highly related. The coefficients of relationship in the two stands investigated by them were estimated to be 0.23 and 0.30, i.e., equivalent to half-sibs. Inbreeding is almost universally harmful. One of its consequences is the increase in homozygosity with greater accumulation of recessive alleles which tend to be deleterious (Falconer 1960, Crow 1948). Crossing between unrelated provenances will produce progenies in which many of the deleterious genes would be rendered ineffective by dominant alleles from one parent provenance and would result in improved hybrid performance. The results of this study support the suggestion that a substantial portion of the improved growth of progenies from the seed orchard over progenies of wind-pollinated seed from natural stands may result from reduced inbreeding rather than genetic superiority of the parents (Coles and Fowler 1976).

The greater height growth of provenance hybrids of white spruce compared with trees grown from seed from a general collection in the local stand appears to be much higher than has been found for a number of other conifers (Nilsson 1973, Woessner 1972, Rehfeldt 1976, Orr-Ewing 1966). Crosses in most studies of other conifers, however, involved parent provenances from a much wider geographic area than in this one. As a result of some earlier studies in maize, it is widely believed that heterosis increases with increased genetic divergence of parent populations. More recent studies, however, have shown that heterosis increases with increased genetic diversity within a rather limited range of divergence and decreases beyond that (Moll *et* al. 1965). All the provenances involved in this study are from a relatively limited geographic range, but even within this limited range the growth of the widest crosses was relatively poor (*Figure* 1). Crosses between extremely distant provenances may cause genic imbalance in the hybrid populations and may not be beneficial (Falconer 1960).

Results of this study indicate that substantial improvement in height growth can be achieved by a reduction of inbreeding combined with selection. This could be achieved rapidly through combined progeny-provenance tests and sedling seed orchards developed on a regional basis, e.g. the ecological regions described by Hills (1961). Only the trees of the best families from the best stands would be retained to cross with each other for final seed production.

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Provenance Study of Douglas-fir in the Pacific Northwest Region

III. Field Performance at Age Twenty Years

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(Received September I November 1978)

Summary

Height growth and DBH of 20-year-old trees from 14 seed sources of Douglas-fir were assessed and analyzed in 10 outplanting areas located on the west side of the Cascade Range in the Pacific Northwest. Differences in traits measured from trees of various provenances were statistically significant at $P < 0.01$. The analysis indicated a significant interaction between seed sources and the outplanting locations. Almost all of this interaction is because of provenance G, originated from Shelton, Washington. Future research of similar genetic studies should be designed to separate the experimental and genetic influences among provenances.

Key words: Douglas-fir, provenance, genotype-environment interaction.

Zusammenfassung

Höhenwachstum und Brusthöhendurchmessern von 20 Jahre alten Douglasien, die 14 verschiedene Herkünfte repräsentieren, wurden auf zehn Versuchsflächen auf der Westseite der Kaskaden im Pazifischen Nordwesten erhoben und ausgewertet. Unterschiede zwischen den untersuchten Eigenschaften der Bäume der verschiedenen Provenienzen waren statistisch hoch signifikant mit $P < 0.01$. Die Analyse machte eine signifikante Interaktion zwischen Herkünf-

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[1]) Dr. Hinz is on leave from the Department of Statistics, Iowa State University, Ames. This is paper 1282, For. Res. Lab., Oregon State Univ., Corvallis.

ten und Versuchsflächen erkennbar. Der größte Teil der Interaktion ist auf Provenienz G zurückzuführen, die von Shelton, Washington stammt. Weitere Arbeiten an ähnlichen genetischen Studien sollten so geplant werden, daß sie eine Trennung zwischen versuchstechnischen und genetischen Einflüssen zwischen Herkünften erlauben.

Introduction

In 1954, the Forest Research Laboratory of Oregon State University and several cooperators initiated a Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) provenance study based on seed collected from 16 locations throughout the west side of the Cascades in the Pacific Northwest. Two-year-old seedlings of these provenances were outplanted in plantations established at or near each seed collection site in 1959.

