The Effect of Outcrossing Rate on the Growth of Selected Families of *Eucalyptus grandis*

By I. P. Burgess, E. R. Williams, J. C. Bell, C. E. Harwood and J. V. Owen

CSIRO, Division of Forestry, Canberra, Australia

(Received 24th November 1995)

Summary

Seed collections were made in natural *Eucalyptus grandis* forests in the region of Coffs Harbour, New South Wales, following a year of good flowering and seed set. Outcrossing rates were determined for individual parent trees by isozyme analysis of progeny arrays. For each of 10 local provenances, 2 parent trees having the highest outcrossing rates and 2 having the lowest outcrossing rates were selected, and seedlings raised for field planting in a designed experiment with 4 replicates of 5-tree plots for each of the 40 selected families, on an effluent-irrigated planting site at Dubbo, New South Wales. Height and diameter were assessed 2.5 years after planting out. Preliminary analysis showed there was substantial variation across the planting site, so mixed-model analyses were carried out on plot mean values of height and diameter to recover family information from the incomplete blocking structures used and generate family means for these variates. The effects of family outcrossing rates and provenance were then examined using parallel line regression. Outcrossing rate strongly influenced all variates while differences between provenances were not significant. Families with higher outcrossing rates generally displayed faster growth and better survival. The interaction between outcrossing rates and provenance was significant for height and diameter. These results support the view that in this species, variations in outcrossing between parent trees have a large influence on differences in growth rate among natural provenances and progenies within provenances.

Key words: Outcrossing, variability, provenance, progeny, growth rates, survival, *Eucalyptus grandis.*

FDC: 165.4; 232.11; 232.12; 176.1 *Eucalyptus grandis;* (944).

Introduction

Field trials have demonstrated considerable variation in productivity of collections of *Eucalyptus grandis* Hill ex Maiden from relatively small geographical areas and year by year collections from much the same site (Burgess, 1988; Matheson and Mullin, 1987). Eucalypts have a mixed mating system (Eldridge *et al.,* 1993) which has been found to vary both spatially and with time (Brown *et al.,* 1975; Moran and Brown, 1980). Also there is substantial evidence for the deleterious effects of selfing or related mating for a range of species (Eldridge *et al.,* 1993). Variation in vigour between provenances, or between the progenies of individual parent trees within a provenance, could be due to differences in genetic potential (breeding value) or to more ephemeral differences in the degree of inbreeding in the progenies (Yeh *et al.,* 1983), or a combination of these 2 effects. Open-pollinated seed typically derives from selfing, mating with relatives (neighbourhood inbreeding) and outcrossing with unrelated trees (Griffin and Cotterill, 1988) and the proportions of these 3 categories will vary both within and between populations (Potts, 1994). The response of individual progenies to inbreeding may differ due to random variations in the genetic load of deleterious recessive alleles. Hodgson (1976) compared the growth of progenies from controlled cross-pollination (outcrosses), controlled self-pollination (selfs) and from open pollination of 12 different clones of *E. grandis* in experimental plantings in South Africa. For the 12 clones, the mean heights of selfed progeny were from 8% to 49% smaller than the corresponding controlled outcrosses, 11 months to 18 months after planting. The overall mean decrease in height from selfing, relative to outcrossing, was 31%. The corresponding reductions in height of open-pollinated progenies of the clones, relative to outcrosses, ranged from 2% to 26%. The percentage of selfing in the open-pollinated progenies of the clones used in Hodgson's experiments was estimated from proportions of deviant seedlings to range from 10% to 38% for the different clones.

