of species and source Research with particular reference to Tropics. Tropical Forest Paper No. 10 Canadian Forest Institute, Oxford (1976). — CHAUHAN, S., NEGI, A.K. and TODARIA, N.P.: Effect of provenance variation and temperature on seed germination of *Alnus nepalensis.* Plant physiology and Biochemistry **23** (1): 94–95 (1996). — DANGASUK, O. G., SEUREI, P. and GUDU, S.: Genetic variation in seed and seedling traits in 12 African sources of *Faidherbia albida* (Del.) A. Chev. At Lodwar, Kenya. Agroforestry systems **37**: 133–141 (1997). — GERA, M., GERA, N. and GINWAL, H. S.: Seed source variation in germination and early growth among ten indigenous populations of *Dalbergia sissoo* Roxb. Indian Forester **125** (12): 1190–1197 (1999). — GINWAL, H. S., MOHIT GERA and SRIVASTAQVA, R. L.: Seed source variability in some seed and seedling characteristics of twenty sources of *Acacia nilotica.* Range Management and Agroforestry **17** (1): 49–59 (1996). — GUTTERMAN, Y.: Maternal effects on seeds during development. *In:* The ecology of regeneration in plant communities (eds.) M. FENNER (Willingford, CAB International), pp. 27–59 (1992). — International Seed Testing Association. International rules for seed testing. Seed Science and Technology 24 (Supplement). Zurich, Switzerland, pp (1996). — ISIK, K.: Altitudinal variation in *Pinus brutia* Ten: Seed and seedling characteristics. Silvae Genetica **35**: 58–67 (1986). — JOHNSON, H. W., ROBINSON, H. F. and COMSTOCK, R. F.: Estimates of genetic and environmental variability in soybean. Agronomy Journal **47**: 314–318 (1955). — KHALIL, M. A. K.: Variation in seed quality and some juvenile characters of white spruce (*Picea glauca* (Moench) Voss). Silvae Genetica **35**: 78–85 (1986). — KUMAR, N. and TOKY, O. P.: Variation in pod and seed size among *Albizia lebbek* provenances. Nitrogen Fixing Tree Research Report **11**: 64–67 (1993). — MEHTA, M. and SEN, D. N.: Seed characteristics and growth pattern of seedlings in some trees of Indian Arid Zone. Annals of Arid Zone **34**: 65–66 (1995). — MURALI, K. S. and SUKUMAR, R.: Reproductive phenology of a tropical dry deciduous forest in southern India. Journal of Ecology **82**: 759–767 (1994). — RANGASWAMI, R.: A Text Book of Agricultural Statistics. pp-260–262 (1995). — SAKLANI, K. P.: Altitudinal and seasonal variation in relation to fodder quality of Oak in Garhwal Himalaya. Ph. D. Thesis (1999). — SALAZER, R.: Genetic variation in 16 sources of *Acacia mangium* at nursery level in Turrialba, Costa Rica. Commonwealth Forestry Review **68** (4): 263–272 (1989). — SEKARAN, S., RANGARAJAN, T. N. and PONNAMMAL, N. R.: Source variance in pod length, seed characters and germination of *Cassia auriculata* Linn. Journal of Tree Science **16** (1): 59–61 (1997). — SHARMA, N. K., BURMAN, U., TEWARI, J. C., BOHRA, M. D. and HERSH, L. N.: Variability studies in pod and seed characteristics of *Prosopis juliflora* (s.W.) DC Indian Journal of Forestry **17** (2): 161–165 (1994). — SHARMA, R. K. and SHARMA, S. S.: Heritability and association analysis in *Grewia optiva.* Indian Forester **121** (4): 318–320 (1995). — SHIV KUMAR, P. and BANERJEE, A. C.: Provenance trials of *Acacia nilotica.* Journal of Tree Science **5** (1): 53–56 (1986). — SINGH, SARJAN, OMBIR SINGH and VIRENDRA SINGH.: Effect of seed weight on germination. Survival and initial growth of *Aesecutus indica.* Indian Forester **119** (8): 627–629 (1993). — SOULE, M.: The epistesis cycle. A Theory of marginal populations. Ann. Rev. Ecology and systematic **4**: 165–187 (1973). — SRIVASTAVA, D. P., SRIVASTAVA, P. K., GOEL, A. K. and THANGAVELA, K.: Genetic Variability in *Terminalia arjuna* Bedd. Indian Journal of Forestry **16** (3): 223–225 (1993). — SUBRAMANIAN, K. N., MANDAL, A. K., GOVINDRAJ, P. and SASIDHRAN, K. R.: Provenance trials in *Eucalyptus grandis* and its implication to forestry programmes. Silvae Genetica **41** (4–5): 239–242 (1992). — SUBRAMANIAN, K. N., MANDAL, A. K. and NICODEMUS, A.: Genetic variability and character association in *E. grandis.* Annals of Forestry **3** (2): 134–137 (1995). — TODARIA, N. P. and NEGI, A. K.: Effect of elevation and temperature on seed germination of some Himalayan tree species. Plant Physiology and Biochemistry **22** (2): 178–82 (1995). — TROUP, R. S.: Silviculture of Indian Trees. Vol. 11 (Revised). Controller of Publications Delhi India 783 P. (1921). — VAKSHAYA, R. K., RAJORA, O. P. and RAWAT, M. S.: Seed and seedling traits of *Dalbergia sissoo* Roxb. Seed source variation studies among ten sources in India. Forest Ecology and Management **48**: 265–275 (1992). — VOLKER, P. W., DEAN, TIBBITS, W. N. and RAVENWOOD, I. C.: Genetic parameters and gain expected from selection in *Eucalyptus globulas* in *Termania*, Silvae Genetica **39** (1): 18–21 (1990). — WOLFE, L. M.: The genetics and ecology of seed size variation in a biennial plant *Hydrophyllum apendiculatum.* Oecologia **101**: 343–352 (1995). — ZOBEL, B. J. and TALBERT, J.: Applied Tree Improvement. John Wiley and Sons. 521 pp. (1984).

Identification of the origin of Portuguese Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] provenances

By L. FONTES[1], P. SAVILL, J. S. LUIS[2] and S. HARRIS

Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

(Received 10th September 2003)

Summary

Douglas-fir was introduced to Portugal in the 19th century and the first plantations were established at the beginning of the 20th century. Since then, it has been planted in the mountainous areas of the centre and north of Portugal. The Portuguese Forestry Service has generally accepted that the establishment of younger plantations has been carried out mainly using seed from existing plantations. Unfortunately, the native North American sources of seeds used for establishment of the older plantations are unknown.

Isozyme analysis (seven loci) of megagametophyte tissue, from 10 Portuguese provenances sampled from across their introduced range (277 trees) and 17 native provenances, was used to investigate genetic variation: (i) among Portuguese Douglas-fir provenances; and (ii) between Portuguese and North American provenances of the 'coastal' variety, with the aim of identifying the putative source provenances for exotic Portuguese provenances. Among the Portuguese provenances, the expected heterozygosity (H_e) was 0.254, which was similar to previous investigations which sampled a wider range of the natural distribution. Therefore, these results reflect a considerable level of genetic diversity within Portuguese Douglas-fir provenances and may be evidence that the Portuguese material has come from more than one source.

