205	72.6	85.5	41.2	26.4	74.1	51.5	82.3	75.7	5.39	5.53
	S.D.	1.5	1.7	4.6	5.3	.08	.11	34.5	25.7	17.3	11.3	11.0	6.3	22.9	16.4	.95	.53
Ohio	Mean	11.3	12.7	13.9	16.4	.100	.144	71.5	87.3	50.0	25.0	91.2	75.5	84.4	77.7	4.74	5.86
	S.D.	1.2	1.6	4.0	4.0	.06	.07	32.3	24.0	18.3	13.1	6.1	6.1	22.0	16.2	1.69	.50
Iowa	Mean	10.5	12.2	15.4	19.2	.107	.191	65.7	76.9	54.5	39.7	66.2	43.5	73.1	64.7	3.79	4.99
	S.D.	1.4	1.5	4.8	5.2	.07	.11	32.9	31.2	19.4	12.1	8.5	5.4	23.1	13.2	.48	1.70
Minnesota	Mean	11.7	13.9	15.3	19.0	.113	.209	70.7	79.7	54.4	36.8	78.2	58.4	78.3	73.6	4.77	6.55
	S.D.	1.3	1.8	4.5	4.8	.06	.11	30.6	27.9	22.7	7.4	5.6	3.5	20.2	13.9	.38	1.15
Wisconsin	Mean	12.5	13.7	16.9	18.7	.131	.191	66.5	80.9	55.9	36.8	71.3	54.2	81.4	74.0	5.01	4.93
	S.D.	1.4	1.5	4.4	4.7	.07	.09	32.5	26.6	15.6	5.6	7.2	7.0	22.2	13.7	.59	1.09
Nova Scotia	Mean	12.9	14.0	17.1	18.2	.143	.185	79.4	90.0	33.8	16.0	81.0	49.3	80.0	78.1	6.42	6.43
	S.D.	1.0	1.5	4.0	4.2	.07	.09	30.5	24.7	7.4	10.0	2.8	6.3	18.5	12.7	.52	.77
Quebec	Mean	11.5	13.1	15.2	18.3	.116	.185	69.2	83.7	53.0	25.0	69.6	52.8	76.0	70.8	4.36	5.20
	S.D.	1.3	1.6	4.6	4.6	.07	.10	31.9	24.5	18.6	19.5	4.6	10.1	18.7	12.6	1.21	.96
Ontario	Mean	11.4	13.2	15.1	18.0	.107	.164	66.1	81.9	55.9	36.8	84.2	58.8	79.2	77.5	4.89	5.16
	S.D.	1.1	1.7	4.6	4.5	.06	.10	33.4	24.7	12.2	11.1	3.6	7.0	21.5	14.4	.65	.76

<sup>a</sup> H:D = [Height (m)/DBH (cm)] x 100.      <sup>b</sup> F:H = [forking height/height] x 100

Table 5. Provenance mean scores for branching characteristics, stem form and weevil attack at Ganaraska Forest (1) and Turkey Point (2)

Provenances	Branch Angle						Branch Diameter						Branches/Whorl			Stem Form		Weevil Attack	
	1		2				1		2				1	2	1	2	1	2	
	50%	>75% <sup>a</sup>	50%	>75%	50%	>75%	50%	>75%	50%	>75%	50%	>75%	50%	>75%	50%	>75%	50%	>75%	50%
Georgia	472	457	438	459	454	421	387	393	398	398	358	385	412	348	364	381	377	391	378
Tennessee	409	407	415	372	406	383	404	372	376	397	417	420	395	388	468	382	382	463	384
Pennsylvania	375	395	403	406	399	431	382	408	404	343	385	367	484	370	409	324	417	336	
New York	356	352	367	378	387	377	429	441	413	448	437	435	439	489	434	486	431	489	
Maine	365	382	404	402	411	395	375	394	409	414	418	410	427	390	382	384	389	394	
Ohio	410	397	392	395	411	395	384	409	400	364	399	370	390	380	417	372	411	378	
Iowa	419	430	414	410	412	417	454	402	418	465	429	404	331	392	450	464	447	462	
Minnesota	416	413	398	488	473	475	447	433	425	480	437	441	428	451	362	396	367	389	
Wisconsin	430	410	408	337	359	371	426	397	433	418	425	444	466	395	421	388	421	384	
Nova Scotia	329	371	373	335	367	365	434	474	439	381	370	410	467	451	421	480	431	473	
Quebec	447	404	431	435	425	450	382	380	369	446	428	430	377	423	375	362	385	363	
Ontario	474	485	459	431	396	420	401	398	418	387	372	362	347	409	381	487	348	471	

<sup>a</sup> 50%, 75% and >75% indicate the percentage of total tree height

Table 7. Kruskal-Wallis  $X^2$ , Friedman's  $X^2$  and percentage of total variance (V%) based upon variance component

Characters	Kruskal-Wallis	Friedman's	V%	
	$X^2$	$X^2$	Provenances	Within Provenances
Branch angle*				
50%	64.72***	74.88***	32.38	67.62
75%	38.49***	45.94***	26.23	73.77
>75%	41.59***	48.87***	22.79	77.21
Branch diameter*				
50%	32.04***	34.83***	15.59	84.41
75%	9.06	10.78	0	100.00
>75%	13.72	15.83	3.56	96.44
Branches/whorl	39.28***	--	17.47	82.53
Stem form	34.70***	36.52***	16.00	84.00
Weevil attack	28.53**	29.44**	11.47	88.53

Significance level: \*\* 1%, \*\*\* 0.1%

\* at 50%, 75% and >75% of total tree-height

Table 8. Wricke's ecovalence for the different provenance

Provenances	DBH		Volume/plot	
	$V_i^*$	Percent Variation	$V_i^*$	Percent Variation
Georgia	0.2249	.62	0.0664	2.03
Tennessee	0.504	1.39	0.9945	30.41
Pennsylvania	1.6057	4.44	0.0068	0.21
New York	0.3649	1.01	0.1537	4.70
Maine	0.2425	0.67	0.4148	12.68
Ohio	0.2249	0.62	0.0281	0.86
Iowa	1.1426	3.16	0.0724	2.21
Minnesota	0.9809	2.71	0.4640	14.19
Wisconsin	6.4250	17.76	0.4680	12.44
Nova Scotia	5.4100	14.96	0.3293	10.07
Quebec	9.3640	25.89	0.0010	0.03
Ontario	9.6842	26.77	0.3329	10.18

\* contribution to genetic x environment interaction sum of squares

Figure 1. Variation among provenances for different traits at two sites. Lines connect ranks for each provenance. Differences in elevation of the lines denote genetic variation and non-parallelism indicates provenance-environment interaction.  $r_3$  is the Spearman's rank correlation coefficient across environments. Letters indicate provenances (see Table 1). Significance level: 5%, 1%

