

## STABILITY ACROSS SITES OF DOUGLAS-FIR PROVENANCES IN NORTHERN SPAIN

R. Zas\*, E. Merlo, R. Díaz & J. Fernández-López

Dpto. Producción Forestal, Centro de Investigaciones Forestales y Ambientales de Lourizán. Apdo. 127, 36080 Pontevedra, Spain; phone: +34 986 805067; fax: +34 986 856420; E-mail: rzas@sfp.cifl.cesga.es.

\* to whom correspondence should be addressed.

Received October 3, 2002; accepted May 8, 2003

### ABSTRACT

This study examines the stability across sites for height growth at different ages in a multi-site Douglas-fir provenance test in Northern Spain in order to study its implications for provenance use recommendations and breeding strategies. A total of 89 IUFRO provenances, planted on 16 sites, were analysed. Type B correlations were used to examine the relation among geographical differences between sites and their site-to-site correlations. The site index differences between sites was the only variable that explained the Type B correlation variation, indicating that  $P \times E$  interaction arises from site index differences between test sites. The lack of significant correlations between geographical differences and Type B correlations between sites, suggested that all the studied area should be considered as a unique breeding zone. Despite the relative high Type B correlation within all the studied area ( $r_B > 0.6$ ), the analyses of variance indicated a high relative importance of the  $P \times E$  interaction, especially at the earlier stages. This interaction must be considered for selection and provenances recommendation. It was concluded that selection should be made for stability and overall good performance within the whole area. Provenance stability over the site index variation was analysed by the joint regression analysis. Significant stability differences were found among provenances. Considering both the overall performance and the stability, 11 provenances were recommended for Northern Spain. Most of these provenances come from North Oregon and South Washington, from latitudes north of 45° N.

**Key words:** *Pseudotsuga menziesii*, provenances, genotype  $\times$  environment interaction, stability, breeding zones.

### INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) was introduced in Northern Spain several decades ago. Although the area planted with this species is not more than 30,000 ha (VEGA *et al.* 1998), the interest of foresters in Douglas-fir is increasing during the last years due the high growth rates found in some stands (GARCÍA *et al.* 1996). In Northwest Spain, Douglas-fir became the fourth most important species planted during the last decade. Most of these old and new plantations used improved seed from breeding programs developed in neighbouring European countries or seeds from American provenances. Adaptedness of these and other seed sources to our especial environment conditions should be tested (MERLO 2002).

During the 1970's several provenances tests of the IUFRO collection were planted in Northern Spain. Some results and recommendations about the best seed sources for reforestation have been published (TOVAL *et al.* 1993, VEGA *et al.* 1993, 1998). However, little attention has been paid toward analysing the magnitude and the consequences of the genotype  $\times$  environment

( $G \times E$ ) interaction in these provenances trials. The concept of  $G \times E$  interaction is defined as the varying relative performance of genotypes with environment (BURDON 1977). If  $G \times E$  is present and is quantitatively important, two major alternatives could be considered. The first approach is to define breeding zones that fit groups of genotypes with a similar pattern of  $G \times E$  interaction and to select, independently within each zone, specifically adapted genotypes (ERIKSSON & EKBERG 2001). The second approach is to identify good general performers to use across a wide range of sites (FINLAY & WILKINSON 1963). However, as BURDON (1977) pointed out, it is debatable whether the emphasis in tree breeding should be on producing genotypes suitable for specific conditions or genotypes suited to a wide range of environments. Both alternatives have their own disadvantages. Regionalisation is costly and the additional expense may not be recouped by increased gains (JOHNSON & BURDON 1990). Moreover, environmental variability within a region can be as high as between regions (MATHESON & COTTERILL 1990). On the other hand, the evidence for the existence of truly broadly adapted genotypes is questionable

(PSWARAYI *et al.* 1997).

In this paper, we analyse the stability across sites of 89 Douglas-fir provenances in Northern Spain using several statistical methods in order to study its implications for provenance use recommendations and breeding strategies.

## MATERIAL AND METHODS

### Genetic material, test design and assessment

A series of trials was planted at 16 locations in Northern Spain (Figure 1) between 1978 and 1981 to evaluate a total of 89 IUFRO Douglas-fir provenances from Southern British Columbia, Washington, Oregon and Northern California. Figure 2 shows the geographic origin of the provenances, and details of the sites included in this study are presented in Table 1. A complete description of the 89 seed sources and the 16 plantation sites can be found in TOVAL *et al.* (1993). Two of the plantation sites (Hermida and Carballa) contained 87 of the 89 provenances. The remaining 14 sites received a subset of the seed source collection varying between 4 and 31 provenances per site (Table 1). Each plantation, with the exception of Conforcal, followed a randomized complete block design with 3 replications. The two principal plantations (Hermida and Carballa) had 25-tree plots, whereas the remainder had 81 tree-plots. Conforcal followed a randomized complete block design with 9 replications and 5-tree plots. However, these 9 replications in Conforcal were grouped into 3 blocks to homogenize the analysis procedure among all sites. Spacing was 3 × 3 m in all sites.

Height ( $H$ ) and diameter at breast height ( $DBH$ ) were measured in all plants at each site at different ages

(Table 1). Height and diameter measurements were adjusted to the standard reference ages of 3, 5, 10 and 16 years old by adding or subtracting the average annual plot growth between the measured age and the nearest ages. For example, the 11-year measurement at Carballa was corrected by subtracting the average annual plot growth between ages 5 and 11, and 11 and 18, whereas the 9-year measurement at Hermida was corrected by adding the average annual plot growth between ages 5 and 9.

All analyses were based on mean values per plot. As part of the data preparation, a plot of height ×  $DBH$  was inspected visually when both variables were measured at the same ages. Trees which were outliers (*i.e.*, those which had abnormal height-diameter ratios) were deleted from the data set.

### Analytical methodology

Three types of statistical analyses were carried out: single-site analyses, paired-site analyses and multi-site analyses.