Assessments for survival, height growth, and some phenological data at ages of 1, 2, 5, and 9 years from seed were reported by Ching (1965), Ching and Bever (1960), and Rowe and Ching (1973). These reports have emphasized the fact that trees from seed source C (Sugarloaf Mountain, Vancouver Island, B.C.) are generally superior in total growth in all plantations surveyed. From the early stage of growth in the nursery up to age 9 years, the tallest trees grew from the same four seed sources — B (Courtenay area, Vancouver Island, B.C.), C (Sugarloaf Mountain, Vancouver Island, B.C.), D (Mesachie Lake, Vancouver Island, B.C.), and G (Shelton, Washington). Trees from A (Nimpkish Forest, Vancouver Island, B.C.), L (Salem area, Oregon), N (Oakridge area, Oregon), and P (Butte Falls, Oregon) are still four of the five shortest ones. More recently age-related variations in growth patterns have been demonstrated in ponderosa pine and Douglas-fir (Namkoong *et al.*, 1972; Namkoong and Conkle, 1976; Campbell and Sorensen, 1978).

This paper reports the pattern of growth of these provenance trees, which are now 20 years of age in 10 different locations.

Procedure

Seed sources. — Ching and Bever (1965) gave information on location of seed collection sites and method of sampling. The main objective was to determine the natural variations in Douglas-fir in relation to climatic and geographical changes.

Plantations. — Ching (1965) showed that a reciprocal design for planting was adopted in this experiment, namely, at each seed collection site. Two plantations were established where the trees of local source were grown and compared with trees from other sources. Rowe and Ching (1973) mentioned that various disasters reduced the number of plantations available for analysis. For example, plantations were damaged by animals at locations I, J, and N; by fire at location G; by frost at locations D and H; and by drought at location P. Nevertheless, this report covers a great variety of environments, such as plantations established from the northern area of Vancouver Island, British Columbia, to Oakridge area in Oregon; from a low elevation site, such as Haney in British Columbia, to a high elevation site, such as White River Tree Farm of Weyerhaeuser Company in Washington; and from a summer droughty site at the Willamette Valley in Oregon to a high rainfall area at the Cascades.

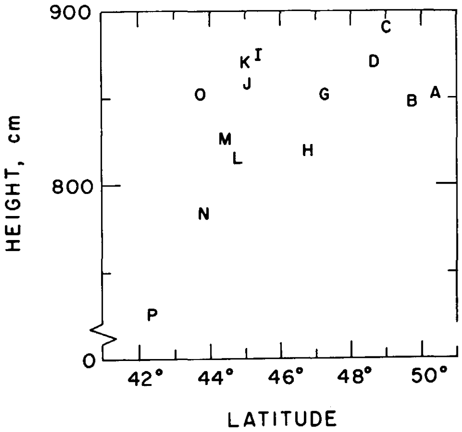

Figure 1. — Relation of height to latitude of origin for 14 provenances of Douglas-fir seedlings at age 20 years. Plotting symbols are used to denote provenances.

Table 1. — Analysis of variance of height of 20-year-old Douglas-fir seedlings planted in 10 locations and representing 14 provenances.

Source	Degrees of freedom		Mean square	F-ratio
Locations	9		1,448,285	
Elevation		1	10,188,248	28.64**
Remainder		8	355,790	
Provenances	13		17,503	4.90**
Latitude		1	81,736	6.46*
Remainder		12	12,651	
Locations by provenances	117		4,276	
Slopes		13	9,892	2.77**
Concurrence		1	27,302	3.23
Nonconcurrence		12	8,441	2.36**
Remainder		104	3,574	1.16
Pooled error	312		3,093	

** Statistically significant at 0.01.

* Statistically significant at 0.05.

Assessments. — In previous assessments, total height of all undamaged trees in plantations was measured but, by the end of the 1976 growing season, because of the time involved in taking height and diameter data from these much larger and taller trees in the plots, as well as from the silivicultural standpoint, we decided to measure diameter at breast height (DBH) and the total heights of only 25 from the original 49 trees in each plot. These 25 trees will be the final crop trees. They are undamaged and well formed and chosen to be well spaced.