UPGMA clustering of Nei's genetic distances for Portuguese and native provenances showed the majority of Portuguese Douglas-fir provenances fell into a single, poorly resolved group together with provenances from across the native range of Douglas-fir. Whether all the Portuguese provenances in this group are the product of a single introduction from the native range and then separate establishment in different parts of Portugal

[1] Current address: Dep. Engenharia Florestal, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda 1399 Lisboa Codex, Portugal.

[2] Secção Florestal, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Apartado 1013 – 5000 - 911Vila Real Codex, Portugal.

or represent separate introductions cannot be resolved using the current data. Vila Flor, the only Portuguese stand of known native provenance (California), grouped as a distinct group with Soajo suggesting it may also have been derived from California but as a separate, earlier, introduction. The provenances from Lousã and Leomil form a distinct group, suggesting that the latter may have been derived from the former. This group is most similar to provenances originating from between Oregon and British Columbia and suggests that provenances from this region may have been introduced into Portugal. Overall these data suggest that at least three separate North American introductions of Douglas-fir seed may have been used to establish plantations in Portugal, two from California and one from Oregon, Washington or British Columbia. For future investigations of the origin of Portuguese Douglas-fir provenances in the native range, sampling of provenances in the native range must be much denser than presently undertaken and high resolution markers are likely to yield better results over the identities of the originating native provenances.

Key words: Pseudotsuga menziesii (Mirb.) Franco, isozymes, provenance, Portugal, genetic variation.

Introduction

Pseudotsuga menziesii (Mirb.) Franco (Douglas-fir) is one of two western North American *Pseudotsuga* species. Douglas-fir, one of the dominant trees in the coniferous forests of the Pacific North West, occurs in 11 western states of the USA and the two western-most Canadian provinces[1]. The north-south range of the species extends approximately 3,400 km, from central British Columbia to sparse populations in the Sierra Madre in Mexico, whilst the east-west distribution covers approximately 1,600 km, from east of the Rocky Mountains in Colorado to the Pacific Ocean[1]. Two Douglas-fir varieties are recognised. *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco ('interior' variety) occurs from British Columbia to southern and central Mexico, whilst *P. menziesii* var. *menziesii* ('coastal' variety) occurs in a broad coastal strip to the west of the Coastal Range of British Columbia, the Cascade Range of Washington and Oregon and the Sierra Nevada of northern California. The two varieties co-occur in southern British Columbia and north-eastern Washington. Furthermore, based on terpene and protein markers, var. *glauca* can be divided into northern and southern subgroups[2].

In Europe, during the second half of the 19th century, the search for fast-growing tree species showed that the 'coastal' variety outperformed native Norway spruce [*Picea abies* (L.) Karst.] and Scots pine (*Pinus sylvestris* L.), whereas the 'interior' variety was unsuitable for European cultivation[3]. Despite the successful introduction of the 'coastal' variety to Europe, it soon became obvious that Douglas-fir provenances had variable adaptive behaviours[4]. Thus, the correct seed origin must be selected for successful Douglas-fir introduction to Europe[5].

The 'coastal' variety was introduced to Portugal in the 19th century, when it was planted at Sintra in about 1846[6] and at Buçaco in 1871[7]. The first plantations were established at Estrela in 1904[8] and at Gerês in 1906[9]. There are references to very small planted areas at Sintra and Gerês of the 'interior' variety[10], although this was unsuccessful compared to the 'coastal' variety[7]. Since the beginning of the 20th century, the 'coastal' variety has been planted in the mountainous areas of central and northern Portugal, and today the estimated area of Douglas-fir plantation in Portugal is approximately 4,200 ha[11]. One of the main problems with Portuguese Douglas-fir is the unknown origin of the seed from which the plantations were established[7,10,12–14]. With the exception of the plantation at Vila Flor (known to be from El Dorado County, California 38° 47' N, 120° 22' W, altitude 1,067 m[15]), there are no records of the seed sources from which the Douglas-fir plantations were established. Despite the absence of records, it is generally accepted by the Portuguese Forestry Service that the establishment of young plantations was carried out using seed from existing Portuguese plantations.

For a genetic marker to be useful for the identification of introduced provenance origins, it must be sufficiently variable across the species' native range, have multiple alleles at a locus and identify geographical regions within the native range. Analyses of allozyme variation within regions[16–19] or across the native range of Douglas-fir[2,20] indicate that these markers possess sufficient variation to identify provenance origins. Allozymes have been used to compare native and introduced Polish provenances of Douglas-fir[21], and to investigate exotic and native Douglas-fir provenances[22–24].

The aims of this study were to investigate genetic variation: (i) among Portuguese Douglas-fir provenances; and (ii) between Portuguese and North American provenances of the 'coastal' variety.

Material and Methods

Sampling strategy

Douglas-fir plantations were sampled from across their introduced ecogeographical range in Portugal. Cones were collected from 10 sites (*Figure 1; Table 1*) between 3rd August and 9th September 1999. The stands included Penhas Douradas (90 years-old), one of the oldest stands in Portugal, Vila Pouca de Aguiar (61 years-old), one of the stands where most seeds have been collected by the Portuguese Forestry Service (CENACEF) for replanting, and Vila Flor (28 years-old), the only stand of known origin. Cones were collected from 30 trees at each stand. For cone collections, a 3 m pruning pole[25] was used for young trees, whilst a climbing team from the Portuguese Forestry Service collected material from mature trees. Since 1999 was not a good seed production year in many Portuguese Douglas-fir plantations, some trees were sampled along plantation edges where cones were more numerous. Douglas-fir seed from the native range was obtained from commercial Douglas-fir sources (tree seed zones: 012, 030, 042, 202 and 412 from

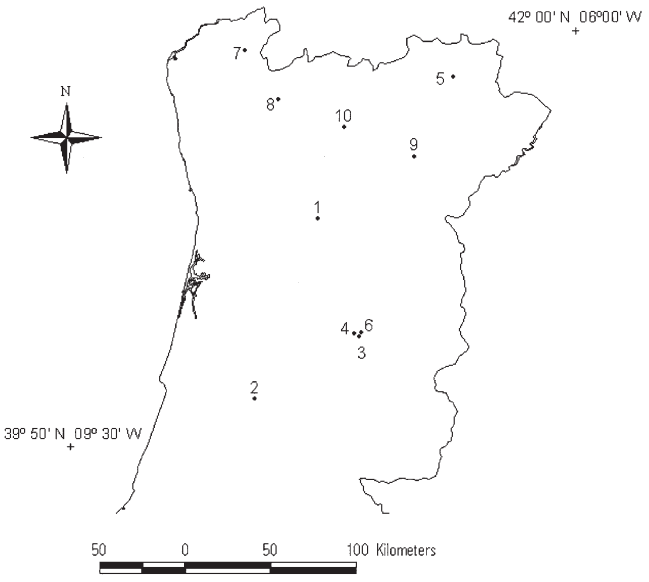

Figure 1. – Location of the 10 sites used in cone collection for the study of Douglas-fir genetic variation in Portugal. Provenance designations as in *Table 1.*

Table 1. – Portuguese and North American provenances of *Pseudotsuga menziesii* investigated for allozyme variation.