(1) Single site analyses were conducted for each test and age. The linear model was:

$$Y_{ij} = \mu + P_i + B_j + E_{ij}$$

where  $Y_{ij}$  is the value of the  $i^{\text{th}}$  provenance at block  $j$ ,  $\mu$  is the site mean,  $P_i$  is the random effect of the  $i^{\text{th}}$  provenance,  $B_j$  is the random effect of the  $j^{\text{th}}$  block, and  $E_{ij}$  is the experimental error. Variance components were estimated using the PROC VARCOMP METHOD = TYPE1 in SAS (SAS 1989). Provenance was considered a random effect in order to compare provenance variation among sites. Provenance variation was estimated using the estimator  $P_b^2$  (HODGE & DVORAK

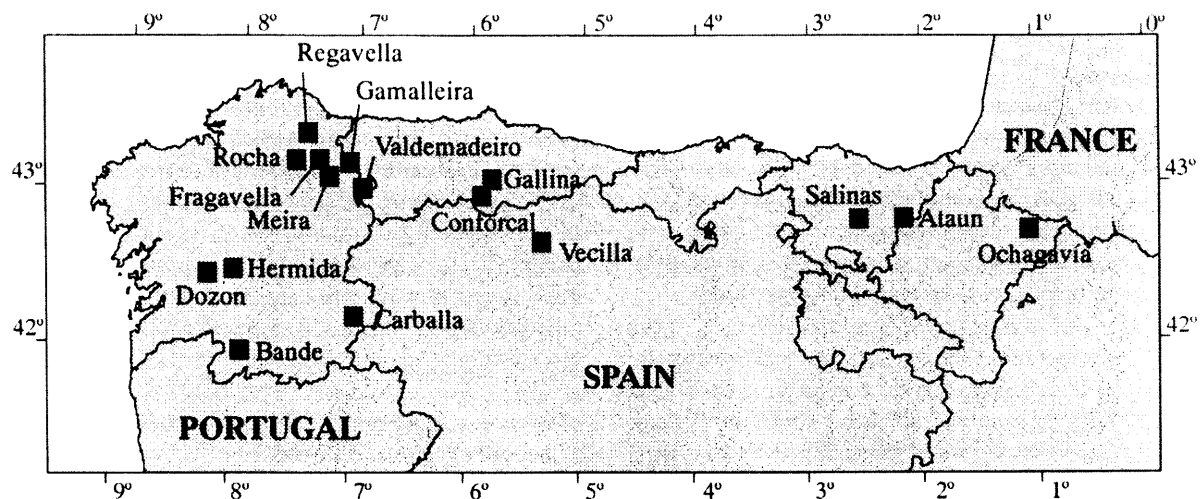
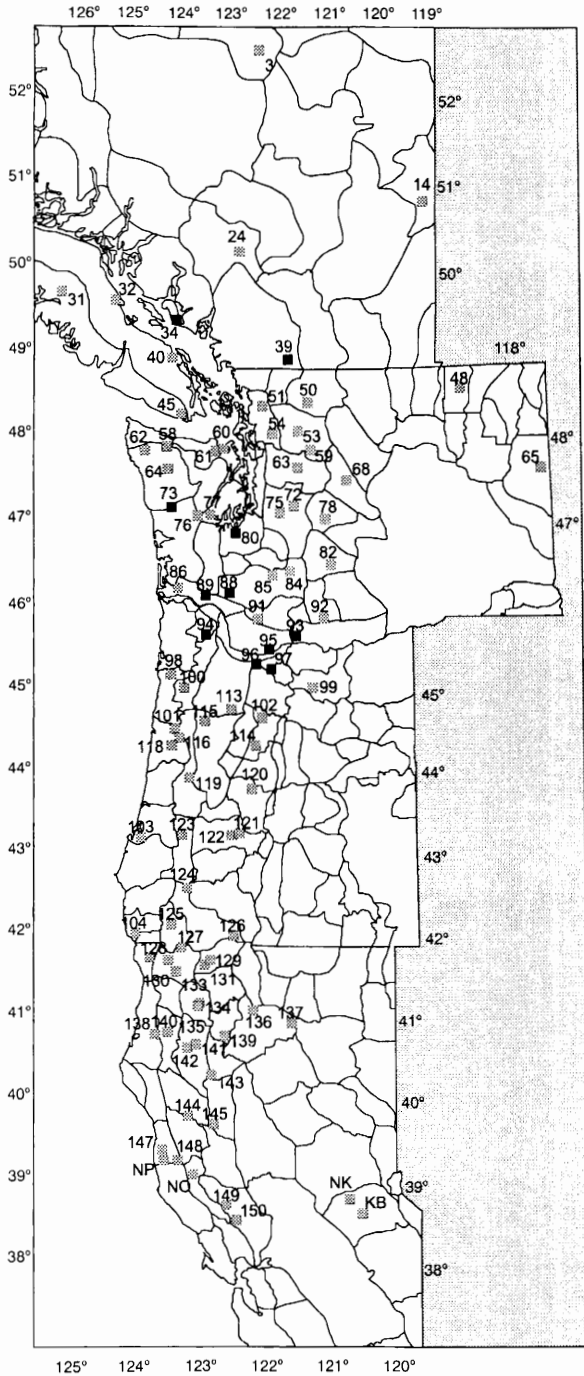


Figure 1. Location of test sites.



**Figure 2.** Geographic origin of the IUFRO's Douglas-fir provenances assessed in Northern Spain. Black squares are the recommended provenances for North Spain based on the results of this paper.

variation among sites. Provenance variation was estimated using the estimator  $P_b^2$  (HODGE & DVORAK 1999):

$$P_b^2 = \frac{\sigma_p^2}{\sigma_T^2}$$

where  $\sigma_p^2$  is the provenance variance and  $\sigma_T^2$  is the phenotypic variance. The  $b$  subscript indicates that the provenance variance was estimated on a single-site basis, and may be biased by the presence of provenance  $\times$  environment interaction. Specifically,  $\sigma_p^2 = \sigma_p^2 + \sigma_{p \times e}^2$ , where  $\sigma_p^2$  and  $\sigma_{p \times e}^2$  are the provenance and provenance  $\times$  environment variance in a multiple-site model (HODGE & DVORAK 1999).

(2) Paired-site analyses were conducted for all possible pairs of tests with at least 3 provenance in common in order to quantify the provenance  $\times$  environment interaction between sites. The analyses were carried out including only the common provenances. The linear model was:

$$Y_{ijk} = \mu + P_i + S_j + PS_{ij} + B(S)_{jk} + E_{ijk}$$

Where  $Y_{ijk}$  is the value of the  $ijk^{\text{th}}$  plot,  $P_i$  is the random effect of the  $i^{\text{th}}$  provenance,  $S_j$  is the random effect of the  $j^{\text{th}}$  site,  $PS_{ij}$  is the interaction between the  $i^{\text{th}}$  provenance and the  $j^{\text{th}}$  environment,  $B(S)_{jk}$  is the random effect of the  $k^{\text{th}}$  block within the site  $j$ , and  $E_{ijk}$  is the experimental error. Variance components were estimated using the PROC VARCOMP METHOD = TYPE1 in SAS (SAS 1989).

For each pair of sites, estimates of the Type B genetic correlation ( $r_B$ ) at the provenance level was calculated as follows (BURDON 1977):

$$r_B = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_{p \times e}^2}$$

where  $\sigma_p^2$  and  $\sigma_{p \times e}^2$  are the provenance and provenance  $\times$  site interaction variance components, respectively (negative variance components were set to zero). Type B correlations range between 0 and 1 and measure the provenance  $\times$  environment interaction between the two sites; a  $r_B \sim 1$  indicates a strong correlation between performance in the two sites, i.e., parallel reaction norms. Type B correlations were only estimated if the estimator  $P_b^2$  exceeded 0.05 in both sites of the pair. Use of sites with extremely low  $P_b^2$  estimates can result in seemingly very imprecise Type B correlation (HODGE & DVORAK 1999).