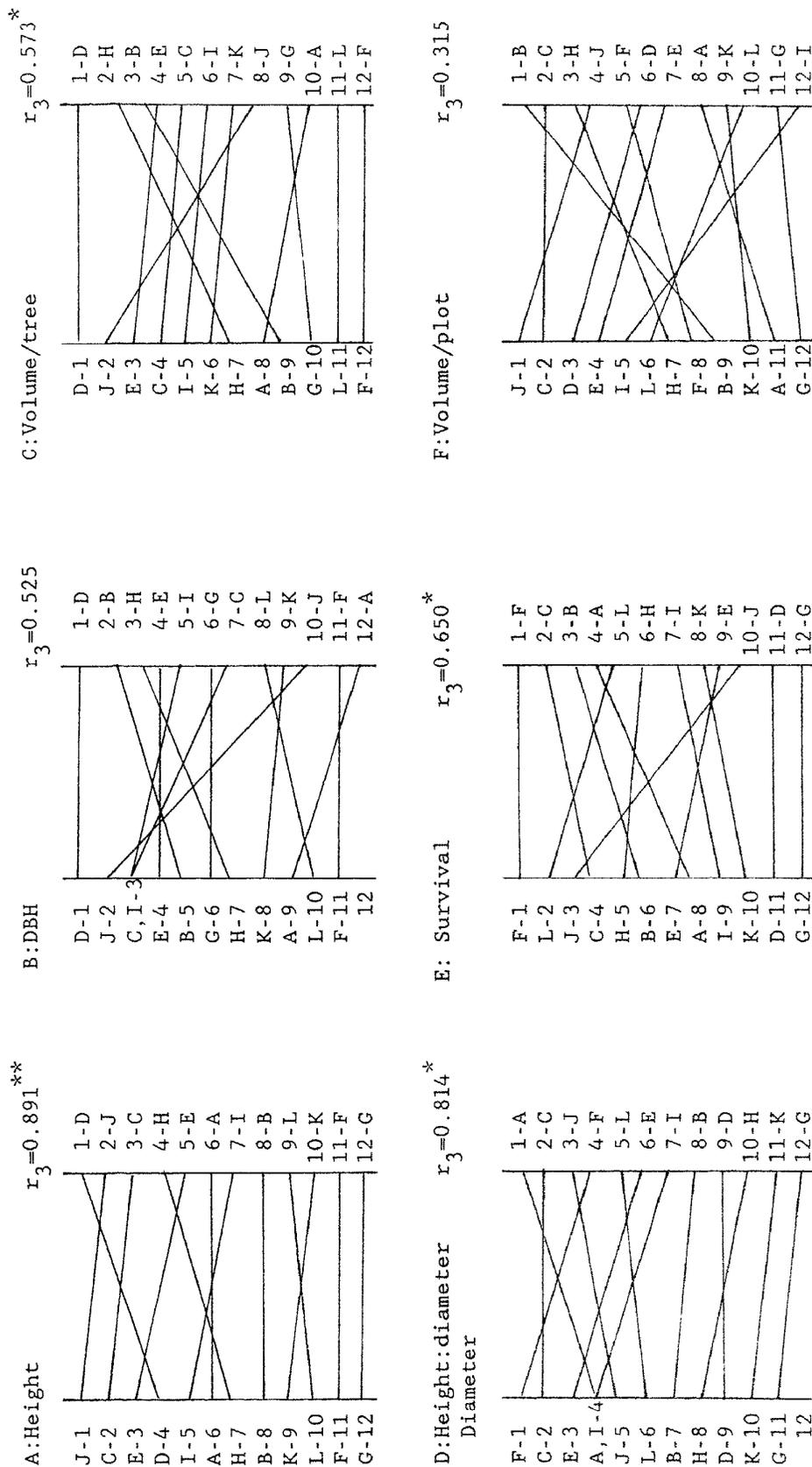
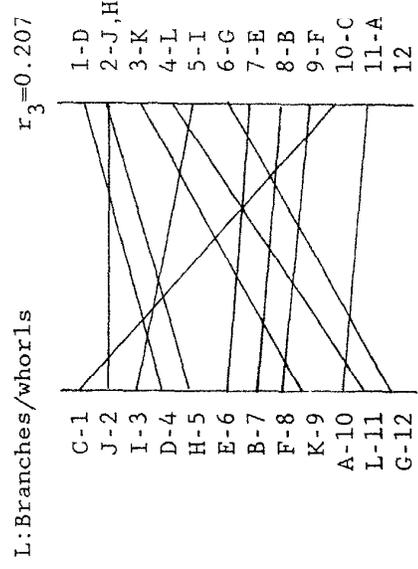
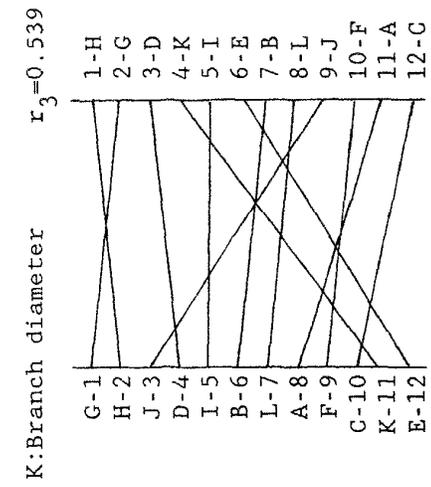
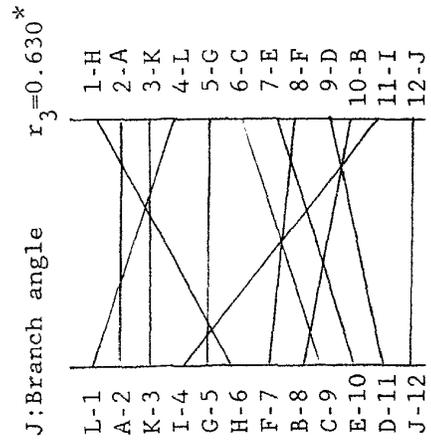
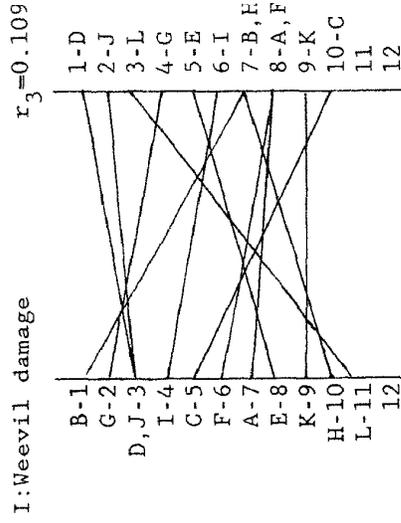
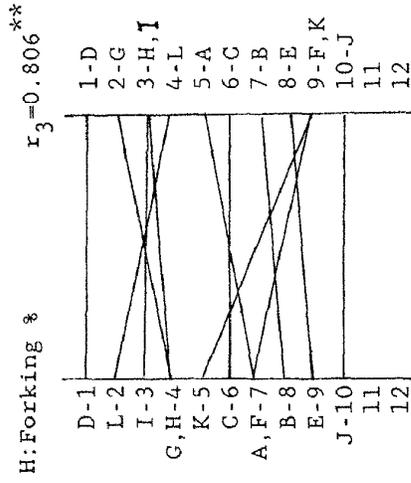
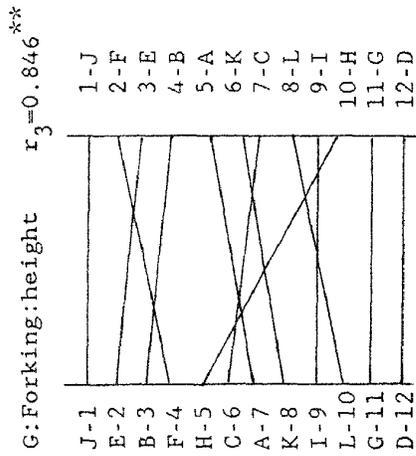