Code	Locality	Tree seed zone	Seed source location		
			Altitude (m)	Latitude (N)	Longitude (W)
Portugal					
1	Leomil	na	945	41º 01'	07º 49'
2	Lousã	na	700	40º 04'	08º 15'
3	Moitas	na	960	40º 23'	07º 31'
4	Penhas Douradas	na	1300	40º 24'	07º 33'
5	Poulos de Formil	na	1020	41º 45'	06º 52'
6	São Lourenço	na	995	40º 24'	07º 30'
7	Soajo	na	740	41º 54'	08º 18'
8	Vieira do Minho	na	460	41º 38'	08º 05'
9	Vila Flor	na	600	41º 20'	07º 08'
10	Vila Pouca Aguiar	na	865	41º 30'	07º 37'
Oregon					
11	Unknown	062	150 - 305	44º - 45º	123º - 124º
12	Unknown	072	0 - 150	42º - 43º	124º - 125º
13	Unknown	262	150 - 305	44º - 45º	122º - 123º
14	Unknown	261	150 - 305	45º - 46º	122º -123º
15	Butte Falls	502	610 - 760	42º - 43º	122º - 123º
California					
16	Sonoma County	095	305 - 460	38º - 39º	123º - 124º
17	Grouse Mountain	303	610 - 760	40º - 41º	123º - 124º
Washington					
18	Sedro Woolley	202	0 - 150	48º - 49º	121º - 122º
19	Forks	012	150 - 305	47º - 48º	124º - 125º
20	Wind River	042	150 - 305	45º - 46º	122º - 123º
21	Enumclaw	412	305 - 460	47º - 48º	121º - 122º
22	Hoquiam	030	0 - 150	46º - 47º	123º - 124º
British Columbia					
23	Owl Creek	107	210	50º 20'	122º 43'
24	Chilliwack Low	105	170	49º 04'	121º 48'
25	Courtenay	102	70	49º 41'	125º 03'
26	Sooke Low	101	50	48º 24'	123º 44'
27	Sechelt	104	190	49º 30'	123º 52'

Washington; 502, 261, 062, 262, and 072 from Oregon; 095 and 303 from California; *Table 1*). Unfortunately, no information about the location of some of the seed lots was available, although there was always reference to the tree seed zone and the altitude where the seed was collected. Twelve provenances from the USA (five from Washington, west of the Cascade Range; five from Oregon, west of the Cascade Range and two from California) and five provenances from British Columbia were sampled (*Figure 2; Table 1*). ADDIN Allozymes were analysed from bulked collections of megagametophytes harvested from at least 30 individuals per provenance.

Allozyme procedures

To activate the enzymes, the testa was cut and the seeds placed on wet cotton wool for approximately 12 hours before the megagametophyte was separated from the diploid embryo. Allozymes were separated on horizontal 12% starch gels[26] and provenance samples screened using 20 enzyme systems: α-esterase (α-Est; E. C. 3.1.1.-), β-esterase (β-Est; E. C. 3.1.1.-), aspartate aminotransferase (AAT; E. C. 2.6.1.1.), malate dehydrogenase (MDH; E. C. 1.1.1.37), malic enzyme (ME; E. C. 5.3.1.8), alcohol dehydrogenase (ADH; E. C. 1.1.1.1), shikimate dehydrogenase (SDH; E. C. 1.1.1.25), aconitase (ACO; E. C. 4.2.1.3.), isocitrate dehydrogenase (IDH; E. C. 1.1.1.42.), acid phosphatase (ACP; E. C. 3.1.3.2), 6-phosphogluconate dehydrogenase (6-PGD; E. C. 1.1.1.44), glucose-6-phosphate dehydrogenase (G6PDH; E. C. 1.1.1.49.), sorbitol dehydrogenase (SOR), lactate dehydrogenase (LDH; E. C. 1.1.1.27), formate dehydrogenase (FDH; E. C. 1.2.1.2), aldolase (ALD; E. C. 4.1.2.13), phosphoglucose mutase (PGM; E. C. 2.7.5.1.), phosphoglucose isomerase (PGI; E. C. 5.3.1.9.), glutamate dehydrogenase (GDH; E. C. 1.4.1.2), and glyceraldehyde-3-phosphate dehydrogenase (G-3-PD; E. C. 1.2.1.12).

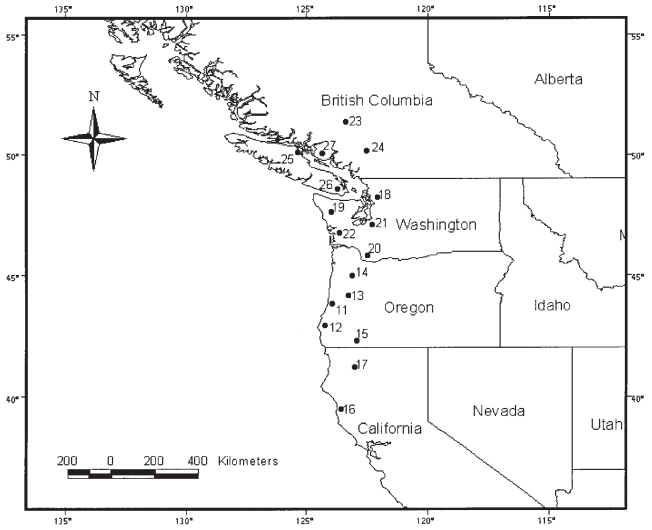

Figure 2. – Map showing locations of North America provenances. Provenance designations as in *Table 1.*

Data analysis

Enzyme nomenclature follows international conventions; upper case letter codes refer to enzyme systems and mixed upper and lower case codes refer to enzyme loci. For systems controlled by more than one locus, isozymes are numbered from anode to cathode and each allozyme is lettered from anode to cathode. Calculations of genetic variation were made using the computer program POPGENE (version 1.32)[27], and the mean numbers of alleles per locus (A) and observed (H_o) and expected (H_e) heterozygosities calculated. Nei's G_{ST}[28] was calculated as a measure of population differentiation. NEI's[29] genetic distances were calculated between pairs of provenances and the data clustered using unweighted pair-group mean analysis (UPGMA;[30]).

Results

Isozyme phenotypes and interpretation

Nine enzyme systems (AAT, ACO, GDH, G6PDH, IDH, 6-PGD, PGI, PGM, SOR) produced clear banding patterns at 15 loci. However, GDH and SOR were monomorphic, during an extensive initial screen of the Portuguese material, and ACO and 6-PGD were inconsistent between replicates. Thus, the final data set comprised seven loci (*Aat*-1, *Aat*-2, *Pgi*-1, *Pgi*-2, *Pgm*-1, *Idh*-1, *G6pdh*-1).