Environmental differences between each pair of sites were examined to find explanations for differing correlations among sites. Each pair of sites was classified as 'same' or 'different' in relation to the differences (lower or higher than the mean differences, respectively) in environmental variables between both sites. The environmental variables considered were: site index differences (overall site height mean) at age 5, geographic zone (see Table 1), and distance, latitude, longitude and altitude differences between sites.

Table 1. Features of field trials.

Geographic zone / site	<i>N<sub>p</sub></i>	Plantation date	Altitude (m)	Aspect	Slope (%)	Geology	Soil depth (cm)	Topsoil pH	Soil texture	<i>P</i> (mm)	<i>T</i> (°C)	Ages measured
<b>South Galicia</b>												
Bande	22	Dec 78	900	N	30	Slate	55	4.5	Sandy loam	1294	10.1	5,10,16
Carballa	87	May 78	1360	SW	10	Sandstone	50	4.6	Sandy loam	1379	8.2	5,11,18
Dozon	5	Nov 80	520	NE	5	Schist	75	4.3	Sandy loam	1324	11.9	3,5,10
Hermida	87	Nov 78	700	N	30	Schist	85	4.6	Loam	1677	10.9	4,5,9
<b>North Galicia</b>												
Fragavella	20	May 79	580	NW	13	Granite	90	4.5	Sandy loam	1146	11.1	5
Gamalleira	21	May 80	660	NE	30	Slate and sandstone	85	4.7	Sandy loam	926	11.0	5,10
Meira	19	Apr 80	850	SE	8	Slate and sandstone	100	4.0	Sandy clay loam	1131	10.2	5
Regavella	21	Dec 78	450	-	-	Schist	60	5.0	Sandy clay loam	1501	11.6	5
Rocha	4	Dec 78	520	NE	35	Slate	120	5.0	Loam	989	11.5	5,10
Valdemadeiro	19	Jan 80	920	SW	10	Slate	100	4.5	Sandy loam	1207	10.0	5,10
<b>North central Spain</b>												
Conforcal	18	Apr 81	850	N	55	Salte	75	4.3	Clay loam	1230	9.8	2,6,10,17
Gallina	12	Apr 81	600	N	10	Slate	50	4.8	Clay loam	1230	10.7	2,6,10,17
Vecilla	9	Apr 81	1160	N	30	Conglomerate	75	4.8	Sandy loam	1120	8.7	6,10,17
<b>North east Spain</b>												
Ataun	15	Mar 80	750	E	30	Calcareous clay	90	4.4	Sandy clay loam	1468	10.1	3,5,11,16
Ochagavia	33	Mar 80	900	W	40	Sandstone	100	5.3	Clay loam	1009	10.3	5,15
Salinas	15	Mar 80	900	W	10	Flysch series	90	4.4	Sandy loam	1500	9.3	5,11,16

Note: *N<sub>p</sub>*: number of provenances assessed at each site; *P*: annual mean precipitation; *T*: annual mean temperature.

Differences in  $r_B$  between two different groups of pairs ('same' versus 'different') were tested by the mean comparison t-test analysis. In addition, correlation analysis was used to find associations between Type B correlations and environmental differences between sites.

A second approach was used to estimate the mean Type B correlation within a group of pair of tests. This approach was based on the mean of the variance components estimated from pairs of tests of a given group (HODGE & DVORAK 1999):

$$r_B^* = \frac{\bar{\sigma}_p^2}{\bar{\sigma}_p^2 + \bar{\sigma}_{p \times e}^2}$$

where  $\bar{\sigma}_p^2$  and  $\bar{\sigma}_{p \times e}^2$  are the mean of the provenance and provenance  $\times$  environment variance components estimated from paired test analyses within a group of pairs of tests. Estimating an average genetic parameter based on the mean components instead of averaging the single parameter estimates has been shown to be more precise, especially when provenance and provenance  $\times$  environment variance components are estimated with only a few degrees of freedom (PEDERSON 1972, HODGE & DVORAK 1999).

(3) Multi-site analyses were carried out selecting those 32 provenances assessed in at least 3 sites at ages 5, 10 and 16. These analyses were done in order to evaluate the evolution over time of the provenance and provenance  $\times$  environment interaction effects. The linear model was the same as the one described for the paired-site analyses. Variance components were calculated using the PROC GLM, type IV mean square estimations due the extreme imbalance (SAS, 1989). Furthermore, joint regression analyses were done to analyse the provenances stability upon the site index variability. These analyses were carried out selecting the 82, 70 and 32 provenances assessed in at least 3 sites at ages 5, 10 and 16, respectively. Provenance mean heights at each site were regressed upon a site index (FINLAY & WILKINSON 1963):

$$PS_{ij} = \beta_i I_j + \delta_{ij}$$

where  $\beta_i$  is the departure of the linear regression coefficient of the  $i^{\text{th}}$  provenance from the overall linear regression coefficient,  $I_j$  is the environmental index of the site  $j$ , and  $\delta_{ij}$  is the deviation from the regression line of the  $i^{\text{th}}$  provenance at the  $j^{\text{th}}$  site.

The regression coefficient of each provenance upon the site index,  $b_i$ , where this coefficient estimates  $1 + \beta_i$  (FINLAY & WILKINSON 1963), was considered as a stability estimator of the given provenance in relation to the site quality variability. We may say that prove-

nances are highly stable and better adapted to poor sites if  $b_i < 1$ . A provenance with  $b_i = 1$  was considered to be an average stability and equally adapted to poor and good sites. If  $b_i > 1$ , the provenance was of low stability and better adapted to good sites. As suggested by FINLAY & WILKINSON (1963), this stability estimator was plotted against the mean provenance height across all the sites to select those provenances with good overall performance and high stability.

For estimating the coefficients  $b_i$ , first, the overall site height mean was considered as a site index. Because not all the same provenances were present in all the sites, a reiterative procedure was used to adjust the site indices estimates. This procedure consisted in (i) estimation of the regression coefficients  $b_i$ , (ii) estimation of the provenance heights in those sites where the provenance is absent using the preceding regression equations, and (iii) recalculation of the overall site mean. These three steps were successively carried out until the convergence of the coefficients  $b_i$  of all the provenances included in the analysis (it was considered that the coefficients  $b_i$  converged when the differences between its estimates in two consecutive steps were less than 0.0001).

## RESULTS AND DISCUSSION

### Provenance variation

Height growth was extremely variable across sites. Mean height at age 16 varied between 2.5 m and nearly 12 m (Table 2). Growth was generally lower than that observed in other Douglas-fir plantations in Northern Spain (GARCÍA *et al.* 1996). Sites are included in French site index classes II and III. Some sites, as Carballa, can be considered as off-site for Douglas-fir establishment. The inclusion of such off-sites may be useful for interpreting the genotype  $\times$  environment interaction (PSWARAYI *et al.* 1997). However, the lack of high quality sites in the trial series can be a handicap for an adequate interpretation of the P  $\times$  E interaction.