Figure 1. Continued:



Ganaraska  
Forest

Turkey  
Point

Ganaraska  
Forest

Turkey  
Point

Ganaraska  
Forest

Turkey  
Point

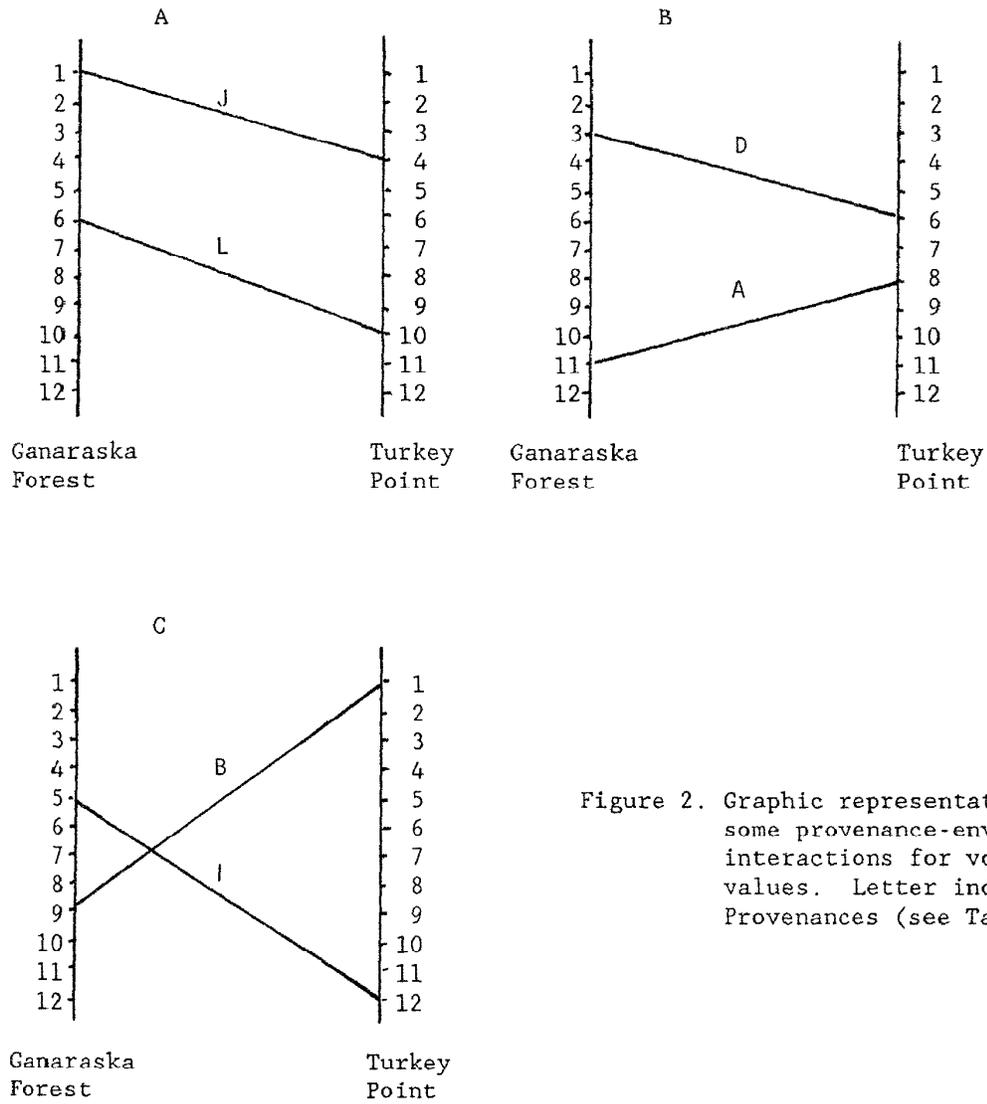


Figure 2. Graphic representation of some provenance-environment interactions for volume/plot values. Letter indicate Provenances (see Table 1).

JUVENILE GROWTH OF OPEN-POLLINATED PROGENIES OF 57 KOREAN WHITE PINE  
FAMILIES IN RELATION TO THEIR CONE AND SEED CHARACTERISTICS

Drs. S.U. Han, H.M. Kwon, G.S. Jhun, S.I. Sohn and K.J. Lee

SUMMARY

Open-pollinated cones of Korean white pine (Pinus koraiensis S. et Z.) were collected from 57 plus trees. Cone and seed characteristics were measured and seeds were sown at the nursery in Suwon. 2-0 stocks were transplanted in the same nursery and seedling heights were measured at the end of the 4th growing season. Cone and seed size were significantly different among families at the 1% level. Height growth was also different among families during the first four years. Estimates of individual tree heritability for height growth were high, 0.661 for 1-year-old seedlings and 0.553 for 4-year-old seedlings. However, these estimates were over estimated due to seed size effect. Spearman rank correlation coefficients among heights and seed and cone characteristics were all statistically high implying that seed size affected the first 4 years' height growth. Even though the nursery data are too premature to draw any decisive conclusions, these early results can provide basic information for breeding Korean white pine.