Three regions of AAT (*Aat*-1, A*at*-2, A*at*-3) activity were identified, although the slowest region (*Aat*-3) was usually very faint and therefore not scored; three alleles were observed at each of *Aat*-1 and *Aat*-2. Two regions of PGI (*Pgi*-1 and *Pgi*-2) activity were identified and three alleles were observed at each locus. One region of PGM (*Pgm*-1) and IDH (*Idh*-1) activity, each with four alleles, was identified. One region of G6PDH *(G6pdh*-1) activity, with five alleles was identified.

Analysis of native provenances

Twenty-three alleles, at seven loci, were detected among the native Douglas-fir provenances sampled. All populations had similar distributions of alleles, that is, at each locus the most frequent allele was the same for each population, although there was some variation in the frequencies of the rare alleles (*Table 2*). The only private allele found was in population 15 (*Aat*-1c). Measures of genetic diversity were very similar

Table 2. – Allele frequencies in 17 Douglas-fir North American provenances. Provenance numbers as in *Table 1.*

Locus		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Aat-2	A	0.05	0.1	0.025	0.025	0.075	0.075	0.1	0.075	0	0.025	0.075	0.15	0	0	0.025	0	0.075
	B	0.925	0.875	0.925	0.975	0.925	0.925	0.9	0.925	0.975	0.95	0.9	0.8	0.975	0.95	0.975	1	0.9
	C	0.025	0.025	0.05	0	0	0	0	0	0.025	0.025	0.025	0.05	0.025	0.05	0	0	0.025
Aat-1	A	0.05	0.025	0.025	0.05	0.025	0	0	0.025	0	0	0	0.025	0	0	0	0.05	0
	B	0.95	0.975	0.975	0.95	0.95	1	1	0.975	1	1	1	0.975	1	1	1	0.95	1
	C	0	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0	0
Pgm-1	A	0.1	0.225	0.15	0.25	0.125	0.1	0.075	0.175	0	0.075	0.15	0.125	0	0.1	0	0.025	0
	B	0.875	0.7	0.825	0.725	0.8	0.9	0.9	0.775	0.975	0.825	0.725	0.85	0.875	0.8	0.975	0.975	0.95
	C	0.025	0.075	0.025	0.025	0.075	0	0.025	0.05	0.025	0.1	0.125	0.025	0.125	0.1	0.025	0	0.05
Pgi-2	A	0.025	0.05	0	0.075	0.05	0.025	0	0.025	0.025	0	0.05	0.075	0.275	0	0.2	0.025	0.025
	B	0.975	0.875	1	0.925	0.95	0.975	0.925	0.925	0.975	1	0.95	0.925	0.725	0.95	0.775	0.975	0.975
	C	0	0.075	0	0	0	0	0.075	0.05	0	0	0	0	0	0.05	0.025	0	0
Pgi-1	A	0.175	0.025	0	0	0	0	0	0.05	0	0	0.225	0	0	0	0	0	0
	B	0.825	0.975	1	0.975	1	1	1	0.95	1	1	0.775	1	1	0.95	1	1	1
	C	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0.05	0	0	0
Idh-1	A	0.225	0.125	0.15	0.2	0.2	0.075	0.1	0.1	0.05	0.175	0.15	0.15	0.125	0.125	0.225	0.175	0.15
	B	0.725	0.85	0.825	0.8	0.75	0.925	0.825	0.9	0.85	0.825	0.825	0.825	0.85	0.825	0.625	0.775	0.675
	C	0	0	0.025	0	0.025	0	0.075	0	0.1	0	0.025	0.025	0.025	0.025	0.15	0.05	0.175
	D	0.05	0.025	0	0	0.025	0	0	0	0	0	0	0	0	0.025	0	0	0
G6pdh-1	A	0.575	0.356	0.175	0.475	0.1	0.15	0.225	0.55	0.5	0.5	0.35	0.475	0.356	0	0.25	0.3	0.111
	B	0.425	0.608	0.75	0.525	0.675	0.575	0.75	0.35	0.5	0.425	0.55	0.525	0.608	0.975	0.75	0.575	0.889
	C	0	0.036	0.075	0	0.15	0.275	0.025	0.075	0	0.075	0.1	0	0.036	0.025	0	0.025	0
	D	0	0	0	0	0.075	0	0	0.025	0	0	0	0	0	0	0	0.1	0

among the native populations; mean A = 2.3 (standard error = 0.070; range 1.8–2.7) and mean H = 0.192 (standard error = 0.009; range 0.13–0.275), and there was a significant linear relationship between these two measures of diversity (R = 0.566; p = 0.017). Genetic differentiation among provenances was low (G_{ST} = 0.08), with approximately 92% of variation being found within provenances. UPGMA clustering of Nei's genetic distances showed four major groupings (*Figure 3*): (i) provenances 12 (Oregon) and 23 (British Columbia); (ii) provenance 18 (Washington); (iii) provenances 24 (British Columbia) and 27 (British Columbia); and (iv) all other native provenances. However, no strong geographical grouping of allozyme variation was found. Furthermore, no private alleles were found in these groups. However, despite the absence of geographical structure to the genetic variation, grouping can be detected with the seven allozyme loci used here.

Analysis of Portuguese provenances

Twenty-five alleles, at seven loci, were detected across the 10 Portuguese provenances sampled (*Table 3*). The additional alleles (*Pgm*-1d, *G6pdh*-1e), compared to the native sample, were private alleles found in the Vieira do Minho provenance, together with the private allele *Idh*-1d. The majority of alleles showed similar distributions across loci, that is the most frequent allele was the same at each locus for each population. However, for loci *G6pdh*-1, *Pgi*-2 and *Pgm*-1 different alleles were more frequent in different populations. Measures of genetic diversity were very similar among the exotic populations; mean A = 2.4 (standard error = 0.12; range 1.6–3.1) and mean H = 0.238 (standard error = 0.009; range 0.191–0.277), although the range of values for A was much greater for exotic than native populations and mean H was also greater for exotic compared to native populations. In contrast to the native populations, there was no significant linear relationship between these two measures of diversity (R = 0.488; p = 0.152). Genetic differentiation among exotic provenances was high (G_{ST} = 0.112) compared to the native provenances, with approximately 89% of variation being found within provenances. UPGMA clustering of NEI's genetic distances showed three major groupings (*Figure 4*): (i) provenances 4 (Penhas Douradas) and 8 (Vieira do Minho); (ii) provenances 7 (Soajo) and 9 (Vila Flor); and (iii) all other exotic provenances.

Regression of neither A nor H on the date of plantation establishment was significant. However, if the outlying Vieira do Minho provenance was excluded from the regression analysis, a significant (p = 0.049) negative relationship between date of plantation establishment and A was found; there was still no significant relationship with H.