Provenance variation for height growth was also very variable across sites (Table 2) probably due to the different subsets of provenances assessed at each site and the important site index differences among sites. The percentage of the phenotypic variation attributable to the variation between provenances ranged from 0 to 92 % and averaged 43 %. In Southwest Oregon, breeding zones accounted for 47.2 % of the total variation for total height (LOOPSTRA & ADAMS 1989).

A slight trend of higher provenance variation at the better sites can be observed (Table 2). The estimated parameter  $P_b^2$  was significantly correlated with the

**Table 2. Mean, standard deviation (std) and provenance variation ( $P_b^2$ ) for height measured at ages 3, 5, 10 and 16 (H3, H5, H10, H16) in all the sites analysed.  $N_p$  = number of provenances assessed at each site.**

Site	$N_p$	H3 (cm)			H5 (cm)			H10 (cm)			H16 (cm)		
		Mean	Std	$P_b^2$	Mean	Std	$P_b^2$	Mean	Std	$P_b^2$	Mean	Std	$P_b^2$
Ataun	15	78.4	22.2	0.92	165.2	38.6	0.69	511.1	141.4	0.78	1157.4	175.4	0.66
Bande	22				89.1	28.1	0.63	259.8	90.6	0.50	559.3	189.5	0.45
Carballa	87				38.0	13.5	0.12	92.1	55.6	0.00	243.8	166.2	0.00
Conforcal	18	89.7	22.1	0.63	141.5	33.4	0.64	397.8	66.8	0.45	747.2	105.5	0.52
Dozon	5	47.1	11.8	0.06	72.8	17.0	0.73	132.9	38.3	0.29			
Fragavella	20				37.1	13.5	0.51						
Gallina	12	64.4	20.4	0.78	97.1	31.5	0.59	247.0	75.0	0.40	567.0	149.8	0.26
Gamalleira	21				103.1	36.3	0.58	251.3	105.1	0.36			
Hermida	87	35.5	12.1	0.42	68.2	18.6	0.36	192.4	64.9	0.09			
Ochagavia	33				142.7	26.4	0.63				1181.1	109.8	0.51
Regavella	21				42.9	10.3	0.21						
Rocha	4				82.2	28.0	0.40	302.6	82.2	0.63			
Salinas	15				107.8	28.3	0.00	227.1	116.3	0.00	685.9	239.6	0.00
Meira	19				53.8	14.0	0.14						
Valdemadeiro	19				61.2	12.8	0.75	85.3	23.9	0.22			
Vecilla	9				59.3	17.3	0.45	242.4	55.9	0.53	517.8	99.9	0.59

**Table 3. Mean Type B provenance correlation estimates for height at ages 3, 5, 10 and 16 in pairs of tests grouped in relation to several criteria.**

	Mean	Geographic zone			Distance			Altitude			Site index (age 5)		
		Same	Diff.	$p < T$	Same	Diff.	$p < T$	Same	Diff.	$p < T$	Same	Diff.	$p < T$
<b>Age 3</b>													
$N$	8	1	7	-	1	7	-	6	2	-	2	6	-
$r_B$	0.63	0.91	0.59	-	0.91	0.59	-	0.53	0.95	-	0.57	0.66	-
SE ( $r_B$ )	0.12	0.91	0.13		0.91	0.13		0.14	0.05		0.15	0.16	
$r_B^*$	0.77		0.73			0.73		0.70	0.94		0.65	0.82	
<b>Age 5</b>													
$N$	47	14	43	n.s.	30	27	n.s.	32	25	n.s.	25	32	**
$r_B$	0.64	0.59	0.66	n.s.	0.64	0.65	n.s.	0.69	0.58	n.s.	0.74	0.56	**
SE ( $r_B$ )	0.03	0.08	0.04		0.05	0.06		0.04	0.06		0.05	0.05	
$r_B^*$	0.66	0.62	0.68		0.63	0.70		0.67	0.63		0.77	0.61	
<b>Age 10</b>													
$N$	15	3	12	n.s.	8	7	n.s.	9	6	n.s.	6	9	n.s.
$r_B$	0.70	0.78	0.68	n.s.	0.68	0.72	n.s.	0.72	0.67	n.s.	0.78	0.65	n.s.
SE ( $r_B$ )	0.07	0.17	0.06		0.11	0.07		0.07	0.13		0.07	0.10	
$r_B^*$	0.62	0.83	0.59		0.77	0.57		0.70	0.52		0.71	0.57	
<b>Age 16</b>													
$N$	10	3	7	n.s.	3	7	n.s.	5	5	n.s.	3	7	n.s.
$r_B$	0.72	0.60	0.77	n.s.	0.56	0.79	n.s.	0.75	0.69	n.s.	0.73	0.71	n.s.
SE ( $r_B$ )	0.09	0.30	0.04		0.29	0.03		0.03	0.18		0.04	0.13	
$r_B^*$	0.73	0.67	0.74		0.59	0.75		0.76	0.68		0.74	0.72	

Note: Type B genetic correlations estimated for a pair of test only in  $P_b^2 > 0.05$  in both tests. Abbreviations are as follow:  $N$  = number of pairs,  $r_B^*$  = ratio of means,  $r_B$  = mean of ratios (negative variance components set to zero), SE ( $r_B$ ) = empirical standard error of the mean of the ratios. Significant levels: \*\*:  $p < 0.05$ , n.s.: no significant. Diff. = different.

overall height site mean at age 5 ( $r = 0.50$ ,  $p < 0.001$ ). The explanation behind this pattern may be that differences between genotypes and heritability estimates are likely to be larger in the best rather than the poorest environments (HILL *et al.* 1992). However, site index did not affect the heritability in Douglas-fir progeny tests in Western Oregon (JOHNSON *et al.* 1997).

Results from Table 2 also show that provenance variation becomes less important with age. Mean  $P_b^2$  was 0.56, 0.46, 0.35 and 0.37 at ages 3, 5, 10 and 16, respectively. However, this trend was not consistent from site to site. Some sites, such as Vecilla showed the opposite trend whereas, in others, no clear trend can be observed. Other Douglas-fir studies show that there is no clear trend in heritability before age 12 whereas, after age 12, heritability shows a consistent increasing trend (NAMKOONG *et al.* 1972, JOHNSON *et al.* 1997).

### Paired-site analyses and genetic correlation

Mean Type B correlations ( $r_B$ ) for different sub-classification of test pairs were examined to try to identify patterns of  $P \times E$  interaction (Table 3).