Introduction

Korean white pine (Pinus koraiensis Sieb. & Zucc.) is one of our native conifers and is very important for reforestation in Korea. In 1987, 28% of the total reforestation area was planted with Korean white pine (Anom., 1988). Korean white pine ranges throughout Korea and eastern Manchuria into southeastern Siberia with outlying populations on the Japanese islands. Breeding of Korean white pine was initiated in 1959 and 300 "plus" trees have been selected to be included in seed orchards of 91 hectares. In 1989 a total of 3,458 kg of seed were produced from the seed orchards. Korean white pine is famous for its red colored wood as well as for pine nuts which are edible and considered a traditional delicacy in Korea. Sometimes economic return from the pine nuts exceeds the timber value by itself.

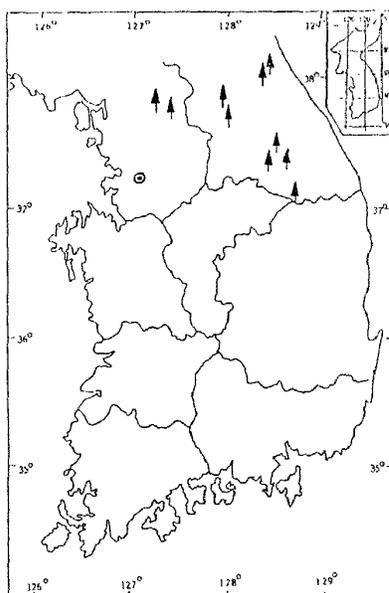
There is not sufficient information on the genetics of Korean white pine. Limited geographic sources available in South Korea have made genetic studies more difficult. Progeny tests of this species have been delayed until plus tree selection and seed orchard establishment were completed. This paper reports results from the first large scale open-pollinated progeny test in Korea. Even though measurements were taken from seed, cone and nursery experiments only, this paper will provide valuable information on the relationship of seed size to juvenile growth.

Drs. Han, Kwon, Jhun and Sohn are on the staff of the Institute of Forest Genetics, Suwon, Korea. Dr. Lee is with the Department of Forestry, Seoul National University, Suwon, Korea.

Materials and methods

Cones were collected from 58 plus trees in Kongwon-do and Kyonggi-do in 1981 (Fig. 1). They were brought to the Institute of Forest Genetics (IFG) at Suwon and carefully processed. Morphological characteristics were measured on five randomly selected cones from each family. Seed characteristics from five seed per cone were examined and their mean values were used for statistical analysis.

Figure 1. Location of plus trees and nursery site.



↑ = plus tree  
⊙ = nursery test

In the spring of 1982, seeds were sown in a completely randomized block design with three replications in the nursery at the IFG. Two years later the 2-0 stock was transplanted at the same nursery. Seedling height was measured through the fourth growing season for 25 trees of each family. All data, including cone and seed characteristics and seedling height at age 4 for 57 families, was statistically analyzed. One family was excluded due to poor germination and survival. Variance components and heritability were calculated for each characteristic. Spearman rank correlation coefficients were also computed among seedling height and seed and cone characteristics.

## Results and discussion

Seed length and seed weight were the most significantly different characteristics. Seed length ranged from 10.0 to 18.4 mm and the range of seed weight was between 0.33 and 1.17 g (Table 1). Family differences in cone and seed characteristics were all statistically significant at the 1% level (Table 2). Abundant yields of pine nuts as well as large nut size can be considered as one of the breeding purposes in Korea. Therefore, significant differences in seed size and weight imply the possibility of successful breeding of large nuts in this species.

One of the limiting factors in establishing Korean white pine plantations in Korea is retarded early growth. One-year-old seedling height was only 6 cm and the tallest 4-year-old seedling height averaged only 27 cm (Table 3). Seedling height differences were highly significant among families during the first four growing seasons (Table 4). Estimate of heritability for individual tree was 0.553 after the 4th growing season. Estimates of family mean heritability decreased gradually from 0.8 for 1-year-old seedlings to 0.605 for 4-year-old seedlings.

Individual tree heritability for seedling height was over-estimated due to seed size effects still present in the fourth year height measurement. Estimates of heritability for height increment during the fourth year from seed in western white pine were less than 0.1 (Squillace et al., 1967). Family mean heritability estimated from 16-year-old height growth of Korean white pine was still high - over 0.8 (Han et al., 1987).

Rank correlation coefficients among ages for height growth were high enough to be statistically significant at the 1% level except the one between the first year and the fourth year height (Table 5). Extremely good correlations between second and third year seedling heights can be explained from the minimum annual height increment in the third year after transplanting of 2-0 stock.

The good rank relationship among ages is expressed again in the comparison of family rankings. The two poorest families in the first year height growth were constantly the poorest families throughout the four year period (Table 6). But the best 5 families in the first year performance were not included in the best five families of the later years. Family rankings of the second year growth performance lasted relatively long.

In general, seed size has strong influence on growth performance of seedlings (Spurr, 1944). It might be especially true with Korean white pine because of its large seed size. As we expected, cone and seed characteristics were strongly correlated with seedling heights during the first four years (Table 7).

In addition to cone length, all characteristics of cone and seed were strongly correlated with heights of the first four years from seed. It will be interesting to know how long the seed size effects will last in Korean white pine. Seed size effect on height growth was observed in many conifers and it is generally accepted that seed size effect diminishes gradually with no significant difference (Squillace et al., 1967; Chon, 1976; Robinson and van Buijtenen, 1979).

The 2-2 stock was out-planted in test sites and height growth was measured continuously. It usually takes another three or four years after outplanting to express normal annual height increment. Therefore comparison of the earlier years' height growth with later years' results will produce much valuable information. Early testing of Korean white pine is especially important to overcome the shortcoming of the slow start of this species. Hybridization of Korean white pine with eastern white pine (Pinus strobus L.) has been attempted with the hope of combining the faster growth in the seedling stage of eastern white pine with the good later growth and pine nut production of Korean white pine.