Results

Height. — An analysis of the relative performance of the various provenances at different locations is shown in *Table 1*. The statistical test of the main effect of provenances shows that differences in mean heights are statistically significant at $P < 0.01$. In addition, a linear relationship between average height and provenance latitude is significant at $P < 0.05$. The slope of this relationship is 9.93 cm per degree of latitude, and has a standard error of 3.83. Inspection of the plot of height compared to latitude (*Fig. 1*) shows that this relationship is weak, and removal of only one provenance, namely provenance P, from the data will result in a nonsignificant relationship. No significant relationships of height with provenance elevation or distance from ocean were found.

The presence of a provenance by planting location interaction was tested by methods given in Mandel (1961) and Eberhart and Russell (1966). In the analysis, the slopes mean square is significant at $P < 0.01$ when tested against the remainder mean square. This implies the existence of a significant provenance by planting location interaction. Further subdivision of the sums of squares because of slopes (not shown) reveals that almost all of this interaction is because of provenance G. Trees of this provenance were shorter than average at the most severe planting locations, but taller than average at the best locations. Apart from this one exception, no statistical evidence suggested that the provenances ranked differently at the various planting

Table 2. — Overall average height and DBH of 20-year-old Dougals-fir seedlings by provenances and plantation locations. Proportions of surviving trees are for age 14 years.[1]

	Provenance means				Location means			
Provenance	Height[2] (cm)	Height adjusted for survival (cm)	DBH[3] (cm)	Survival	Location	Height (cm)	DBH (cm)	Survival
C	881.0	873.0	11.577	0.805	L	1,360.4	17.836	0 .741
I	874.1	854.6	11.604	.836	E	1,312.8	16.796	.898
K	872.2	860.0	11.538	.817	A	1,049.4	14.231	.963
D	870.0	840.3	11.242	.864	Q	921.4	11.357	.690
J	857.4	841.6	11.381	.826	B	819.4	9.907	.968
A	852.4	900.8	10.716	.653	C	691.1	9.920	.873
G	852.0	868.0	10.875	.740	M	665.3	9.059	.522
O	851.1	845.2	11.396	.800	O	633.6	8.205	.642
B	848.5	844.8	11.078	.794	K	517.5	7.164	.858
M	826.9	828.6	11.139	.779	F	408.1	6.866	.680
H	819.9	836.8	11.254	.738				
L	815.2	814.5	10.943	.786				
N	782.5	788.8	10.843	.767				
P	727.1	733.3	10.294	.767				

[1] See *Figure 2* for plantation locations.

[2] 95% LSD for height is 53.0.

[3] 95% LSD for DBH is 0.693.

locations. Thus, in the discussion of Eagles *et al.* (1977), the relative performances of the provenances can be estimated from the overall mean heights given in *Table 2* where a 95% least significant difference (LSD) also can be used to compare provenance means (Snedecor and Cochran, 1967). For example, provenances B and C do not differ significantly at the 95% level because their means differ by 32.5, which is less than the LSD value of 53.0.