Outcrossing rate of a single seed crop on an individual tree will depend upon such factors as self-fertility, abundance of flowers, flowering phenology, weather conditions and pollinator activity (Griffin and Cotterill, 1988). Most previous work in this area has concentrated on estimating mean outcrossing rates of local populations. Little attention has been paid to variability in individual tree outcrossing rates and the effect this may have on progeny growth and the repeatability of provenance collections. An exception is the recent study by Potts et al. (1995), who found that differences in stand type (isolated trees, remnant isolated small stands, continuous open stands and continuous closed stands) from which seed collections were made accounted for much of the variation in performance in provenance/progeny trials of *E. globulus* in Tasmania. These stand effects were assumed to be the consequence of different levels of outcrossing, and the importance of outcrossing differences in determining growth rates was confirmed by comparing the growth of open-pollinated progenies and controlled crosses involving the same female parents in a subsequent experiment. The work reported here examines the relationship between outcrossing and growth in progeny collections from natural forests of *E. grandis.* It investigates whether selection of progenies according to their estimated outcrossing rate has an impact on survival and growth.

Methods

Collection

To investigate the variation in outcrossing rates in individual trees in natural populations over time, seed was collected from 183 trees from 18 provenances in the general region of Coffs Harbour (latitude 30°S, New South Wales, Australia) in late 1988. The collections were designed to cover the altitudinal range of the species in that area and to sample as wide a range of sites as practical. Each tree was labelled and its location mapped. This collection was considered to originate from a good to excellent flowering some months earlier. Further seed collections were made in 1989 from 54 of the 183 trees originally sampled. This was judged a poor flowering year.

Isozyme analysis

Thirty seedlings from each tree of the 1988 collection were scored for electrophoretic variants at 13 loci using standard techniques (MORAN and BELL, 1983). Multi-locus estimates of individual tree outcrossing rates were made (data of J. C. BELL) using MLTF (RITLAND, 1990). Cases where convergence did not occur were discarded.

Experiment

In 1990 an opportunity arose to conduct an experiment on a limited selection of the 1988 collection to investigate the effect on growth of different outcrossing rates and, to a lesser extent, provenance differences.

Ten provenances were subjectively chosen as a sample of the whole collection (*Table 1*). Among the trees within each provenance, the 2 trees with the highest outcrossing rate and the 2 trees with the lowest outcrossing rate were selected. Open-pollinated seed from these trees provided the 40 families for the field trial. Details of the trees selected and estimated outcrossing rates are given in *table 2*. Note that estimates of single tree outcrossing rates can exceed one (see CHELIAK *et al.*, 1983; RITLAND and EL-KASSABY, 1985; and EL-KASSABY, *et al.*, 1993 for discussion).

Table 1. – Details of the 10 *E. grandis* collections.

Provenance Number	Forest	Location	Latitude	Longitude	Altitude (m)
16435	Orara East	Sherwood Rd/Duck Trail	30° 09’	153° 07’	120
16436	Orara East	Sherwood Rd/Creek Trail	30° 10’	153° 07’	100
16437	Conglomerate	Waihou Trig	30° 05’	143° 01’	300
16440	Orara East	Eastern Boundary Rd/Por. 113	30° 10’	153° 09’	5
16443	Orara East	Eastbank Rd/Burra Trail	30° 13’	153° 02’	130
16445	Nana Ck	Bushmans Range Rd	30° 12’	152° 55’	590
16447	Orara West	Langleys Rd	30° 13’	152° 56’	630
16448	Bagawa	12/60 Flora Reserve	30° 07’	152° 34’	400
16451	Bagawa	Black Mt./Bellbird Rd	30° 07’	152° 50’	510
16454	Pine Creek	Gordons Rd	30° 09’	153° 00’	150

Table 2. – Calculated outcrossing rate for each parent tree.