The overall mean Type B correlation increased with time from  $0.63 \pm 0.12$  at age 3 to  $0.72 \pm 0.09$  at age 16, which may suggest a reduction of the  $P \times E$  interaction with time. JOHNSON *et al.* (1997) found similar trends in Douglas-fir progeny tests in Western Oregon. However it must be noted that mean Type B correlations are relatively imprecise due to many few degrees of freedom, resulting in high standard errors. Differences between the mean Type B correlation ( $r_B$ ) and the 'function of the mean' estimations ( $r_B^*$ ) were variable and relatively high, especially when a low number of pairs were used in its estimations. As HODGE & DVORAK (1999) pointed out, further breakdowns of the data become somewhat less reliable as number of pairs decreases. In this sense, data from age 5, available for all the 16 sites, is apparently the most useful for comparing Type B correlations between different pair groups.

Differences in mean Type B correlation between pairs 'same' or 'different' were only significant at age 5 when the site index difference was the discriminatory criteria (Table 3). Both, mean  $r_B$  and  $r_B^*$ , were higher for pairs of tests of similar site index ( $r_B = 0.74 \pm 0.05$ ,  $r_B^* = 0.77$ ) than for pairs of tests with different site index ( $r_B = 0.56 \pm 0.05$ ,  $r_B^* = 0.61$ ). These differences are maintained with time but they become insignificant probably due to the decrease in the number of pairs considered. On the other hand, there were no consistent differences in  $r_B$  or  $r_B^*$  between pairs of tests from the same or from different geographic zone.

**Table 4. Pearson correlation coefficients between Type B genetic correlations estimated at ages 3, 5, 10 and 16 ( $r_{B3}$ ,  $r_{B5}$ ,  $r_{B10}$  and  $r_{B16}$ ) and features differences between the sites of each pair.**

Type B genetic	Differences between sites				
	Long.	Lat.	Distance	Altitude	SI
$r_{B3}$	-0.68	-0.21	-0.68	0.31	-0.22
$r_{B5}$	0.02	0.10	0.03	-0.12	-0.38*
$r_{B10}$	-0.14	0.08	-0.13	-0.09	-0.34
$r_{B16}$	0.22	0.04	0.23	-0.18	-0.64*

Note: \*: Significant at  $p < 0.05$ ; SI: Site index.

These results agree with those found in Table 4. Type B correlation at age 5 and 16 were significantly and negatively correlated with the site index differences between both test of each pair, but there were no trends indicating that correlations between sites varied with distance or longitude, latitude or altitude differences. Test locations ranged over 600 km,  $7^\circ$  longitude,  $2^\circ$  latitude and more than 900 m altitude but Type B correlation did not seem to follow the expected pattern as judged from the environmental variation among sites. Site quality was the only variable that has some influence in the correlations. These results underscore the difficulties in delineating breeding units for Douglas-fir in Northern Spain. Two other important conclusions can be drawn from these results. First, the relationship between the  $P \times E$  interaction and the site quality suggests that large site index differences between test sites and commercial production land will decrease the reliability of the selection results obtained in these tests. In this sense, the lack of high quality sites in our trial series can be interpreted as a handicap for good provenance recommendation for Northern Spain and thus, this recommendation may only be useful for low to mid quality sites. BASTIEN & ROMAN-AMAT (1990) and HILL *et al.* (1992) suggest that genetic tests can be planted on higher quality sites, such as abandoned agricultural land or that more intensive cultural practices, such as fertilisation or competition control, can be used in genetic tests. The intent is to increase environment uniformity, allow genetic differences to be more readily observed and increase heritability of the trait measured (HODGE & WHITE 1992). However, if  $P \times E$  interaction is related to the site index, as is the case here, the increase genetic gain may be offset by the reduction of genetic correlation between the trait measured (e.g. growth on a high quality site) and the target trait which the breeder ultimately wants to improve (i.e. growth on commercial quality land).

Secondly, the difficulty of a reliable site index

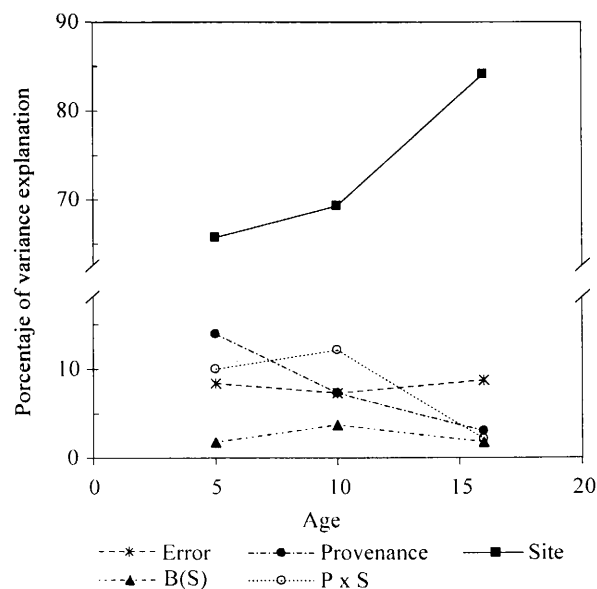
prediction for Douglas-fir in Northern Spain, suggests that for most environments, selections should preferably be made for stability and overall good performance. HANNERZ *et al.* (1999) and STCLAIR & KLEINSCHMIT (1986) give similar conclusion for Norway spruce (*Picea abies* Karst.) in Sweden. The mean Type B correlations for the overall studied area were always over 0.6 (Table 3). These values are similar to those found in Douglas-fir within each breeding zone of Western Oregon (JOHNSON 1997, JOHNSON *et al.* 1997). The joint regression analysis appears as an interesting technique to analyse the provenance stability across the site index variation.

The variation pattern of  $G \times E$  interaction depends on the species, the genetic material and the environment variation among the test sites. Some authors show an important geographical effect on  $G \times E$  interaction (ADAMS *et al.* 1994, HODGE & DVORAK 1999), whereas others do not find any geographical influence on the  $G \times E$  interaction (BENTZER *et al.* 1988, HANNERZ *et al.* 1999, SONESSON 1999). In Douglas-fir, JOHNSON (1997) find that very few variables significantly affected the correlation between sites. The environmental variables significantly correlated with Type B genetic correlations varied among the breeding zones and were not consistent from age to age in most of them. This author concludes that none of the environmental variables examined indicated that reducing the size or elevation range of the current breeding zones would strengthen the Type B correlations significantly.

Similar to the results found in our study, in *Pinus elliottii* Engelm. a  $G \times E$  interaction pattern is associated with site quality (HODGE & WHITE 1992). Type B genetic correlations are higher between pairs of locations of similar site index than for pairs of locations with very different site index. HODGE & WHITE (1992) suggest that in future progeny tests, families should be tested over a range of site indices, and site quality should be considered when using data to predict breeding values.