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Table 1. Means and other statistics for cone and seed characteristics in 58 plus trees of Korean white pine

	Cone length (cm)	Cone diameter (cm)	Cone weight (g)	No. of seed per cone	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Seed weight (g)
Range	9.7-16.4	5.2-8.2	126-442	93-219	10.0-18.4	7.7-12.7	5.9-9.2	0.33-1.17
Mean	13.2	6.5	241	149	15.2	10.1	7.3	0.67
Standard deviation	0.88	0.24	28.8	16.8	0.47	0.44	0.24	0.057
Coefficients of variation (%)	6.7	3.7	11.9	11.2	3.1	4.3	3.4	8.4

Table 2. Mean squares from the analysis of variance of cone and seed characteristics in 58 plus trees of Korean white pine

Source of Variation	df	Cone length	Cone diameter	Cone weight	No. of seed per cone	Seed length	Seed width	Seed thickness	Seed weight
Families	57	4.40 <sup>**</sup>	0.87 <sup>**</sup>	8796.21 <sup>**</sup>	1233.94 <sup>**</sup>	7.14 <sup>**</sup>	2.56 <sup>**</sup>	1.10 <sup>**</sup>	0.061 <sup>**</sup>
Error	232	0.78	0.06	831.80	285.53	0.22	0.19	0.06	0.003

Table 3. Means and other statistics for height growth of 57 open-pollinated progenies at different ages

	1 year	2 years	3 years	4 years
Range	4.3 - 7.0	8.1 - 13.9	10.2 - 16.1	15.3 - 26.7
Mean	6.0	12.0	14.1	21.3
Standard deviation	0.53	1.08	1.09	1.40
Coefficients of variation (%)	8.9	9.0	7.6	11.3

Table 4. Mean squares, variance components and heritabilities determined from the analysis of variance for height growth at different ages

Source of variation	df	1 year	2 years	3 years	4 years
Replications	2	8.43**	157.88**	560.25**	1324.31**
Families	56	21.33**	86.84	89.68	403.56
Rep x Fam	112	4.23	29.80	30.94	162.49
Within plot	4104	1.02	4.68	4.31	14.10
$\sigma^2$		1.023	4.676	4.312	14.100
$\sigma^2$		0.128	1.005	1.065	5.936
$\sigma^2$		0.228	0.761	0.783	3.214
$h^2$		0.661	0.473	0.508	0.553
$h^2$		0.800	0.657	0.655	0.605

Table 5. Spearman's rank correlations among ages for height growth of 57 open-pollinated families

Age	1	2	3	4
1	1.0000**			
2	0.5597**	1.0000**		
3	0.5326**	0.9449**	1.0000**	
4	0.2056	0.6295**	0.7174	1.0000

Table 6. Ranking in height growth of 57 open-pollinated progenies at different ages

Rank	1 year	2 years	3 years	4 years
1	1030	1031	1003	212
2	1057	1056	212	1003
3	1060	1057	1056	1086
4	1120	212	1031	1061
5	1063	1030	1030	1056
.	.	.	.	.
27	1088	1085	1066	1072
28	1085	1083	242	1097
29	1067	241	1083	1048
30	1109	1048	1062	1067
31	1116	1067	1104	256
.	.	.	.	.
.	.	.	.	.
53	1087	243	1102	1009
54	1110	1110	1087	1064
55	243	1110	1087	1064
56	1108	1108	1108	1108
57	1090	1090	1090	1090

Table 7. Correlation coefficients between cone and seed characteristics and height growth at different ages in nursery

Age	Cone length	Cone diameter	Cone weight	No. of seeds per cone	Seed length	Seed width	Seed thickness	Seed weight
Height 1 - 0	0.2412	0.3915**	0.3205*	-0.2195	0.3998**	0.4218**	0.3525**	0.4660**
Height 2 - 0	0.0456	0.5354**	0.3925**	-0.3444**	0.5814**	0.4975**	0.4116**	0.5349**
Height 2 - 1	-0.0328	0.5093**	0.3659**	-0.3816**	0.5924**	0.4871**	0.4353**	0.5529**
Height 2 - 2	-0.1271	0.3369*	0.2643*	-0.3530**	0.3738**	0.2958*	0.3002*	0.3621**

GROWTH PHENOLOGY OF VARIOUS TREE MODULES IN A  
PINUS KORAIENSIS S. ET Z. PLANTATION

Drs. J.H. Shin and D.K. Lee

SUMMARY

The growth pattern of bud-shoot-needle of isolated 15-year-old trees, and seasonal changes in litter-falls and fine root dry weights in the unthinned 28-year-old plantation were investigated to understand the growth phenology of Pinus koraiensis. Shoot growth was observed until 7 June when buds appeared, while current needle growth was observed until 19 July when bud growth started. Most of the litter-falls occurred in October but many needles fell in July and August due to storms. Fine roots were produced mostly in autumn (1004 kg/ha), and were dead during winter and spring.

Key words: Growth phenology, Pinus koraiensis

Introduction

Korean white pine (Pinus koraiensis Sieb. et Zucc.) grows in Korea and in the Amur and Maritime provinces of Russia (Mirov, 1967). Korean white pine is known as a shade-tolerant tree at young stages but gradually becomes intolerant as it ages (Hyun, 1969; Kim, 1968). The trees start to bear cones at around age 15 but do not produce large quantities of cones until they are near age 30 (Hyun, 1969). Because of the value of both nuts (seeds) and timber, Korean white pine is one of the major tree species that have been widely planted in Korea,

Architectural approach may be useful in evaluating not only ecological growth processes such as competition, differentiation and growth strategies, but also silvicultural treatments such as pruning and thinning (Halle et al., 1978). It was found that the architectural development of Korean white pine conformed to Rauh's architectural model (Halle et al., 1978). In order to interpret this process quantitatively, however, it is necessary to express the phenology of such growth modules as bud-shoot-needle or fine roots quantitatively. The growth phenology of the modules may markedly influence the tree architecture.

The objective of this study was to examine quantitatively the phenology of growth modules in Korean white pine.

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Materials and methods

Three 15-year-old Korean white pine trees growing alone were selected, and their diameter and length of buds, needle length, and diameter and length of shoots at each whorl were measured in Suwon plantations weekly from April 12 to November 10, 1988. Five 50 x 50 cm nylon screens (0.5 mm mesh) were placed in the unthinned plantation of 28-year-old trees and litter-falls were collected monthly from October 1987 to October 1988.

The amount of fine roots distributed in the top 30 cm of soil were estimated from thirty sample cores (2 cm in diameter) sampled in the same unthinned plantation where litter-fall was collected. Core samples were collected bimonthly from October 1987 to October 1988. In this study the fine roots were defined as unignified roots less than 3 mm in diameter and their associated root tips. The distinction between living and dead roots was made using the same methods that McClaugherty et al. (1982) used in a 53-year-old red pine (*Pinus resinosa*) stand.

A decision matrix (Fig. 1) modified from the method described by McClaugherty et al. (1982) was used to estimate the total amount of fine root products.