Provenance	Parent tree	Outcrossing rate
16435	1663	0.64
	1665	0.69
	1669	1.14
	1670	1.20
16436	1684	0.34
	1677	0.39
	1688	0.99
	1687	1.04
16437	1696	0.63
	1844	0.72
	1689	1.06
	1692	1.13
16440	1724	0.36
	1722	0.58
	1737	1.13
	1725	1.36
16443	1757	0.59
	1754	0.62
	1753	1.08
	1759	1.37
16445	1773	0.45
	1769	0.53
	1839	1.11
	1838	1.31
16447	1792	0.52
	1794	0.62
	1796	1.24
	1795	1.48
16448	1801	0.46
	1803	0.57
	1805	0.98
	1806	1.00
16451	1823	0.47
	1817	0.58
	1822	0.94
	1821	1.00
16454	1857	0.49
	1852	0.49
	1853	1.04
	1851	1.13

Dubbo City Council’s sewerage plant on the Bunglegumbie Road was chosen as the trial site (lat. 32°15’S, long. 148°37’E, altitude 260 m, maximum temperature recorded 46 °C, minimum recorded –8 °C, averaging 11 frosts per year). The site was sprayed to control all weeds, ripped and fenced against rabbits. The treated effluent sprinkler system was controlled to maintain near field capacity at all times.

The experiment consisted of 4 contiguous replicates of 40 families (10 provenances each with 2 parent trees with high outcrossing rates and 2 with low outcrossing rates) in 5-tree row plots. The configuration of the plots and the direction of the irrigation lines is shown in *figure 1*. The tree spacing was 3 m between irrigation lines and 2 m between trees along the lines. No attempt was made to select the largest, most vigorous seedlings for outplanting – germinants were pricked out at random for potting up, and seedlings were selected at random from among the healthy stock available from the nursery at the time of outplanting.

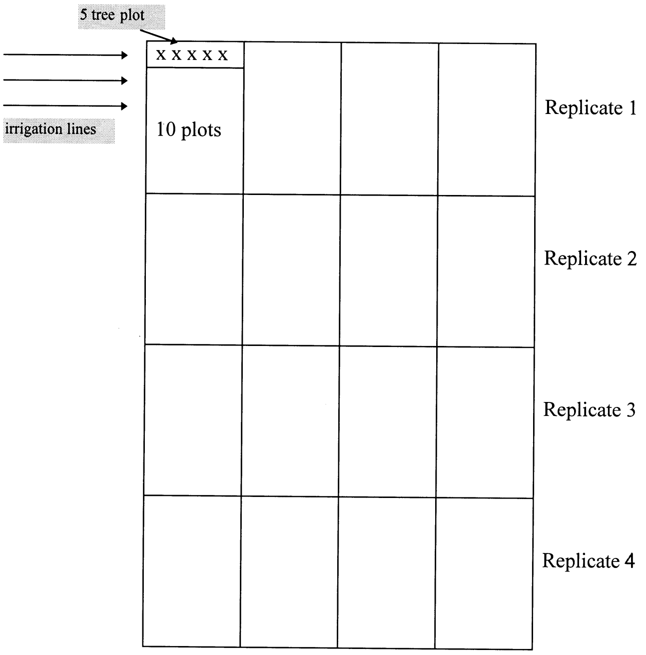

Figure 1. – Layout of field trial at Dubbo.

The experimental design was obtained from ALPHA+ (WILLIAMS and TALBOT, 1993) using an efficient latinized row-column design chosen to have desirable factorial properties as discussed by WILLIAMS and JOHN (1995). The design was generated such that each long column of 40 plots constituted a replicate of the 40 families (the latinized property); each column of 10 plots within replicates consisted of one representative of each of the 10 provenances and 5 replications of the 2 levels of outcrossing rate (the optimal factorial main-effects property for columns within replicates). In addition each row of the design consisted of 2 replications of the 2 levels of outcrossing rate. This latinized 2-factor row-column design was used to effectively accommodate anticipated site variation. Previous experience (WILLIAMS, 1986) had indicated that considerable site variation can arise from the use of irrigation, through differences in the performance of irrigation lines.

The experiment was assessed for individual tree height (*ht*) and diameter at breast height (*dbh*) in April 1994, 2.5 years after planting.