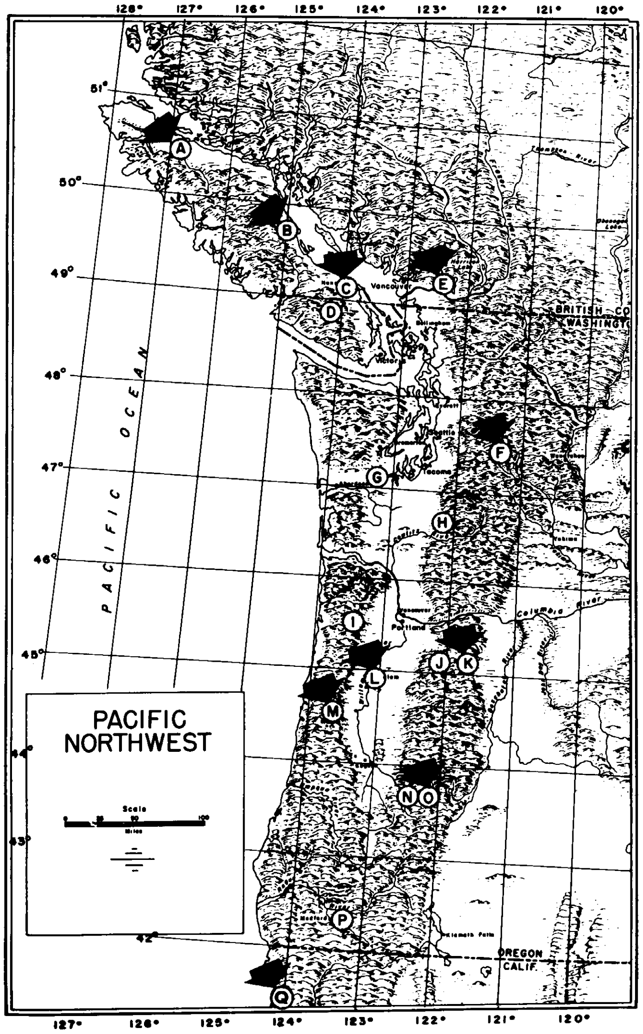

Figure 2. — Locations of plantations in provenance study of Douglas-fir. Arrows indicate those reported here. A, Nimpkish Forest; B, Courtenay area; C, Sugarloaf Mountain; E, Haney area; F, Snoqualmie National Forest; K, Molalla area; L, Salem area; M, McDonald Forest; O, High Prairie, Oakridge; Q, Korbel.

Average tree heights by planting locations are given in *Table 2.* The linear relation between average tree height and the elevation of planting location is significant at $P < 0.01$ *(Table 1).* The average height decreases 699 cm for every 1000-m increase in elevation with a 95% confidence interval from 398 to 1000 cm.

DBH. — The statistical methods used to analyze the trait DBH were the same as those used to analyze the height data. No evidence of an interaction between provenances and planting locations was found (analysis not shown). The main effect of provenances was significant at $P < 0.01$ and the 95% LSD for differences between two provenance means is 0.693. No statistically significant relation was found between average DBH of provenances and the latitude, elevation, or distance from ocean where the seeds were collected. A strong relation ($P < 0.01$) between planting elevation and DBH exists. The average DBH is estimated to decrease at a rate of 0.00843 cm per 1000-m increase in elevation with a 95% confidence interval of 0.100473 to 0.01213.

Survival. — An analysis of survival percentages was performed on data of age 14 years, using the same methods as for height (analysis not shown). An analysis of survival of age 20 years would have been more relevant but, unfortunately, the data were only partially available because thinnings were done before total counts were made in some locations. Nevertheless, interesting and useful information was obtained from the data for age 14 years. Differences among provenances were significant at $P < 0.01$. Also, the source of variation because of concurrence was significant ($P < 0.01$), indicating the presence of a location by provenance interaction; this was interpreted as a scale effect, which resulted from measuring survival as a percentage rather than provenances changing rank at different locations (Eagles *et al.*, 1977). Analysis of survival at ages 5 and 9 years leads to the same inferences as data for age 14 years. Average survivals at age 14 years by provenances and locations are given in *Table 2.*

Relation of height with survival. — An analysis of covariance of height with survival at age 14 years showed the presence of a significant ($P < 0.01$) relationship between these two variables. The regression coefficient of height on survival is $b = 369.1$, and the correlation between the variables equals 0.44. Height means adjusted to the average survival percentage of 0.7835 are shown in *Table 2.*

Table 3. — Correlations among 14 provenances for height at ages 2, 5, 9, and 20 years.

	Age (years)			
Age	2	5	9	20
2	1.00	0.68	0.69	0.46
5	0.68	1.00	0.97	0.69
9	0.69	0.97	1.00	0.75
20	0.46	0.69	0.75	1.00

Height at several ages. — The relative performances of the 14 provenances at various ages can be used to gain information on the ability to make early selections of superior genetic stock. Average height measurements at ages 2, 5, and 9 years were reported in Ching and Bever (1960), Ching (1965), and Rowe and Ching (1973) and can be used for this purpose. The data at age 2 years differ from those of the other years because the trees were still in the nursery, but later data came from 10 different outplanted locations. Also, data at age 2 years are based on only 4 replications in the nursery in contrast with 4 replications of many more trees at each of the outplanted locations 2). Although the data at age 2 years would seem incompatible with the other ages, they are still useful for comparisons. Correlations among the average heights are given in *Table 3*.