### Multi-site and joint regression analyses

In order to quantify the relevance of the  $P \times E$  interaction, a combined analysis of variance was carried out at ages 5, 10 and 16 for those 32 provenances assessed in at least three sites in the three ages. Results of these analyses are summarised in Figure 3. Most of the total variance was explained by the site effect ( $\sigma_s^2 > 65\%$ ) whereas the provenance and the  $P \times E$  interaction effects explained less than the 15% of the total variance. Both, provenance and  $P \times E$  variances tended to decrease with time but remained significant at the three

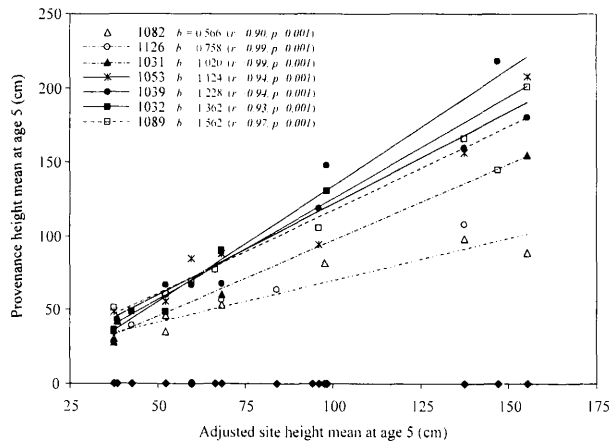


**Figure 3.** Evolution of components of variance estimates for height measurements at ages 5, 10 and 16. Only the 32 provenances assessed in at least 3 sites at age 16, and the 8 sites measured at age 16 were included in the analyses.

ages. The components of variance for the provenance and the  $P \times E$  interaction together accounted for only 5.2% of the total variation at age 16. In contrast the site component tended to increase and reached nearly 85% at age 16. As found previously, it seems that the performance differences between provenances tended to become more uniform with time. The  $P \times E$  interaction component is as important or even more important than the provenance variance component. The ratio between both components was always over 70% and increased to more than 165% at age 10. This later ratio indicated a high relative importance of the  $P \times E$  interaction in this trial series that should be taken into account for selection and provenance recommendations. ERIKSSON & EKBERG (2001) suggested that with a ratio above 100% there is a need for delineation of different breeding zones with separate breeding in each zone. However, as described before and as found previously by JOHNSON (1997), there was no evidence that dividing the studied area into different breeding zones would improve the Type B correlation and, thus, reduce the  $P \times E$  interaction.

The heterogeneity of the regressions upon the site index (corrected overall site height mean) were highly significant ( $p < 0.001$ ) at the three ages, indicating that the site quality has a notable influence on the  $P \times E$  interaction. The deviations from the regression lines were not significant at any age. Figure 4 shows the regression lines of 7 provenances for height at age 5. Differences in the slope of the regression lines among provenances can be observed, indicating differences in

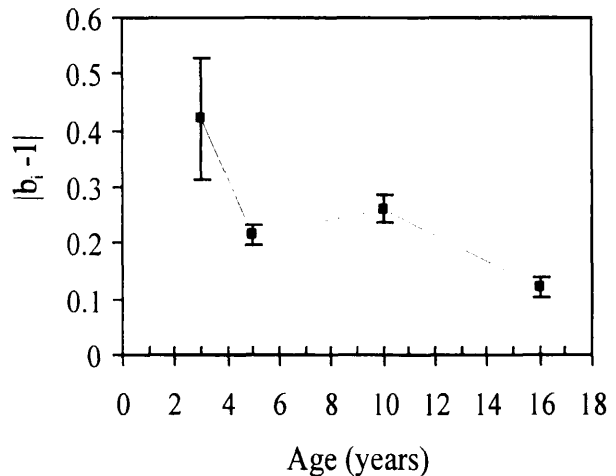




**Figure 4.** Plot of provenance height means against adjusted site mean at age 5 and estimated regression lines for 7 provenances.

the provenance stability in relation to the site quality variation. The estimated regression coefficients  $b_i$  ranged from 0.12 to 1.57 at age 5, from 0.20 to 1.68 at age 10 and from 0.72 to 1.27 at age 16. The coefficients  $b_i$  tended to be closer to 1 with age. Figure 5 shows the evolution of the deviations of the coefficients  $b_i$  from the average stability ( $b = 1$ ). The homogenisation of the provenance stability upon the site index agrees with the decrease of the  $P \times E$  interaction with age found previously.

The coefficients  $b_i$  at age 5 were significantly and negatively correlated with the altitude of the seed source origin ( $r = -0.42, p < 0.001$ ) indicating that the high-elevation provenances tend to be more stable with respect to the site quality than the low-elevation seed sources. However, a significant correlation was also observed between the provenance elevation and the overall provenance performance at age 5 across all sites ( $r = -0.38, p < 0.001$ ). Thus, low-elevation provenances



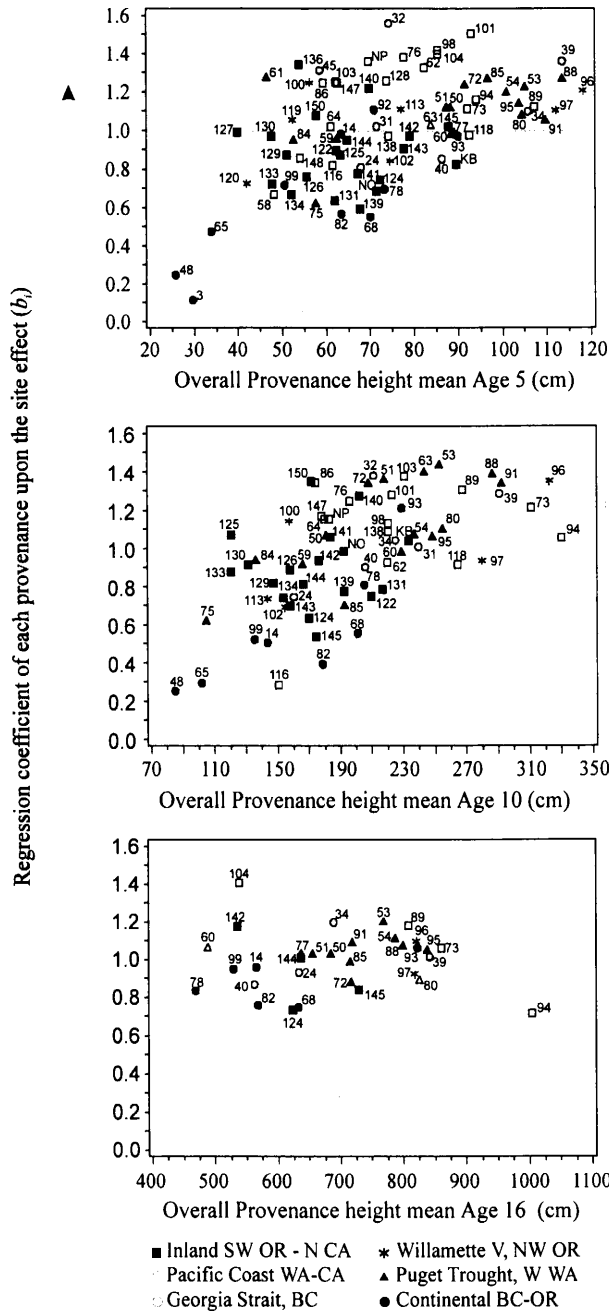
**Figure 5.** Evolution of the coefficients  $b_i$  deviations from the average stability ( $b = 1$ ) for height measurements at ages 5, 10 and 16. At each age, only the provenances assessed in at least 3 sites were considered, i.e. 82, 70 and 32 provenances at ages 5, 10 and 16, respectively.

tended to perform better in Northern Spain but tended to have low stability and were better adapted to high-quality sites. KLEINSCHMIT *et al.* (1990) also find stability differences among Douglas-fir provenances in Germany. In this work, provenances originating from British Columbia are more stable than the Washington sources. However there are considerable differences in stability within regions.