Results

The individual-tree data for *ht* and *dbh* were converted into a plot summary file following the methods of WILLIAMS and MATHESON (1994, Chapter 3), i.e. a new data file was constructed containing means (*mn*), tree counts (*cnt*) and the between-tree-within-plot variances (*var*) for each 5-tree plot. This plot summary file was screened for data entry errors as discussed by WILLIAMS and MATHESON (1994). None of the checked, individual tree values were excluded from the data set.

The plot counts were converted to percentage survival (*pcnt* = 100 x *cnt / 5*) and the plot variances converted to logarithms (*lvar* = log (*var* + 1)); both these transformations were carried out to stabilise variances for the purpose of analysis of variance. The plot means and transformed plot counts and plot variances were then analysed using non-orthogonal analysis of variance in the statistical package GENSTAT. This analysis allowed investigation of the effectiveness of the multiple blocking structures built into the design. Results are included in *table 3*. It can be seen that for the plot mean data, there is considerable variation across the blocking structures. Consequently a mixed-model analysis (using REML in GENSTAT) was performed for plot means in order to recover family information from the incomplete blocking structures. Estimated family means for *mn, lvar* and *pcnt* were assembled into a new file; these are included in *table 4*.

Table 3. – Mean squares from non-orthogonal analyses of variance for height (*ht*), diameter at breast height (*dbh*) and survival (*pcnt*) incorporating multiple blocking structures; d.f. = degrees of freedom, *mn* = mean, *lvar* = log of (variance + 1).

		ht		*dbh*		
Source	d.f.	*mn*	*lvar*	*mn*	*lvar*	*pcnt*
replicate	3	3.00*	1.50*	0.62	0.70	203
column	3	6.13***	0.78	2.34	0.72	83
replicate.row	36	1.68**	0.33	2.24**	0.42	153
replicate.column	9	1.89*	0.35	1.22	0.21	225
family	39	3.35***	0.71*	3.64***	0.58	207
residual	69	0.79	0.42	1.16	0.42	188

---

*) = P < 0.05, **) = P < 0.01, ***) = P < 0.001

Table 4. – Family means and variances for height (*ht*), diameter at breast height (*dbh*), and survival (*pcnt*) at age 2.5 years; *mn* = mean, *lvar* = log of (variance + 1).

		ht		*dbh*		
Provenance	Family	*mn* (m)	*lvar*	*mn* (cm)	*lvar*	*pcnt*
16435	1663	10.09	0.77	8.87	1.15	90
	1665	11.02	0.53	9.71	0.94	90
	1669	9.65	0.73	8.46	1.44	85
	1670	10.40	0.41	9.76	1.10	95
16436	1684	7.11	1.77	6.18	2.10	75
	1677	7.94	2.20	6.93	2.12	80
	1688	10.25	0.62	9.08	0.87	90
	1687	10.44	0.51	9.63	0.58	100
16437	1696	10.47	0.94	9.9	1.09	95
	1844	9.34	1.34	8.23	1.46	95
	1689	9.57	1.18	8.51	1.45	85
	1692	10.26	0.41	9.12	0.95	95
16440	1724	6.58	1.56	5.85	1.83	80
	1722	9.05	1.81	8.55	2.01	70
	1737	10.70	0.91	9.37	1.01	100
	1725	11.00	0.73	10.49	1.07	100
16443	1757	9.51	1.33	8.65	1.53	95
	1754	9.44	1.05	8.50	1.35	80
	1753	10.63	0.39	9.43	0.65	100
	1759	10.91	0.66	9.82	0.79	85
16445	1773	7.61	1.76	6.94	1.82	85
	1769	10.47	0.83	8.59	1.21	100
	1839	11.06	0.64	10.12	1.10	95
	1838	10.13	0.51	8.76	1.27	100
16447	1792	9.36	1.66	7.73	1.89	95
	1794	9.90	1.25	8.76	1.51	95
	1796	9.37	1.21	8.89	1.68	95
	1795	10.52	0.63	10.24	0.87	100
16448	1801	8.92	1.17	7.97	2.01	85
	1803	9.88	0.98	9.27	1.43	90
	1805	10.41	0.61	9.01	1.38	95
	1806	9.63	1.16	9.38	1.29	100
16451	1823	9.70	0.68	8.99	0.99	75
	1817	9.54	1.16	8.63	1.43	95
	1822	10.38	0.56	9.96	0.94	90
	1821	10.12	1.02	10.04	1.50	95
16454	1857	8.75	1.18	7.76	1.20	90
	1852	9.48	1.35	8.77	1.56	95
	1853	10.80	0.55	9.60	0.94	90
	1851	10.53	0.62	9.66	0.98	95