The possibility exists that some provenances might have rates of growth that are slower than average at early ages, but faster than average at older ages so that the total growth compensates or even surpasses the other provenances at later ages. If this situation were present in these data, the correlations in *Table 3* could be misleading. This question was investigated, and no statistical evidence of this tendency was found.

Discussion

The overall impression gained from the analysis of height, DBH, and survival data is that provenances differ in all traits measured, but that the interactions between provenances and locations are not of practical importance at this stage of growth. The provenance by location interaction was not significant for DBH and, although some interaction was detected for survival, no evidence was found that provenances ranked differently, depending on location. The presence of an interaction between provenance G and locations for height would appear to be an anomaly of the data. This judgment is reached by noting that the other provenances do not exhibit meaningful interaction for height, and that the other traits lack important interaction.

The relation between height and survival data is both interesting and perplexing. The significant association of height with survival found in the analysis of covariance is, of course, a relationship that applies within locations. The association is unlikely to imply cause and effect because increasing survival by better management would not increase height growth as might be suggested by the positive regression coefficient. Rather, we interpret survival as a proxy variable for, or index of, site quality. For example, better quality sites have higher survival and better growth and, therefore, height and survival exhibit a positive relationship. This interpretation would suggest that provenance mean height adjusted for survival would be a better measure of provenance performance than unadjusted mean height. Survival has been shown to differ significantly among provenances, however. Thus, provenance mean height should not be adjusted for site differences when survival differences among provenances may be because of genetic influences.

One way to assess the importance of genetic compared to site (or environmental) influences in adjusted means is to separate the data into groups that have similar survival. The uniformity of survival would tend to eliminate differences among sites. Thus, the data were divided into two groups, one with high overall survival (0.96, 0.97, 0.87, 0.90, 0.86 for locations A, B, C, E, K) and one with low overall survival (0.68, 0.74, 0.52, 0.64, 0.70 for locations F, L, M, O, Q). The average heights for provenances at high survival locations were correlated with those from low survival locations, resulting in $R^2 = 0.25$. A similar correlation was calculated, using adjusted provenance means. In this instance, the value of R^2 improved to 0.51. This improvement suggests that the adjustment has reduced the experimental errors associated with measuring provenance performance. Thus, overall survival of provenances seems to be a proxy variable for unknown variables associated with the complex activities involved in establishing plantations, such as seed collection, nursery management, lifting practices, and planting practices. Adjustment for survival improves correlations among provenances and, hence, can be regarded as partially eliminating experimental errors associated with differences among provenances. This conclusion presents a contradiction. On one hand, a standard analysis suggests that survival differences among provenances is because of genetic factors but, on the other hand, survival differences are a measure of experimental errors among provenances. Most likely both aspects are involved. Additional evidence suggesting the experimental error role of survival data is obtained by noting that R^2 between mean provenance height and latitude is 0.36, but improves to 0.53 when adjusted means are used. Obviously, data from this experiment can not be used to separate the experimental and genetic influences among provenances. Future research, however, to resolve these questions or identify these influences seems imperative. Unless this can be done, results of similar studies will lead to erratic results when the unknown influences are not controlled.

This study provides no evidence to suggest at the 20th year of growth that a provenance is "best adapted" to the location from which it came. The lack of rank changes implies that the same provenances perform best, regardless of the planting location. This conclusion apparently contradicts other evidence of local adaptation in traits, such as drought resistance, time of bud burst, and bud set. Evidently, at this stage of development, the overall impact of local differences in these traits has not been sufficiently important to change rankings of provenances. Plantations established at other times could lead to different results and conclusions because of different climatic conditions. A wide variety of conditions was included in this study, however, as evidenced by the vastly different average height growth at locations F (408 cm) and L (1360 cm). These different locations provide some measure of the effects of broad variations in climate and tend to diminish the practical importance of planting "locally adapted" provenances.

