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ORIGINAL PAPER

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Effects of inbreeding on coastal Douglas fir growth and yield in operational plantations: a model-based approach

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Abstract In advanced generation seed orchards, tradeoffs exist between genetic gain obtained by selecting the best related individuals for seed orchard populations, and potential losses due to subsequent inbreeding between these individuals. Although inbreeding depression for growth rate is strong in most forest tree species at the individual tree level, the effect of a small proportion of inbreds in seed lots on final stand yield may be less important. The effects of inbreeding on wood production of mature stands cannot be assessed empirically in the short term, thus such effects were simulated for coastal Douglas fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco] using an individual-tree growth and yield model TASS (Tree and Stand Simulator). The simulations were based on seed set, nursery culling rates, and 10-year-old field test performance for trees resulting from crosses between unrelated individuals and for inbred trees produced through mating between half-sibs, full-sibs, parents and offspring and self-pollination. Results indicate that inclusion of a small proportion of related clones in seed orchards will have relatively low impacts on stand yields due to low probability of related individuals mating, lower probability of producing acceptable seedlings from related matings than from unrelated matings, and a greater probability of competition-induced mortality for slower growing inbred individuals than for outcrossed trees. Thus, competition reduces the losses expected due to inbreeding depression at harvest, particularly on better sites with higher planting densities and longer rotations. Slightly higher breeding values for related clones than unrelated clones would offset or exceed the effects of inbreeding resulting from related matings. Concerns regarding the maintenance of genetic diversity are more likely to limit inclusion of related clones in orchards than inbreeding depression for final stand yield.

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Introduction

As breeding programs advance, the relatedness of individuals in breeding populations will increase in each generation, as some top-ranking individuals are likely selected from the same families or share common ancestors. Thus, low to moderate levels of inbreeding resulting from matings between relatives, such as full-sibs, half-sibs, and parents and their offspring, etc. may be inevitable in advanced-generation seed orchards without operational modifications, such as sublining (Lowe and van Buijtenen 1986; McKeand and Bridgwater 1998). Negative effects of inbreeding have been observed at different life stages in many conifers, including Douglas fir, in terms of seed production (Griffin and Lindgren 1985; Sorensen and Cress 1994; Woods and Heaman 1989), nursery performance (Sniezko and Zobel 1988; Sorensen 1997; Woods et al. 2002) and survival and growth vigor in early stages of field trials (Durel et al. 1996; Orr-Ewing 1976; Sorensen and Miles 1982; Williams and Savolainen 1996). Therefore, decisions need to be made on tradeoffs between genetic gain obtained by selecting the best, but related, individuals and potential losses due to inbreeding between these individuals.

Although strong inbreeding depression for growth rate has been observed in most forest tree species at the individual tree level, the effects of a small proportion of inbred individuals in seed lots on final yield at the stand level are likely less important, as these individuals may be eliminated from nursery stock due to mortality or culling (Woods et al. 2002), or may be purged in the natural process of self-thinning due to competition-induced mortality in natural stands (Plessas and Strauss 1986). Therefore, the effects of related matings in a seed orchard on realized genetic gain depend on the probability of related matings, the effects of inbreeding on seed production and nursery performance (including seed germination, seedling mortality and growth, and seedling cull rate), and the proportion of inbred trees remaining in mature stands at the time of harvest.

In order to investigate the effects of various levels of inbreeding at different historical life stages for Douglas fir, a large number of controlled crosses were made in 1985 and 1986 (Woods and Heaman 1989), including outcrosses (inbreeding coefficient F=0), crosses between half-sibs (F=0.125), between full-sibs (F=0.25), between parents and offspring (F=0.25), and self-pollination (F=0.5). Seed obtained from these crosses were used for a nursery experiment in 1987, and the seedlings that met nursery culling standards were planted at two test sites in 1988. Results from these studies showed that inbreeding significantly reduces the number of filled-seed per cone (Woods and Heaman 1989) and the probability of producing acceptable seedlings for operational reforestation (Woods et al. 2002). The probabilities of producing acceptable seedlings from inbred matings relative to outcrossed matings are 55%, 41% and 3% for the levels of inbreeding F=0.125, 0.25 and 0.5, respectively. These results, together with other studies, suggest that self-fertility is very low in most conifers including Douglas fir (Prat and Burczyk 1998; Sorenson 1971; Sorensen and Cress 1994; Woods and Heaman 1989), and only a small proportion of selfed seedlings are acceptable for operational planting (Woods et al. 2002). In contrast, low to moderate levels of inbreeding can produce relatively large proportions of viable seed (Sorensen and Cress 1994; Woods and Heaman 1989). Seed with low to moderate levels of inbreeding can also produce relatively large proportions of seedlings that meet nursery culling standards for reforestation. Therefore, low to moderate levels of inbreeding have the potential to result in much greater negative impacts on growth and yield of plantations than self-pollination.