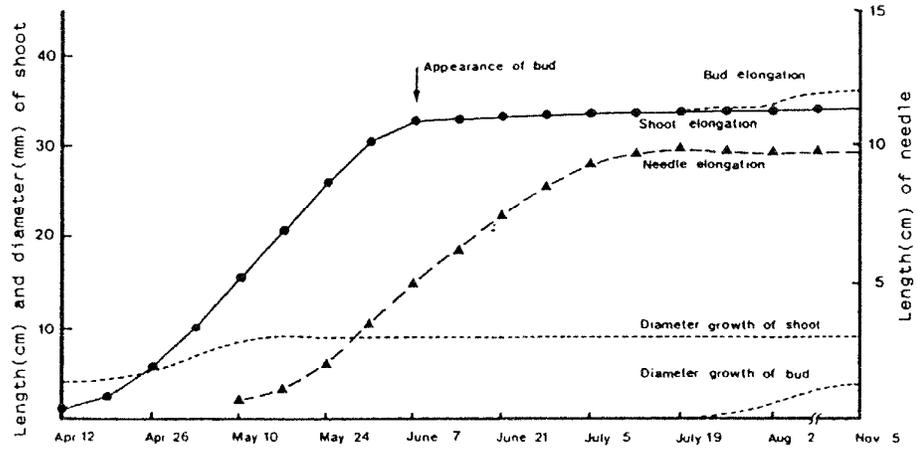
Figure 1. Decision matrix illustrating one method for estimating production, mortality, and decomposition of fine roots. The appropriate quadrant was selected according to the direction of change in live (L), and dead (D) standing crop for the successive two samples. Production (P), mortality (M), and decomposition (C) were calculated from the successive biomass using the equations in a chosen quadrant.

		Live	
		Increase	Decrease
Dead	Increase	$P = \Delta L + \Delta D$ $M = 0$ $C = 0$	$P = \Delta L + \Delta D$ $M = \Delta D$ or $(-\Delta L)$ $C = (-\Delta L) - \Delta D$
	Decrease	$P = \Delta L$ $M = 0$ $C = (-\Delta D)$	$P = 0$ $M = (-\Delta L)$ $C = (-\Delta L) + (-\Delta D)$

Results and discussion

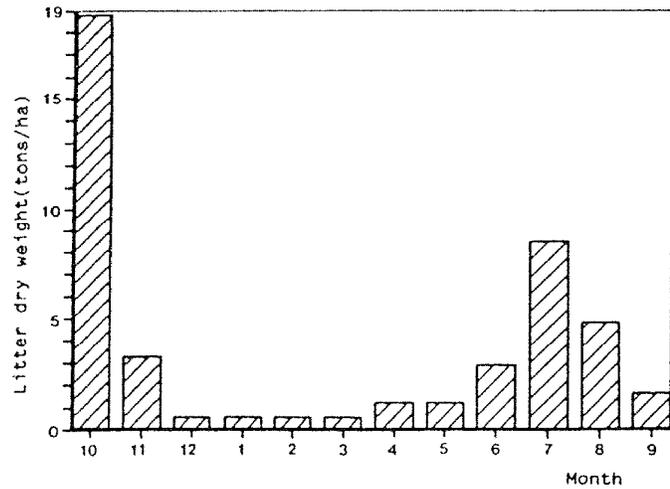
The overall seasonal pattern of module growth in Korean white pine is shown in Figure 2. A bud appeared on the top of the shoot near June 7 in this study. It appeared at the end of shoot elongation and its expansion began with the cessation of needle elongation. Such phenomena may be controlled by innate developmental program of the tree (Kozlowski, 1971), and such patterns may be efficient for allocation of limited nutrients for tree growth.

Figure 2. Growth pattern of module (bud-shoot-needle) of 15-year-old Korean white pine trees growing alone.



Most of the litters were shed during October (Fig. 3). Many litters that fell in July and August in this study were caused by storms and this abnormal litter-fall was also reported by Gwak (1986) in a *Quercus mongolica* stand.

Figure 3. Seasonal changes of litter-fall in the 28-year-old Korean white pine stand.



More living fine roots were found in autumn and early winter than dead ones, but in late winter, spring and summer, more dead fine roots were found than living ones (Fig. 4).

The results estimated from the decision matrix (Fig. 1) indicated that fine root biomass in the stand was produced mostly in the autumn (1004 kg/ha), most of them were dead during winter and spring (1914 kg/ha) and the decomposed during the summer (1398 kg/ha)(Table 1). The decomposition rate might be dependent upon soil temperature (Fig. 4) as Swift et al. (1978) reported.

Figure 4. Seasonal changes of fine root dry weights (Kg/ha) and soil temperatures at 15 cm depth in the 28-year-old Korean white pine stand.

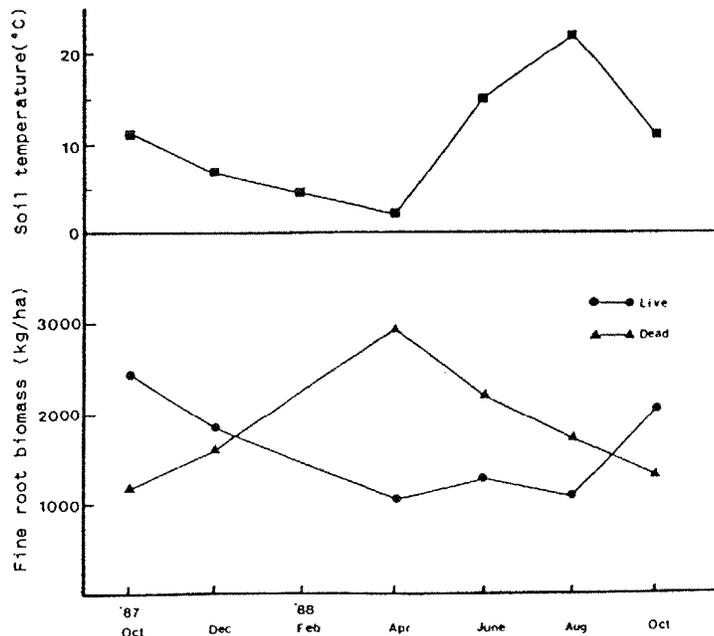


Table 1. Amount (kg/ha) of seasonal production, mortality and decomposition of fine roots in a 28-year-old Korean white pine stand

	Winter	Spring	Early summer	Late summer	Autumn	Total
Production	0	540	222	0	1004	1766
Mortality	583	1331	0	211	0	2125
Decomposition	155	0	721	677	421	1974

Various phenological events of growth modules of Korean white pine are shown in Figure 5. Most of the module growth started in spring and early summer. Kramer and Kozlowski (1979) reported that much energy was demanded during those seasons. However, the amounts of current needles which are energy sources, were the smallest during those seasons since they have not fully expanded and most of the needles also have fallen in the previous autumn. Therefore energy for tree growth was insufficient in the spring and early summer. In late summer and autumn when the needles had not yet fallen (Fig. 3) and after current needles had matured (fig. 2), energy for growth was not lacking in module growth. Surplus energy in this season may be supplied for cambial growth of the stem (Brand and Janas, 1988) and for growth of fine roots (Table 1, Fig. 5), and may be reserved for winter and the next spring (Kramer and Kozlowski, 1979).