The family structure of the 40 estimated means was then investigated following the methods of WILLIAMS and MATHESON (1994, Section 8.5). The effects of outcrossing rate (*ocr*) and provenance (*prov*) on the estimated means were examined using a parallel line regression approach. This allowed the estimated values for *ocr* (*Table 2*) to be included in the analysis and also the possibility of differential responses to ocr for different provenances. The results from the analyses are included in *table 5*; note that the residual mean squares from the *table 3* analyses have been added to *table 5,* following WILLIAMS and MATHESON (1994).

There is a strong relationship between the variates analysed and *ocr* (*Table 5*). For *lvar* the estimated regression coefficients for *ht* and *dbh* are –0.93 and –0.75; and for *pcnt* the value is 12.2. For *ht mn* and *dbh mn*, there are significant differences in the slopes of the *ocr* regression lines for the individual provenances. These ranged from –0.86 to 4.37 for *ht mn* and –0.87 to 4.36 for *dbh mn*. Differences among provenances are not significant for any of the 5 variates analysed. It should be kept in mind that the provenance comparisons are not based on random samples. The relationships between family ocr and *ht* for separate provenances are illustrated in *figure 2* by graphing the individual family *ht* means against *ocr* and using the last two digits of the provenance numbers from *table 2* to identify provenances. A similar situation exists for *dbh.*

Table 5. – Mean squares from parallel line regression analyses of variance of height (*ht*), diameter at breast height (*dbh*) and percent survival (*pcnt*) on outcrossing rate (*ocr*), at age 2.5 years; *mn* = mean, *lvar* = log of (variance + 1).

		ht		*dbh*		
Source	d.f.	*mn*	*lvar*	*mn*	*lvar*	*pcnt*
ocr	1	74.9***	14.5***	84.9***	9.39***	2511***
prov	9	1.64	0.66	2.26	0.41	128
ocr.prov	9	4.29***	0.45	3.23**	0.68	255
family remainder	20	1.94	0.37	1.94	0.29	177
residual	69	0.79	0.42	1.16	0.42	188

) = P < 0.01, *) = P < 0.001

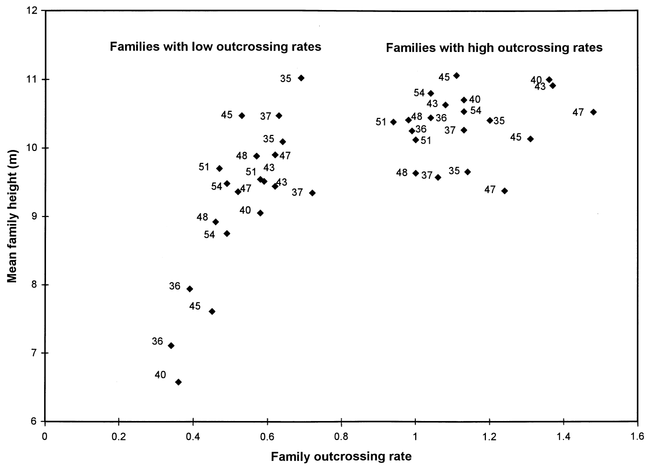

Figure 2. – Graph of family mean height (*ht*) versus family outcrossing rate (*ocr*). The numbers shown for each point are the last 2 digits of the provenance numbers from *table 1.*