A plot of the coefficients  $b_i$  against the overall height mean for ages 5, 10 and 16 is presented in Figure 6. Considering that selections should preferably be made for stability and overall good performance within all the study area, these figures are very useful for selection purposes. Thus, those provenances at the right-end of the graph, which have at the same time the

**Table 5.** Recommended provenances based on the results of the present paper.

IUFRO Number	Tree seed zone	State	Locality	Latitude	Longitude	Altitude
1034	104	British Columbia	Sechelt	49.51	123.88	185
1039	105	British Columbia	Chilliwack	49.07	121.80	170
1073	030	Washington	Humptulips	47.32	123.90	135
1080	232	Washington	Yelm	47.02	122.73	60
1088	430	Washington	Castle Rock	46.32	122.87	150
1089	041	Washington	Cathlamet	46.30	123.27	245
1093	653	Washington	Willard	45.80	121.68	550
1094	052	Oregon	Vernonia	45.77	123.22	215
1095	042	Washington	Prindle	45.62	122.13	455
1096	452	Oregon	Sandy	45.38	122.30	275
1097	451	Oregon	Cherryville	45.32	122.13	730



**Figure 6.** Plot of the stability parameter  $b_i$  against overall height means at ages 5, 10 and 16. At each age, only the provenances assessed in at least 3 sites were considered, *i.e.* 82, 70 and 32 provenances at ages 5, 10 and 16, respectively. Horizontal dotted lines indicates average stability ( $b_i = 1$ ). Vertical dotted line indicates the overall height mean at each age.

lower coefficients  $b_i$  (higher stability) are those to be selected. However, a significant correlation was observed between the overall height mean and the coefficients  $b_i$  ( $r = 0.51, p < 0.001$  and  $r = 0.58, p < 0.001$  at ages 5 and 10, respectively) indicating that the best

overall performing provenances tended to have low stability depending on the site quality. This relationship has been found previously for Norway spruce (SKRØPPA 1984, BENTZER *et al.* 1988) and it is probably due to the own regression analysis procedure: the site index includes the genotypic values which are to be regressed (FREEMAN 1973, SKRØPPA 1984). Furthermore, the modification used in this paper for the adjustment of the site index includes the estimated genotypic values based on the previous regression equations. A strict regression line between the provenance height and the site mean height is assumed for the estimation of the missing entries.

Nevertheless, no significant correlation between the overall provenance mean and the stability parameter was observed at age 16. Thus, results at this age appeared more suitable for selection. Unfortunately, not all the 89 provenances can be analysed at this age. Provenance 94 (IUFRO's 1094, Vernonia), coming from the North Oregon Pacific coast, appeared as the best. This provenance shows the best overall performance at age 10 and at age 16 and has also the lowest coefficient  $b_i$  at age 16 ( $b_{94} = 0.72$ ). Other provenances that show good overall performance and high stability at age 16 are 1073, 1039, 1095, 1080, 1097, 1093, 1096 and 1088. All of these provenances also have good overall performance at ages 5 and 10 but have average to low stability at these ages. Provenances 1089 and 1034 can be also selected from results at age 5. Most of these provenances come from North Oregon and South Washington (Table 5, Figure 2), ranging from 45° to 47° latitude and 60 to 730 m altitude. Two of these selected provenances come from Southern British Columbia (1039 and 1034). All of the 11 proposed provenances, except 1093, were included within the best at age 5 (TOVAL *et al.* 1993) and all, except 1034, 1093 and 1097, were included within the best at age 10 (VEGA *et al.* 1993). All of them presented high survival in almost all the sites where they were assessed (data not presented). Provenance survival was always higher than the site mean in more than 80 % of the sites where each provenance was assessed, except for provenance 1093 which showed a relative high mortality in 3 out of 5 sites. Nevertheless, survival of this provenance was always higher than 70 %.

These 11 provenances are a restricted subset of the previous provenance recommendations for Northern Spain (TOVAL *et al.* 1993, VEGA *et al.* 1993, 1998). TOVAL *et al.* (1993) pointed out that the best provenances in Spain differ from those in other European countries in terms of substantial southward shifts. However, taking into account the stability over the environmental variability, all the selected provenances come from latitudes north of 45 ° N. These subset of

provenances also gave good results in many European countries (KLEINSCHMIT & BASTIEN 1992). Since there are quite drastic climatic differences between these European countries, these authors have remarked the broad adaptability of Douglas-fir provenances from Northern Oregon, Washington and Southwest British Columbia. The low coefficients  $b_i$  of the selected provenances confirm the high stability of these provenances.

In conclusion, provenance  $\times$  environment interaction is an important source of variation in the Douglas-fir provenance test in Northern Spain that should be taken into account for selection and provenance recommendations. None of the geographical variables examined indicated that dividing the study area into different breeding zones would strengthen the type B correlations significantly. Thus, selection should preferably be made for stability and overall good performance for the whole studied area. Because the site quality had some influence on the variation pattern of the P  $\times$  E interaction, the stability with respect to the site index as estimated by the joint regression analysis (coefficient  $b_i$ ) was selected as a stability estimator for each provenance. Taken into account both, the stability and the overall performance, we recommended 11 provenances for Northern Spain (Table 5, Figure 2). These provenances are a subset of the previous provenance recommendations and come from latitudes north of 45° N.

## ACKNOWLEDGEMENTS

This study was financed, during the last years, by the European Projects EUDIREC CT95-0909 and FEDER 1FD97-0585-C-03-01. We thank all the members of C.I.F.A. of Lourizán that have made possible the establishment and measurement of all the test sites considered in this paper. Especially, Guillermo Vega, Gabriel Toval and Fernando Molina, previous co-ordinators of the *Pseudotsuga menziesii* breeding program at Lourizán, are acknowledged.