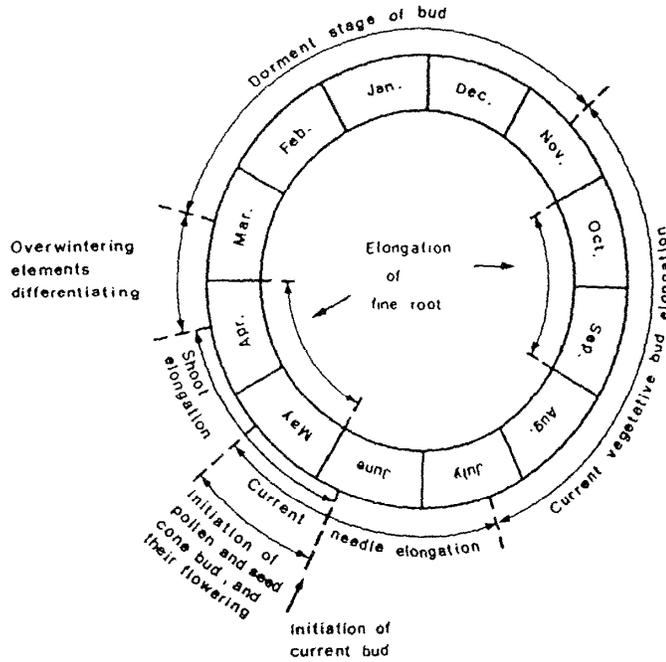
#### Conclusion

Shoot growth was continued by June 7 while current needle was by July 19. Bud appeared on the top of the shoot near June 7 when shoot elongation was ended, and its expansion began with the cessation of needle elongation.

Most of the litter-falls occurred in October but many of them fell in July and August as a result of storms and high winds.

Fine roots were produced mostly in autumn (1004 kg/ha), most of them were dead during winter and spring (1914 kg/ha) and decomposed mostly during summer (1398 kg/ha).

Figure 5. Phenology of vegetative growth module, reproductive growth module, root growth module of Korean white pine in Suwon, Korea.



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GENETIC DIVERSITY IN PINUS STROBUS  
RESULTS OF RANGE-WIDE PROVENANCE STUDIES IN MARYLAND, 1965-1990

Dr. John B. Genys

SUMMARY

About one hundred different geographic sources of eastern white pine (Pinus strobus L.) from Canada and United States were studied for twenty-five years in three Maryland plantations. Results from this long-term study are discussed in respect to diversity in germination, seed coats, cotyledons, seed weights, terminal bud formation, leaf-lengths, foliage color, survival, heights, diameters, susceptibility to ozone and sulfur dioxide, white pine blister rust, white pine weevil, and cambial electric conductivity. Included are some data from plantations in other states and from outside the United States.

Dr. John B. Genys is Professor of Dendrogenetics, University of Maryland, Frostburg, Maryland.

## Introduction

Eastern white pine is one of the most important forest trees of southeastern Canada and eastern United States. Estimates suggest about 15 million seedlings are planted annually in the United States alone. Smaller plantings of this species are established in other countries in Europe and Asia.

Studies of genetic diversity in eastern white pine were started during the post-WW II era. The largest provenance experiments were established by the U.S. Forest Service, the Natural Resources Institute of the University of Maryland, and the Forestry Department of Michigan State University (Garrett et al., 1973; Genys et al., 1978; Wright et al., 1978). The respective project leaders of these experiments were Drs. E.J. Schreiner, J.B. Genys and J.W. Wright.

## Materials and methods

This report is based on data from Maryland's experiment with more than 100 different geographic sources of *P. strobus* in the nursery and in three research plantations during the period of 1965-1990. Also, some information is utilized from plantings of the same sources in Germany, New Zealand, Australia and other states in the United States (Genys et al., 1978).

All individual experiments were arranged in four complete randomized blocks with sources represented by 4-tree plots in each block; a total of 16 trees of each source per experiment. The Maryland plantations are in three physiographic regions: (1) Coastal Plain (CP) near Annapolis, MD; (2) Piedmont Plateau (PP) near Frederick, MD; and Appalachian Mountains (AM) near Oakland, MD. Their respective elevations are 5, 150 and 790 m, and average lengths of growing season are 280, 200 and 140 days. On all sites, soil quality was average. Details on experimental design and study sites were outlined in previous publications (Genys, et al., 1978; Genys, 1987).

## Results

Germination, seed-coats and cotyledons, seed weights: In the Maryland Forest Tree Nursery, 119 seed sources of eastern white pine varied in their speed of germination (Genys, 1968). Sources from low elevations in the northern range germinated sooner than those from high elevations in the north. The advance in seed-coat shedding was significantly correlated with the same factors as the speed of germination. Also, the higher the average number of cotyledons per seedling, the later the seed-coats dropped ( $r = 0.75$ ). Cotyledon numbers, counted for 16 sources, ranged from 8.1 to 9.8. Phenotypic 1000-seed weights from different provenances ranged from 13 to 32 grams, and were inversely correlated with the northern latitude of the seed-source and with the age of mother trees ( $r = -0.58$ ).

Time of setting terminal buds and secondary growth: One-year-old trees varied in the date of terminal bud formation by 45 days (Sept 24 - Nov 8). The lateness of bud-set was inversely correlated with the latitude of the seed source ( $r = -0.77$ ). Two-year-old trees varied significantly in their tendency

to resume secondary growth after original terminal buds were formed. In different sources, the number of trees with such secondary growth ranged from 4% (Minnesota) to 58% (Maryland), and was inversely correlated with the latitude of the seed source ( $r = -0.38$ ).

Needle-length and foliage color: Needle lengths of 2-year-old trees ranged from 5.5 cm (Minnesota) to 10.0 cm (North Carolina), and were inversely correlated with the latitude of the seed source ( $r = -0.65$ ). Also, they were correlated with some other traits typical of southern provenances. Three grades were used to score foliage color of 2-year-old trees, and sources ranged from 1 = yellowish (Manitoba, Minnesota) to 3 = blueish-green (some Ohio sources and some sources in the central part of the range). Since the dark color was not common among the trees from the central range, this characteristic showed no correlation to the latitude of the seed source. Also, foliage color was studied among the 17-year-old trees in the CP and PP plantings in Maryland (Genys et al., 1978). Again, the largest number of trees with a dark green foliage came from various locations in the central range, including those from Prince Edward Island, Canada.

Survival rates: The 12-year-old trees with the poorest survival in the CP planting were from northern Ontario (74), Minnesota (1, 55) and Quebec (774). The highest mortality in the PP planting was for sources from Quebec (774), and in the AM planting, among trees from northern Ontario (73), Quebec (531), New Brunswick (530, 775) and Prince Edward Island (773). Obviously the sources with the poorest survival were from various parts of the northern range of this species.