Discussion

The lower growth and poorer survival of the families with lower outcrossing rates is consistent with the results that HODGSON (1976) obtained studying inbreeding depression in selected genotypes of *E. grandis* in South Africa. VAN WYK (1981) showed for *E. grandis* that selfing and full-sib matings were more deleterious, in terms of vigour reduction, than were half-sib matings. From the outcrossing rates we can estimate the proportion of seed (1.0 minus the outcrossing estimate) which is inbred, but we cannot distinguish selfing and other forms of inbreeding (matings between close relatives with indistinguishable allele profiles). Estimates of outcrossing rates for the different parent trees in our study ranged from 0.34 to 1.48. It might be argued that outcrossing rates greater than one have no biological meaning. However, there is an error estimate associated with each estimate, and values greater than one indicate a greater likelihood that the progeny is fully outcrossed. The analysis presented in *table 5* was also conducted with outcrossing rates greater than one re-set to one, and essentially similar results were obtained.

Growth data presented here derive from a 2.5-year assessment of trees raised in 1990, while outcrossing rates were determined by analysis of germinants in 1989. Because of the reduced fitness of inbred individuals, there may have been some decrease in the proportion of inbred individuals, and particularly of selfs, during seed storage subsequent to the isozyme analysis, and during raising of the seedlings for planting out. Accentuation of growth differences arising from inbreeding would be expected later in the life of the field trial, after intense competition had set in. Selfing effects were not significant in *E. regnans* 2 years after planting, but became clearly apparent at age 4 years (ELDRIDGE and GRIFFIN, 1983). Plot size, initial spacing and whether or not trials have been thinned, will affect the magnitude of later-age growth differences arising from different levels of inbreeding.

Seed collections from different provenances may derive from different flowering seasons, or there may be geographic variation in outcrossing rates in a particular season. Considering the magnitude of the outcrossing rate effects presented here, it is clear that differences in outcrossing rate, resulting from geographic or temporal differences in environmental conditions affecting flowering and pollination, could change the rankings of provenances, as well as the rankings of individual parent trees within provenances. Such effects may explain the inconsistent local variation patterns found in *E. grandis* provenance trials (BURGESS, 1988; MATHESON and MULLIN, 1980). It follows that breeding programs for *E. grandis* should not place overly strong emphasis on the growth of first-generation provenance and progeny trials when determining the composition of advanced-generation breeding populations. It is only after controlled crossing, or after open-pollination under uniform conditions in an even-aged plantation of numerous unrelated trees (ELDRIDGE *et al.,* 1993, p. 197) that candidate provenances and individuals can be accurately ranked. As noted by GRIFFIN and COTTERILL (1988) and POTTS (1994), calculations of heritabilities and other genetic parameters based on open-pollinated progenies from natural forests must also be treated with caution.

Acknowledgements

ROD GRIFFIN and GAVIN MORAN were instrumental in the design of this study. The Dubbo City Council Engineering Department supplied the prepared planting site and has been most co-operative during the life of the trial. Shell Australia financed the collection and laboratory work, much of which was carried out by DEBBIE CLARIDGE and CHRISTINE TOMPKINS. KEN ELDRIDGE and GAVIN MORAN provided valuable comments on an earlier draft.