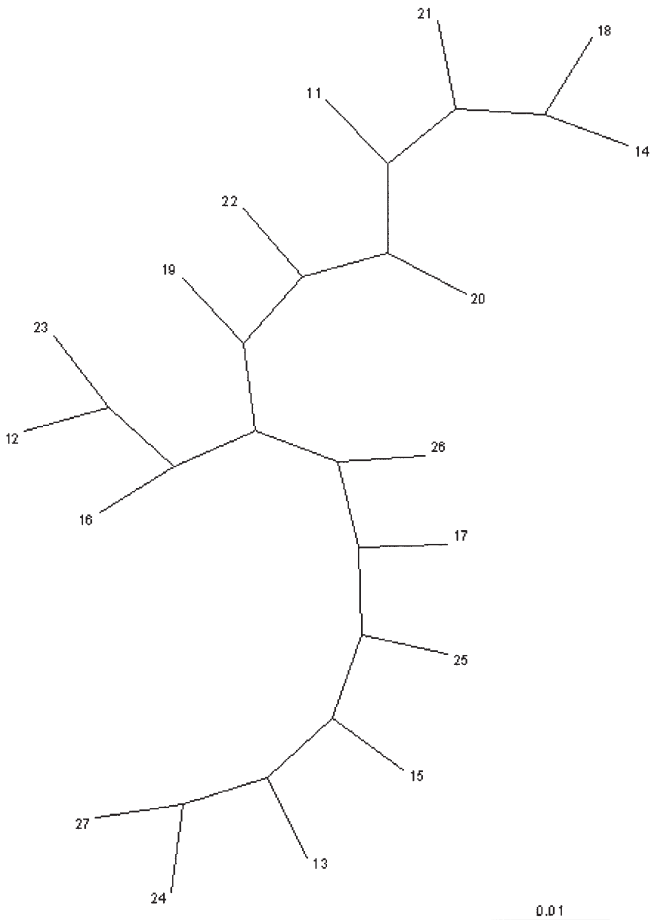

Figure 3. – UPGMA clustering of Nei's genetic distances for Douglas-fir native provenances using TreeView[36]. Numbers refer to provenance codes *(Table 1).*

Analysis of native and Portuguese provenances

Compared to the native provenances sampled, the Portuguese provenances are more diverse in terms of total number of alleles and gene diversity (*Table 4*). Furthermore, the total diversity is greater for the exotic compared to the native provenances [H_T = 0.268 (standard error = 0.013) versus H_T = 0.212 (standard error = 0.007), respectively]. Differentiation between the exotic provenances (G_{ST} = 0.112) is also greater than differentiation between the native provenances (G_{ST} = 0.080). UPGMA clustering of Nei's genetic distances calculated from the total data set showed six clusters (*Figure 5*): (i) native provenances 24 (British Columbia) and 27 (British Columbia); (ii) exotic provenances 7 (Soajo) and 9 (Vila Flor); (iii) native provenance 18 (Washington); (iv) native provenances 12 (Oregon) and 23 (British Columbia); (v) exotic provenances 1 (Leomil) and 2 (Lousã); and (vi) all other exotic and native provenances. These data suggest that there may have been two or three introductions of Douglas-fir into Portugal from the native range, although the use of allozymes limits the resolution found in the largest of the clusters, which may represent single or multiple introductions. The second possible introduction is of genotypes similar to provenances 7 and 9, probably from Washington and British Columbia. The third possible introduction is of genotypes similar to provenances 1 and 2, probably from Oregon and British Columbia.

Table 3. – Allele frequencies in 10 Portuguese Douglas-fir provenances. Provenance designations as in *Table 1*.

Locus		1	2	3	4	5	6	7	8	9	10
Aat-2	a	0.055	0.014	0.067	0.063	0.110	0.086	0.082	0.021	0.119	0.180
	b	0.946	0.857	0.926	0.930	0.890	0.729	0.919	0.964	0.874	0.787
	c	0.000	0.129	0.007	0.007	0.000	0.186	0.000	0.014	0.007	0.033
Aat-1	a	0.000	0.093	0.000	0.014	0.000	0.000	0.008	0.000	0.052	0.007
	b	1.000	0.907	1.000	0.972	1.000	1.000	0.993	1.000	0.941	0.993
	c	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.007	0.000
Pgm-1	a	0.386	0.229	0.333	0.110	0.320	0.243	0.684	0.150	0.824	0.113
	b	0.574	0.721	0.637	0.835	0.680	0.729	0.317	0.800	0.168	0.860
	c	0.040	0.050	0.030	0.055	0.000	0.029	0.013	0.029	0.008	0.027
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
Pgi-2	a	0.018	0.121	0.030	0.083	0.020	0.057	0.987	0.029	0.015	0.027
	b	0.982	0.879	0.970	0.897	0.980	0.921	0.020	0.957	0.985	0.973
	c	0.000	0.000	0.000	0.021	0.000	0.021	0.000	0.014	0.000	0.000
Pgi-1	a	0.000	0.007	0.104	0.000	0.000	0.000	0.140	0.000	0.030	0.100
	b	0.927	0.993	0.896	1.000	1.000	1.000	0.787	0.986	0.970	0.893
	c	0.073	0.000	0.000	0.000	0.000	0.000	0.073	0.014	0.000	0.007
Idh-1	a	0.200	0.229	0.207	0.179	0.287	0.107	0.260	0.236	0.370	0.260
	b	0.727	0.771	0.622	0.786	0.713	0.893	0.720	0.729	0.570	0.740
	c	0.073	0.000	0.170	0.035	0.000	0.000	0.000	0.021	0.059	0.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000
G6pdh-1	a	0.782	0.825	0.437	0.159	0.461	0.573	0.486	0.179	0.528	0.511
	b	0.218	0.175	0.526	0.807	0.478	0.400	0.514	0.707	0.472	0.482
	c	0.000	0.000	0.000	0.028	0.060	0.027	0.000	0.064	0.000	0.000
	d	0.000	0.000	0.037	0.007	0.000	0.000	0.000	0.029	0.000	0.007
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000

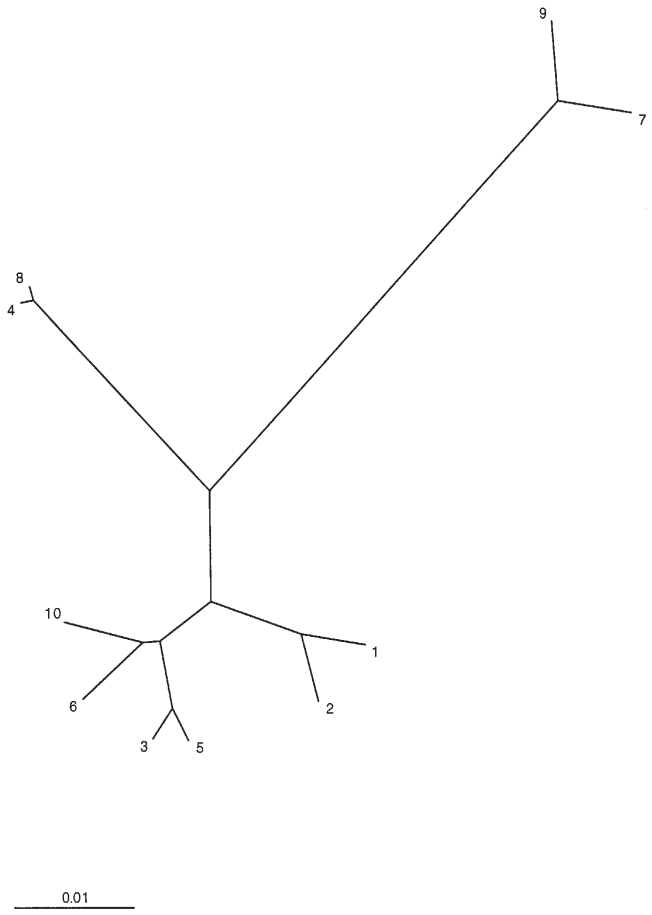

Figure 4. – UPGMA clustering of Nei's genetic distances for Douglas-fir Portuguese provenances using TreeView[36]. Numbers refer to provenance codes *(Table 1)*.