At 16 years the survival rates of different sources in the CP planting ranged from 25 to 100% and in the PP planting from 38 to 100%. Again, the sources showing the poorest survival rates were from northern locations.

Heights: Heights were measured at various ages (1, 2, 12, and 16 years). The 12-year heights were measured in Maryland (3 plantings), Michigan (2), Maine, New York, Pennsylvania, Illinois (2), North Carolina, West Germany (2), New Zealand (2) and Australia. Some of these plantings include less than complete sets of sources. For each site, heights were expressed in percentages of planting means, and averages of these percentages from all plantings were calculated for each source (Genys et al., 1978). These particular height indices ranged from 53% (central Ontario) to 136% (673-Tennessee), meaning that the trees of the best sources were more than twice as tall as trees of the poorest source. Among other populations which were 20% or more taller than average were those from southern Ontario (77), Tennessee (7), North Carolina (84), and from planted parent trees of unknown origin in Virginia (561).

At age 16, in Maryland's CP planting, heights ranged from 48% (531-Quebec) to 124% (77-Norfolk) of the plantation mean (Genys, 1987). In addition to sources listed above, a source with outstanding height was 763 from Tennessee. In the PP planting, heights ranged from 46% (Cochran, Canada) to 125% (84-North Carolina). Other sources with 120% or larger heights were from McKean County, Pennsylvania and from western counties in North Carolina (9).

Diameters: DBH's of 16-year-old trees in the CP and PP plantings were very significantly correlated with the 16-year heights ( $r = 0.93$  and  $r = 0.92$ , respectively). However, diameters showed a broader range of variation than heights, ranging from 44 to 150% of plantation mean in CP and from 37 to 144% in PP. In the CP planting, five sources had diameters 30% or larger than the planting mean; in the PP planting, there were six such sources (Genys, 1987).

Diameters of 17-year-old trees in the AM planting ranged from 37% (76-Canada) to 126% (594-Connecticut), and were correlated with 12-year heights ( $r = 0.81$ ). In total, eight sources had 20% or larger diameters than the planting mean (Bates, 1989).

Susceptibility to ozone and sulfur dioxide: In 1977, eight different "types" of eastern white pine were subjected to increased levels of sulfur dioxide (0.45 to 0.90 ppm), and to increased levels of ozone (0.9 and 1.8 ppm) (Genys and Heggestad, 1978). Among the studied sources, the most resistant to sulfur dioxide were trees from southern Ontario (77) and the most susceptible was P. strobus cv. 'nana Kelsyi'. With respect to ozone, the most resistant was the 'Brigham' clone of unknown origin and the most susceptible was P. strobus cv. 'pumila'.

The second joint research project between the University of Maryland and the U.S. Forest Service included 16 "types" of eastern white pine (Genys and Heggestad, 1983). The specimens were treated with 1.5 ppm of sulfur dioxide for six hours. The most resistant was P. strobus cv. 'fastigiata', and the most susceptible, a source from South Carolina (66).

White pine blister rust and white pine weevil: Susceptibility of different sources to blister rust (Cronartium ribicola Fisch) was studied in Germany (Genys et al., 1978). In the nursery, the most susceptible seedlings were from Garrett County, Maryland, and the least susceptible were from adjacent Allegany County, Maryland. In the planting in Nordhorm, susceptibility ranged from 0 to 8%. The most susceptible sources were from New Brunswick and West Virginia. In Reinhausen, injury ranged from 0 to 25%, and again the most susceptible source was from West Virginia.

Susceptibility of different sources of eastern white pine to the white pine weevil (Pissodes strobi Peck.) was studied in North Carolina, New York and Pennsylvania. In New York, the sources that escaped injury were from Ontario, West Virginia, Maryland, Pennsylvania and Illinois. In Pennsylvania, sources that had less than 10% successful attacks were from Wisconsin, Minnesota, Quebec, Vermont and Pennsylvania. In North Carolina, 0 to 100% of the trees were attacked. The four non-attacked sources were Vermont (585), West Virginia (615, 563), Maryland (562) and Virginia (5). Among the least attacked in all plantings was source 563 from West Virginia.

Cambial electric conductivity: Cambial electrical conductivity of different sources was measured with a Shigometer (Model 02-67) in the AP planting in August 1987 (Bates, 1989). Conductivity levels of 107 sources of 17-year-old trees ranged from 6.4 to 11.8 kilohms. These values were inversely correlated with average diameters ( $r = -0.78$ ) and with height of 12-year-old trees ( $r = -0.78$ ) and with heights of 12-year-old trees ( $r = -0.60$ ), and were positively correlated with the latitude of seed source ( $r = 0.36$ ).

## Discussion

The information presented in this report qualifies as a broad guide on genetic diversity in eastern white pine. For specific data on any discussed characteristic, it will be necessary to consult the references cited.

A valid conclusion is that eastern white pine is a very variable species. Some sources have superior characteristics for production of wood and fiber. Also, some sources may be more resistant to various destructive agents such as ozone, sulfur dioxide, white pine weevil, etc.

## Literature cited

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POSTER SESSIONS

TEMPORAL VARIATION IN NATURAL INBREEDING IN A PINUS MONTICOLA  
POPULATION: EFFECTS ON SEEDLING MORPHOLOGY AND PARENTAL RANK

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Evaluation of parent trees using their open-pollinated progeny is common in tree-improvement programs. Although western white pine (Pinus monticola D. Don) is fairly tolerant of selfing, such progeny generally grow poorer than those from outcrossing. Strong differences in estimated inbreeding by cone parents in a single stand were found for three successive cone crops, using isozyme techniques on seeds. The effects of these differences on 2-year family height, dry weight and growth distribution, and on family rank, are assessed.

WINTER TEMPERATURE FLUCTUATIONS AS A MECHANISM  
CAUSING POLE BLIGHT ON WESTERN WHITE PINE

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The onset of crown dieback and other "poleblight" symptoms on western white pine (Pinus monticola Dougl.) in the Pacific Northwest United States and British Columbia (1936-1958) coincided with marked thaw-freeze and frost events in the fall and winter of 1935-36. In contrast to results of Leaphart and State (Ecology, 1971), an event analysis of meteorological records indicated clearly that the injury mechanism resulting in persistent dieback was not drought but freezing injuries to the xylem (viz. cavitation); hypersensitivity to heat and water stress in 1936 and in subsequent years was likely. Similar winter temperature extremes were present at the onset of forest dieback in Northern Hardwoods (easter North America) and in Norway spruce (Central Europe) suggesting the climate-cavitation-forest dieback mechanism may be universal.