The evaluation of provenance or genotypic performances must necessarily be based on dependable early test results when rotations are long. The analysis of our provenance data gives hope that early evaluation can be used to suc-

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2) Data from some replications at the outplanted locations were not used in the final analysis for reasons such as extremely low survival, slow growth in a frost pocket, and excessive animal damage.

cessfully screen for superior provenances. This conclusion is based on the fact that provenance by environmental interactions (when they exist) are because of scale differences rather than actual changes of rank. This would imply that results of test plots in one environment can be applied at other untested environments. Also, correlations among provenances at different ages are large enough to encourage the use of early results as predictions of future performance. For example, the correlation between height means for ages 5 and 20 years is 0.67. These correlations change little if adjusted rather than unadjusted means are used. The largest changes were from 0.46 to 0.32 between ages 2 and 20 years and from 0.75 to 0.67 between ages 9 and 20 years. All other correlations change by 0.03 or less. (The data at age 2 years were not adjusted because no attempt was made to measure nursery survival.)

Because of the uncertainties involved in evaluating provenances or genotypes, viewing early test results as a means of eliminating poor genetic material may be more profitable than selecting superior stock. The material that is not eliminated would form a mixture of a broadly based source of genetic material that could be used for reforestation. Although some theoretical loss in growth potential may occur by not using the best genotype, the difficulties in determining the best stock combine with the potential for ecological disaster from the use of genetic monocultures to argue against using a narrow genetic base for reforestation.

Acknowledgement

The authors greatly appreciate the assistance in collecting field data provided by foresters from Simpson Redwood Co., Weyerhaeuser Co., University of British Columbia, Canadian Forest Products, Ltd., Crown Zellerbach Canada, Ltd., and MacMillan and Bloedel, Ltd.

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New Methods to Prepare Squashes to Study Microsporogenesis in Pinus resinosa Ait. I. Formulas Based on Glycerin, Water and Dimethylsulfoxide

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(Received August / November 1978)

Abstract

A new method was developed for the preparation of squashes of microstrobili of *Pinus resinosa* for the study of microsporogenesis. The tissues were fixed in ethanol-acetic acid (3 : 1 v/v), and stored in ethanol-glycerin-water (1 : 1 : 1 v/v). To 10 columes of glycerin-water-dimethylsulfoxide (2 : 1 : 7 v/v) (GWD), 1 volume methyl salicylate and 1 volume chloral hydrate stock (20 g of chloral hydrate in 100 ml GWD) were added (GWDMC). The GWDMC was preheated to 170° C, the tissues were dropped into the hot GWDMC and kept at 170° C for 20—40 minutes. If the tissues were stained in carmine, the carmine was added to GWDMC before heating to 170° C. Toluidine blue and nigrosin did not tolerate heating to 170° C, and tissues were stained in these at room temperature after the GWDMC treatment was completed. Cell separation was excellent. Meiotic prophases were well cleared and showed details of chromosomes well. In the stages from metaphase to tetrads, the cytoplasm was often somewhat contracted. Microspores were well cleared and stained.

Key words: Pinus resinosa, microsporogenesis, cytology, squashes, meiosis.

Zusammenfassung

Es wurde eine neue Methode zur Herstellung von Quetschpräparaten von Mikrostrobili auf der Basis von Glycerin, Wasser und Dimethylsulfoxyd bei *Pinus resinosa* Ait. entwickelt, durch welche insbesondere die Phasen der Meiose gut aufgehellt werden.

Introduction

The most commonly used fixative-stain for the preparation of squashes for karyological studies of conifer tissues is aceto-carmine. Because this fixative-stain has some drawbacks, an alternative method was developed using lactic acid, propionic acid, and dimethylsulfoxide as the carmine solvent (Bonga and Venketeswaran, 1976). Later, a new improved method based on glycerin, water, and dimethylsulfoxide was developed for root tip squashes of conifers, which allowed squash preparation at higher temperatures, (Bonga, 1977). However, subsequent investigations showed that although before diakinesis the chromosomes fixed, stained, and spread well, this method was not fully satisfactory for the study of microsporogenesis of conifers,