Discussion

The aims of the present investigation were two-fold, to investigate genetic variation: (i) among Portuguese Douglas-fir provenances; and (ii) between Portuguese and North American provenances of the 'coastal' variety, with the aim of identifying the putative source provenances for exotic Portuguese provenances. The allozyme data shows that three introductions from the native range may have given arise to the current Portuguese provenances.

Analysis of Portuguese and native provenances

Among the Portuguese provenances, the mean number of alleles per locus (2.4) was similar to that estimated for the native provenances (2.3) in this investigation, and estimates from British Columbian provenances[17] and lower than that from MEJNARTOWICZ's[21] study (2.9) across the native range. Gene diversity for the exotic provenances is greater than gene diversity for the native provenances used in this study, and may be a reflection of limited sampling of the native range and the use of multiple native provenances to found the exotic provenances.

The expected heterozygosity (H_e), which has the advantage, compared to A, of being independent of sample size, was 0.254 in the Portuguese provenances, which is similar to the values obtained by Mejnartowicz (H_e = 0.222;[21]) and Stauffer (0.219;[23]) which sampled a wider range of the natural distribution. Therefore, these results reflect a considerable level of genetic diversity within Portuguese Douglas-fir provenances. Given that some of the above studies seem to be based on material of limited provenance sampling, and the low rate of evolution expected at allozyme loci, this might be evidence that the Portuguese material has come from more than one source. If one were seeing the situation that material was collected from one source and then planted at multiple locations, it is unlikely that one would be seeing such high levels of overall diversity. Some populations would be expected to have very much lower numbers of alleles than other populations. For example, one might expect to see a significant negative relationship between the number of alleles and the date of plantation establishment, which is not the case (*Table 2*).

The most diverse provenance, from either the native or the exotic range, is Vieira do Minho and this may be a result of multiple seed sources being planted and sampled at this site. Furthermore, alleles are represented at this locality that are absent from all other provenances and is additional evidence that sampling of the native range is limited.

Table 4. – Genetic diversity measures for seven allozyme loci within 10 Portuguese and 17 native *Pseudotsuga menziesii* provenances.

N°	Provenance	Date of plantation establishment	Mean allele number	Nei's gene diversity
1	Leomil	1961	2.1	0.249 (0.0677)
2	Lousã	1938	2.3	0.223 (0.077)
3	Moitas	1949	2.4	0.244 (0.050)
4	Penhas Douradas	1909	2.9	0.277 (0.088)
5	Poulos de Formil	1971	1.9	0.191 (0.051)
6	São Lourenço	1938	2.3	0.234 (0.088)
7	Soajo	1950	2.3	0.241 (0.080)
8	Vieira do Minho	1963	3.1	0.199 (0.074)
9	Vila Flor	1971	2.6	0.27 (0.076)
10	Vila Pouca Aguiar	1938	2.6	0.249 (0.077)
	Mean (SE)		2.4	0.242 (0.008)

N°	Provenance	Mean allele number	Nei's gene diversity
Oregon			
11	Unknown	2.429	0.244 (0.063)
12	Unknown	2.667	0.210 (0.062)
13	Unknown	2.286	0.169 (0.062)
14	Unknown	2.143	0.223 (0.070)
15	Butte Falls	2.714	0.225 (0.071)
California			
16	Sonoma County	1.857	0.244 (0.063)
17	Grouse Mountain	2.286	
Washington			
18	Sedro Woolley	2.5	0.195 (0.071)
19	Forks	1.857	0.13 (0.071)
20	Wind River	2	0.179 (0.082)
21	Enumclaw	2.429	0.275 (0.074)
22	Hoquiam	2.286	0.226 (0.066)
23	Owl Creek	1.833	0.155 (0.067)
British Columbia			
24	Chilliwack Low	2.667	0.17 (0.050)
25	Courtenay	2	0.195 (0.084)
26	Sooke Low	2.143	0.161 (0.083)
27	Sechelt	2.167	0.169 (0.071)
Mean (SE)		2.251 (0.070)	

Origin of Portuguese provenances

The majority of Portuguese Douglas-fir provenances fell into a single poorly resolved group together with provenances from across the native range of Douglas-fir. Within this group are provenances from Moitas, São Lourenço, Poulos de Formil, Vila Pouca and Penhas Douradas, the primary sources of Douglas-fir seed for plantation establishment in Portugal[31]; Vila Pouca (established 1938) being the main source of Douglas-fir seed. Furthermore, Penhas Douradas (established 1909) is probably the oldest Douglas-fir stand in Portugal; thus this stand may have supplied seed for new plantations before other provenances started to produce seed. Such poor resolution is likely to be a reflection of the variation encountered at the isozyme loci investigated. Whether all the Portuguese provenances in this group are the product of a single introduction from the native range and then separate establishment in different parts of Portugal or represent separate introductions cannot be resolved using the current data. Evidence that plantations may not have been derived from single seed sources is seen in the high mean allele number and occurrence of private alleles in the Vieira do Minho provenance compared to the other Portuguese provenances.

Vila Flor, the only Portuguese stand of known native provenance (California) grouped as a distinct group with Soajo. The most similar native provenances to this cluster are from Washington (Provenance 18) and British Columbia (Provenances 24 and 27), albeit that these provenances are very distinct from this group. The absence of grouping of Vila Flor with California provenances may have been due to the small number of native Californian provenances that were available for sampling; the