## REFERENCES

- ADAMS, W. T., WHITE, T. L., HODGE, G. R. & POWELL, G. L. 1994: Genetic parameters for bole volume in longleaf pine: Large sample estimates and influences of test characteristics. *Silvae Genetica* **43**(5–6): 357–366.
- BASTIEN, J. C. & ROMAN-AMAT, B. 1990: Predicting Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) volume at age 15 with early traits. *Silvae Genetica* **39**(1): 29–35.
- BENTZER, B. G., FOSTER, G. S., HELLBERG, A. R. & PODZORSHI, A. C. 1988: Genotype  $\times$  environment interaction in Norway spruce involving three levels of genetic control: seed source, clone mixture, and clone. *Canadian Journal of forest Research* **18**(9): 1172–1181.
- BURDON, R. D. 1977: Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genetica* **26**(5–6): 168–175.
- ERIKSSON, G. & EKBERG, I. 2001: An Introduction to Forest Genetics. Swedish University of Agricultural Sciences, Uppsala, Sweden, 166 pp.
- FINLAY, K. W. & WILKINSON, G. N. 1963: The analysis of adaptation in a plant breeding programme. *Australian Journal of Agricultural Science* **14**: 742–745.
- FREEMAN, G. H. 1973: Statistical methods for the analysis of Genotype-Environment Interactions. *Heredity* **31**(3): 339–354.
- GARCÍA, S. S., RODRIGUEZ, R. S., ALVAREZ, J. G. G., GONZÁLEZ, R. C. & VEGA, G. A. 1996: Primeros resultados de crecimiento de plantaciones de 15 a 30 años de abeto Douglas (*Pseudotsuga menziesii* Mirb Franco) en el noroeste de España. *Investigación Agraria. Sistemas y Recursos Forestales* **5**(2): 231–243.
- HANNERZ, M., SONESSON, J. & EKBERG, I. 1999: Genetic correlations between growth and growth rhythm observed in a short-term test and performance in long-term field trials of Norway spruce. *Canadian Journal of forest Research* **29**(6): 768–778.
- HILL, J., BECKER, H. C. & TIGARSTEDT, P. M. A. 1992: Quantitative and Ecological Aspects of Plant Breeding. Chapman & Hall, London, United Kingdom, 275 pp.
- HODGE, G. R. & DVORAK, W. S. 1999: Genetic parameters and provenance variation of *Pinus tecunumanii* in 78 international trials. *Forest Genetics* **6**(3): 157–180.
- HODGE, G. R. & WHITE, T. L. 1992: Genetic parameter estimates for growth traits at different ages in slash pine and some implications for breeding. *Silvae Genetica* **41**(4–5): 252–262.
- JOHNSON, G. R. 1997: Site-to-site genetic correlations and their implications on breeding zone size and optimum number of progeny test sites for coastal Douglas-fir. *Silvae Genetica* **46**(5): 280–285.
- JOHNSON, G. R. & BURDON, E. D. 1990: Family-site interaction in *Pinus radiata* implications for progeny testing strategy and regionalised breeding in New Zealand. *Silvae Genetica* **39**: 55–62.
- JOHNSON, G. R., SNIETKO, R. A. & MANDEL, N. L. 1997: Age trends in Douglas-fir genetic parameters and implications for optimum selection age. *Silvae Genetica* **46**(6): 349–358.
- KLEINSCHMIT, J. & BASTIEN, J. C. 1992: IUFRO's role in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tree improvement. *Silvae Genetica* **41**(3): 161–173.
- KLEINSCHMIT, J., SVOLBA, J., WEISGERBER, H., RAU, H. M., DIMPFLEMEIER, R., RUETZ, W. & FRANKE, A. 1990: Results of the IUFRO Douglas-fir provenance experiment in the Federal Republic of Germany at age 20. In: Proceedings of the Joint Meeting of Western Forest Genetics Association and IUFRO Working Parties S 2.02.05. Weyerhaeuser, Olympia, Washington, USA, pp. 2195–2208.
- LOOPSTRA, C. A. & ADAMS, W. T. 1989: Patterns of variation in first-year seedlings traits within and among Douglas-fir breeding zones in southwest Oregon. *Silvae Genetica* **38**(5–6): 235–243.
- MATHESON, A. C. & COTTERILL, P. P. 1990: Utility of genotype  $\times$  environment interactions. *Forest Ecology and Management* **30**(1–4): 159–174.

- MERLO, E. 2002: Optimización del proceso de obtención de semilla y su aplicación en el ciclo de mejora genética de *Pseudotsuga menziesii* Mirb. Franco. Ph. Thesis. Universidad Politécnica de Madrid, Madrid, Spain, 180 pp.
- NAMKOONG, G., USANIS, R. A. & SILEN, R. R. 1972: Age-related variation in genetic control of height growth in Douglas-fir. *Theoretical and Applied Genetics* **42**(4): 151–159.
- PEDERSON, D. G. 1972: A comparison of four experimental designs for the estimation of heritability. *Theoretical and Applied Genetics* **42**: 371–377.
- PSWARAYI, I. Z., BARNES, R. D., BIRKS, J. S. & KANOWSKI, P. J. 1997: Genotype-environment interaction in a population of *Pinus elliottii* Engelm. var. *elliottii*. *Silvae Genetica* **46**(1): 35–40.
- SAS 1989: SAS STAT User guide, Version 6. SAS Institute Inc., Cary, NC, 943 pp.
- SKRØPPA, T. 1984: A critical evaluation of methods available to estimate the genotype  $\times$  environment interaction. *Studia Forestalia Suecica* **166**: 3–14.
- SONESSON, J. 1999: Provenance differences and clone  $\times$  site interaction in five clonal tests with *Picea abies* (L.) Karst. in Central Sweden. In: Genetics and breeding of Norway spruce. (ed. T. Skroppa, L. Paule & D. Gömöry). Arbora Publishers, Zvolen, Slovakia.
- STCLAIR, J. B. & KLEINSCHMIT, J. 1986: Genotype-environment interaction and stability in ten-year height growth of Norway spruce clones (*Picea abies* Karst.). *Silvae Genetica* **35**(5–6): 177–186.
- TOVAL, G. H., VEGA, G. A., PUERTO, G. A. & JENKINSON, J. L. 1993: Screening Douglas-fir for rapid early growth in common-garden tests in Spain. *General Technical Report Pacific Southwest Research Station* **146**: 43 pp.
- VEGA, A. G., RODRÍGUEZ, S. R., ARENAS, R. S. G., GARCÍA, S. S., MANSILLA, V. P., VEGA, A. P. & RUÍZ, G. A. D. 1998: Manual de Selvicultura del pino oregon. Universidad de Santiago de Copostela, Santiago, Spain, 84 pp.
- VEGA, G., PUERTO, G., RODRÍGUEZ, R., VEGA, P. & GONZÁLEZ, M. 1993: La introducción de *Pseudotsuga menziesii* en el norte de España. In: Proceedings of the I Congreso Forestal Español. Vol II, pp. 65–69. Pontevedra, Spain.