References

BROWN, A. D. H., MATHESON, A. C. and ELDRIDGE, K. G.: Estimation of the mating system of *Eucalyptus obliqua* L'HERIT. using allozyme polymorphisms. Aust. J. Bot. **23**: 931–949 (1975). — BURGESS, I. P.: Provenance trials of *Eucalyptus grandis* and *E. saligna* in Australia. Silv. Genet. **37**: 221–227 (1988). — CHELIAK, W. M., MORGAN, K., Strobeck, C., YEH, F. C. H. and DANIK, B. P.: Estimation of mating system parameters in plant populations using the EM algorithm. Theor. Appl. Genet. **65**: 157–161 (1983). — ELDRIDGE, K., DAVIDSON, J., HARWOOD, C. and VAN WYK, G.: Eucalypt Domestication and Breeding. Clarendon Press, Oxford (1993). — EL-KASSABY, Y. A., MEAGHER, M. D. and DAVIDSON, R.: Temporal variation in outcrossing rate in a natural stand of Western White Pine. Silv. Genet. **42**: 131–135 (1993). — GRIFFIN, A. R. and COTTERILL, P. P.: Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. Silv. Genet. **37**: 124–131 (1988). — HODGSON, L. M.: Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (HILLE) MAIDEN at J. D. M. Keet Research Station 2. The fruit, seed, seedlings, self-fertility, selfing and inbreeding effects. S. A. For. J. **98**: 32–43 (1976). — MATHESON, A. C. and MULLIN, L. J.: Variation among neighbouring and distant provenances of *Eucalyptus grandis* and *E. tereticornis* in Zimbabwean field trials. Aust. For. Res. **17**(3): 233–250 (1987). — MORAN, G. F. and BELL, J. C.: *Eucalyptus.* In: Isozymes in Plant Genetics and Breeding. Part B. S. D. TANKSLEY and T. J. ORTON (eds). Elsevier, Amsterdam. Pp. 423–441 (1983). — MORAN, G. F. and BROWN, A. D. H.: Temporal heterogeneity of outcrossing rates in alpine ash (*Eucalyptus delegatensis* R. T. BAKER). Theor. Appl. Genet. **57**: 101–105 (1980). — POTTS, B. M.: Biological constraints to accurate parameter estimation and prediction of breeding values. Eucalyptus Improvement and Silviculture, Newsletter WP S2.02-09 IUFRO (1994). — POTTS, B. M., VOLKER, P. W., HODGE, G. R., BORRALHO, N. M. G., HARDNER, C. M. and OWEN, J. V.: Genetic limitations in the exploitation of base populations of *Eucalyptus globulus* ssp. *globulus.* In: Eucalypt Plantations: Improving Fibre Yield and Quality. B. M. POTTS, N. M. G. BORRALHO, J. B. REID, R. N. CROMER, W. N. TIBBITS and C. A. RAYMOND (eds). Proc. CRCTHF-IUFRO Conf., Hobart 19 to 24 Feb. Pp. 217–221 (1995). — RITLAND, K. and EL-KASSABY, Y. A.: The nature of inbreeding in a seed orchard of Douglas fir as shown by an efficient multilocus model. Theor. Appl. Genet. **71**: 375–384 (1985). — RITLAND, K. and EL-KASSABY, Y. A.: Generalised Multilocus Estimation Program II MLTF. Documentation Manual (1990). — VAN WYK, G.: Inbreeding effects in *Eucalyptus grandis* in relation to degree of relatedness. S.A. For. J. **116**: 60–63 (1981). — WILLIAMS, E. R.: Row and column designs with contiguous replicates. Austral. J. Statist. **28**: 149–154 (1986). — WILLIAMS, E. R. and JOHN, J. A.: Row-column designs for use in agricultural field trials. Appl. Statist. (1996, to appear). — WILLIAMS, E. R. and MATHESON, A. C.: Experimental Design and Analysis for Use in Tree Improvement. CSIRO, Melbourne (1994). — WILLIAMS, E. R. and TALBOT, M.: *ALPHA* +. Experimental design for variety trials, Version 1.0. Design User Manual. CSIRO, Canberra and SASS, Edinburgh (1993). — YEH, F. C., BRUNE, A., CHELIAK, W. M. and CHIPMAN, D. C.: Mating system of *Eucalyptus citriodora* in a seed-production area. Can. J. For. Res. **13**: 1051–1055 (1983).