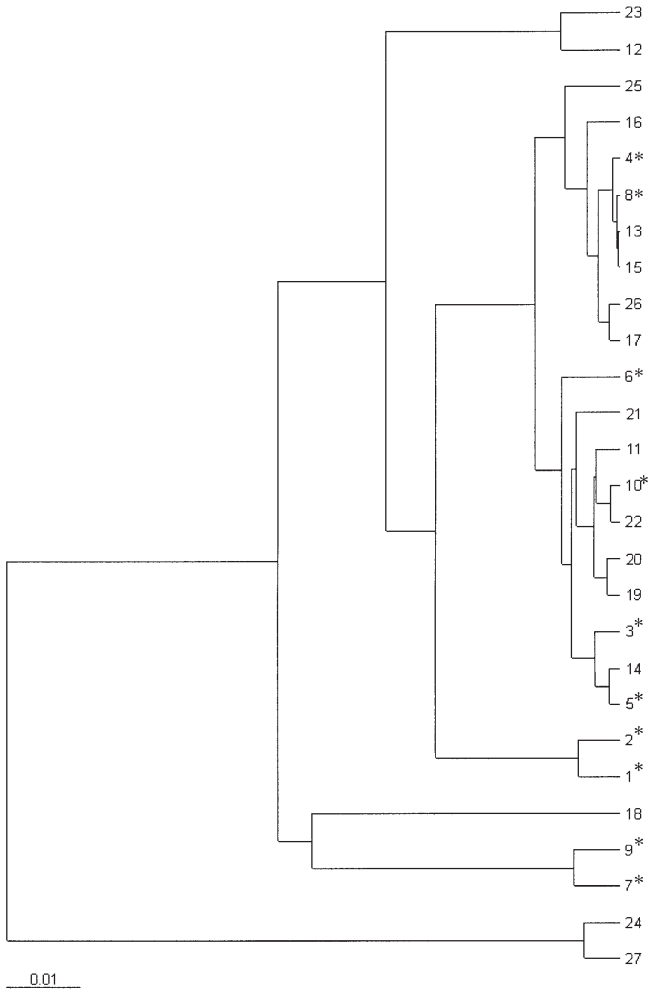

Figure 5. – UPGMA clustering of Nei's genetic distances for all Douglas-fir provenances using TreeView[36]. Numbers refer to provenance codes *(Table 1)*. Numbers 1 to 10 (*) refer to Portuguese provenances.

native Californian provenances were from the 095 and 303 tree seed zones, whilst the seed used to establish Vila Flor came from seed zone 526[32]. A less likely explanation for the failure of Vila Flor to cluster with the Californian provenances is the low resolution of the allozymes used. However, these data suggest that Soajo may also have been derived from California but as a separate, earlier, introduction (c. 1950).

Finally, provenances from Lousã and Leomil form a distinct group, suggesting that the latter may have been derived from the former. This group is most similar to provenances originating from between Oregon and British Columbia and suggests that provenances from this region may have been introduced into Portugal. These data suggest that at least three separate North American introductions of Douglas-fir seed may have been used to establish plantations in Portugal, two from California and a one from Oregon, Washington, or British Columbia.

This investigation has highlighted two important features for future investigations of the origin of Portuguese Douglas-fir provenances in the native range. Firstly, sampling of provenances in the native range must be much denser than presently undertaken, for example the apparent variation that exists between California provenances if the Californian origin of Vila Flor is correct. Secondly, high resolution markers are likely to yield better results over the identities of the originating native provenances, when combined with improved sampling of the native range. Such resolution could be achieved in two ways, through the use of markers that detect numerous putative loci (e.g. AFLPs) or numerous alleles at a locus (e.g. microsatellites;[33]). In addition, analysis of chloroplast DNA pattern across the North American range of the species might also enable increased resolution among the Portuguese provenances, although investigations of cpDNA variation among British Columbia provenances has shown little between provenance variation[34,35].

Acknowledgements

This research project was funded by Fundação para a Ciência e a Tecnologia grant PRAXIS XXI / BD / 18258 / 98, to whom we are grateful. Many thanks to Eng. MARIA TERESA ALVES DA SILVA sub-director from Direcção Geral das Florestas for her encouragement and immense support, which made possible the completion of a large part of the field work. There are a great number of people from the following institutions whom we would like to thank, Direcção Geral das Florestas, COTF, and Serviços Florestais das Direcções Regionais da Agricultura of Entre-Douro e Minho, Trás-os-Montes e Alto Douro, Beira Interior, and Beira Litoral. We are thankful to Eng. CARLOS SILVA and staff of CENACEF for carry out the work of climbing trees for collection cones and for extracting seed.

References

[1.] LITTLE, E. L., JR.: Atlas of United States trees. Volume 1. Conifers and important hardwoods. pp. v + 9 + 202 maps (1971). — [2.] LI, P. and ADAMS, W. T.: Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). Canadian Journal of Forest Research **19**, 149–161 (1989). — [3.] HERMANN, R. K. et al. *in:* Special issue on planted forests: contributions to the quest for sustainable societies. Papers from a symposium, Portland, Oregon, USA, June 1995. New Forests. 1999, **17**: 1 3, 53 70; 4 pp. of ref. (1999). — [4.] KLEINSCHMIT, J. and BASTIEN, J. C.: IUFRO's role in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tree improvement. Silvae Genetica **41**, 161–173 (1992). — [5.] SAVILL, P., EVANS, J., AUCLAIR, D. and FALCK, J.: Plantation silviculture in Europe (Oxford University Press, Oxford, 1997). — [6.] GOMES, M. d. A. and RAPOSO, F.: Estudos dendrológicos. O género "Pseudotsuga" no Parque da Pena (Sintra). Anais do Instituto Superior de Agronomia **10**, 121–135 (1939). — [7.] Goes, E.: A floresta Portuguesa (Portucel, Lisboa, 1991). — [8.] FREITAS, A. S. B.: Perimetro Florestal de Manteigas (Direcção Geral das Florestas, Lisboa, 1989). — [9.] COUTINHO, A. X. P.: 334–340 (DGSFA, Lisboa, 1936). — [10.] GOES, A. *in:* Instituto Superior de Agronomia 88 (Universidade Técnica de Lisboa, Lisboa, 1976). — [11.] MARTINS, L.: (Direcção Geral da Florestas, Lisboa, 1999). — [12.] LOURO, V. and CABRITA, P.: *Pseudotsuga,* Estudo de Sucessos e Insucessos Pos-plantação (Direcção Geral das Florestas, Lisboa, 1985). — [13.] LOURO, V. and CABRITA, P.: *Pseudotsuga,* Contribuição para o conhecimento da sua cultura em Portugal. Estudos e Informação N° 298 (Direcção Geral das Florestas, Lisboa, 1989). — [14.] WOODS, F. W.: 26 (Estudos N° 7. Fundo de Fomento Florestal, Lisboa, 1976). — [15.] Luis, J. F. S.: (Vila Real, Portugal (unpublished), 1992). — [16.] YEH, F. C. H. and O' MALLEY, D.: Enzyme variations in natural populations of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. 1. Genetic variation patterns in coastal populations. Silvae Genetica **29**, 83–92 (1980). — [17.] EL KASSABY, Y. A. and SZIKLAI, O.: Genetic variation of allozyme and quantitative traits in a selected Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) population. Forest Ecology and Management **4**, 115–126 (1982). — [18.] MERKLE, S. A. and ADAMS, W. T.: Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. Canadian Journal of Forest Research **17**, 402–407 (1987). — [19.] MORAN, G. F. and ADAMS, W. T.: Microgeographical patterns of allozyme differentiation in Douglas-fir from southwest Oregon. Forest Science **35**, 3–15 (1989). — [20.] EL KASSABY, Y. A.: Impact of selection and breeding on the genetic diversity in Douglas-fir. Biodiversity and Conservation **5**, 795–813 (1996). — [21.] MEJNARTOWICZ, L. and LEWANDOWSKI, A.: Allozyme polymorphism in seeds collected from a IUFRO-68 Douglas-fir test-plantation. Silvae Genetica **43**, 181–186 (1994). — [22.] HOFFMANN, C. and GEBUREK, T.: Allozyme variation of indigenous Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) populations and their descendants in Germany. Silvae Genetica **44**, 222–225 (1995). — [23.] STAUFFER, A. and ADAMS, W. T.: Allozyme variation and mating system of three Douglas-fir stands in Switzerland. Silvae Genetica **42**, 254–258 (1993). — [24.] PRAT, D. and ARNAL, S.: Allozyme variation and mating system in three artificial stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) planted in Europe. Silvae Genetica **43**, 199–206 (1994). — [25.] DIVISION, F. R. *in:* Collecting plant genetic diversity: technical guidelines (eds. GUARINO, L., RAO, V. R. and REID, R.) (CAB International, Wallingford, UK, 1995).

— [26] WENDEL, J. F. and WEEDEN, N. F. *in:* Isozymes in Plant Biology (eds. SOLTIS, D. E. and SOLTIS, P. S.) (Dioscorides Press, Portland, Oregon, 1990). — [27] YEH, F. C., YANG, R. and BOYLE, T.: (University of Alberta, Edmonton, 1999). — [28] Nei, M. Molecular evolutionary genetics (Columbia University Press, New York, 1987). — [29] Nei, M.: Molecular population genetics and evolution (American Elsevier, New York, 1975). — [30] SNEATH, P. H. A. and SOKAL, R. R.: Numerical Taxonomy: The Principles and Practice of Numerical Classification (W. H. Freeman & Co, San Francisco, 1973). — [31] SILVA, C.: (Centro Nacional de Sementes Florestais (CENACEF), Amarante, 2001). — [32] BUCK, J. M. et al.: (California Region. Forest Service. U S Department of Agriculture., San Francisco, 1970). — [33] KARP, A., ISAAC, P. G. and INGRAM, D. S.: Molecular tools for screening biodiversity: plants and animals (Chapman & Hall Ltd, London; UK, 1998). — [34] PONOY, B., HONG, Y. P., WOODS, J., JAQUISH, B. and CARLSON, J. E.: Chloroplast DNA diversity of Douglas-fir in British Columbia. Canadian Journal of Forest Research **24**, 1824–1834 (1994). — [35] VIARD, F., EL KASSABY, Y. A. and RITLAND, K.: Diversity and genetic structure in populations of *Pseudotsuga menziesii* (Pinaceae) at chloroplast microsatellite loci. Genome **44**, 336–344 (2001). — [36] PAGE, R. D. M. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences **12**, 357–358 (1996).

Shoot Position Affects Root Initiation and Growth of Dormant Unrooted Cuttings of *Populus*

By R. S. ZALESNY JR.[1)], R. B. HALL, E. O. BAUER and D. E. RIEMENSCHNEIDER

Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA 50011 / USDA Forest Service, North Central Research Station, Forestry Sciences Laboratory, Rhinelander, WI 54501

(Received 22nd September 2003)

Abstract

Rooting of dormant unrooted cuttings is crucial to the commercial deployment of intensively cultured poplar (*Populus* spp.) plantations because it is the first biological prerequisite to stand establishment. Rooting can be genetically controlled and subject to selection. Thus, our objective was to test for differences in rooting ability among cuttings from three positions on cutting orchard plants of five genomic groups ([Bartr. ex Marsh × *P. trichocarpa* Torr. & Gray *P. deltoides*] × *P. deltoides* 'BC', *P. deltoides* 'D', *P. deltoides* × *P. maximowiczii* A. Henry 'DM', *P. deltoides* × *P. nigra* L. 'DN', *P. nigra* × *P. maximowiczii* 'NM'). Cuttings, 20 cm long, were randomly planted at 1.2- x 2.4-m spacing across three planting dates during 2001 and 2002 at Ames, Iowa, USA (42.0°N, 93.6°W); Waseca, Minnesota, USA (44.1°N, 93.5°W); and Westport, Minnesota, USA (45.7°N, 95.2°W). We measured root dry weight, number of roots, and total root length from harvested cuttings after 14 d of growth. Rooting traits varied relative to stem position but interactions of genomic groups and positions and genotype x environment interactions existed on multiple-year and single-year bases. Position accounted for the second highest amount of variation (≥ 5%) for all rooting traits. Cuttings from the basal third of the shoot system of the stool plant exhibited nearly two times more rooting as those from middle and apical regions, whereas middle cuttings exhibited similar rooting trends as apical cuttings, for all rooting traits. The percentage of cuttings rooted across years was greatest with basal cuttings for the BC, D, DM, and DN genomic groups (> 50%). Middle cuttings of the NM group survived at a greater rate (88%) than did basal (80%) and apical (72%) cuttings. Single-year analyses of interactions of genomic groups and positions showed rooting was greatest with basal cuttings for BC, D, and DN genotypes. Basal cuttings of the DM and NM genomic groups did not clearly outperform middle and apical cuttings, and differences among all cutting positions were site- and year-dependent.

[1)] Corresponding author is currently a research plant geneticist at: USDA Forest Service, North Central Research Station, Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, WI 54501. Email: rzalesny@fs.fed.us

Key words: Poplar, lateral rooting, adventitious rooting, rooting ability, short rotation intensive culture.

Introduction

There is a predicted shortage of *P. tremuloides* Michx. (quaking aspen) and *P. grandidentata* Michx. (bigtooth aspen) in the North Central United States within 10 to 20 years due to a lack of suitable aspen stumpage within harvestable diameter classes (PIVA, 2003). Thus, recent attention in the North Central United States focuses on increasing production from intensively managed plantations because production from such plantations reduces pressure on native forests (GLADSTONE and LEDIG, 1990). Selected poplar clones (*Populus* spp.) are suited to intensive culture because they are fast-growing, relatively easy to propagate vegetatively, and require shorter harvest cycles than aspen (DICKMANN, 2001; HEILMAN, 1999). Poplar plantations can provide fiber, energy (liquid fuels and biomass for electricity), phytoremediation benefits, raw material for engineered lumber products, cordwood (firewood), riparian stabilization, agroforestry opportunities, wildlife habitat, and aesthetic values (HEILMAN, 1999; JOSLIN and SCHOENHOLTZ, 1997). Four *Populus* species commonly used in North American breeding programs are *P. deltoides* (eastern cottonwood), *P. trichocarpa* (western black cottonwood), *P. nigra* (European black poplar), and *P. maximowiczii* (Japanese poplar).

The ability of poplars to form lateral and adventitious roots from dormant unrooted cuttings is crucial to the commercial deployment of intensively cultured poplar plantations because rooting is the first biological prerequisite to stand establishment. Information is lacking about the genetics and physiology underlying the ability of stem cuttings to root (HAISSIG and DAVIS, 1994; HAISSIG et al., 1992), and an increased knowledge of genetic and environmental covariances between root and shoot developmental systems is desirable (RIEMENSCHNEIDER et al., 1996). Breeding for enhanced rooting ability is a key component of poplar clonal development (RIEMENSCHNEIDER and BAUER, 1997).