Strong inbreeding depression was observed for height and diameter growth in field tests at age 10 years (Wang et al., unpublished data). Height at age 10 differed significantly among the five cross types and was linearly and negatively correlated with the associated inbreeding coefficient of a cross. However, these experiments are still in a relatively early stage of stand development and were not designed for stand-level realized gain assessment per unit area. In our study, the long-term implications of different levels of inbreeding on final stand yields were simulated using the growth and yield model TASS (Tree and Stand Simulator), developed by the Research Branch, British Columbia Ministry of Forests (Mitchell 1975). Simulations are supported by results from two 10-year-old inbreeding field trials. The effect of using related materials on the genetic gain of a seed orchard is evaluated by incorporating the following factors: the probability of mating among related clones in a seed orchard, effects of inbreeding on seed production, nursery performance and growth and yield at final stand, and the higher-than-average breeding value of related trees.

Materials and methods

About TASS

TASS is a height-driven, biologically oriented, spatially explicit individual tree model (Mitchell 1975). It was designed to produce potential growth and yield tables for even-aged managed stands. It simulates the growth of individual trees in a three-dimensional space with competition. Tree height, bole diameter, branch length and crown form and foliage are updated annually. These measures can be affected by inter-tree competition, thinning, pruning, animal damage, site characteristics, defoliation, mortality, and fertilization, as well as genetic variation (or genetic treatments) in tree growth. It has been calibrated to model growth and yield for even-aged stands of coastal and interior species of commercial importance in British Columbia, including Douglas fir (Di Lucca 1999).

In our study, TASS was used to simulate the performance of mature stands comprising different proportions of trees with various levels of inbreeding. Effects of inbreeding are quantified at the stand level in terms of their impact on merchantable volume per hectare. The initial effects of inbreeding on height growth at various levels of inbreeding in the simulated stands were based on the results from two 10-year-old inbreeding field trials (Wang et al. unpublished data).

Inbreeding field trials

A detailed description of the founders and mating design used for producing various levels of inbreds and outcross controls has been described by Woods and Heaman (1989). Nine unrelated founder clones from parent trees selected in natural stands in south coastal British Columbia were crossed in a 4×5 factorial to produce 20 full-sib families to form the outcrossed (OC) population. The same families obtained from previous matings were used to develop the crosses between half-sibs (HS), full-sibs (FS) and parents and offspring (PO). Each of the nine founders was self-pollinated to produce selfed (S1) families. The inbreeding coefficients (F) of these cross types are 0, 0.125, 0.25, 0.25 and 0.5 for OC, HS, FS, PO crosses and S1, respectively.

Progeny tests were established with 1-year-old seedlings in May 1988 at two farm-field sites located in south coastal British

Table 1 Numbers of families in each cross type at the two test sites, Cowichan Lake and Langley. *OC* Outcrosses, *HS* crosses between half-sibs, *FS* crosses between full-sibs, *PO* crosses between parents and their offspring, *S1* self-pollination

Cross type	F	Cowichan Lake	Langley
OC	0	20	20
HS	0.125	67	69
FS	0.25	37	37
PO	0.25	17	17
S1	0.5	7	8
Total	–	148	151

Columbia (Table 1); one at the Cowichan Lake Research Station (Lat. 48° 49′, Long. 124° 20′) and one in Langley (Lat. 49° 06′, Long. 122° 38′). The non-contiguous plot design of Libby and Cockerham (1980) with randomized interlocking replications was used. There were four interlocking replications in each of eight blocks with a single tree in each replication per family.

In order to follow operational forestry practices, seedlings were subjected to nursery culling standards used at the time by the British Columbia Ministry of Forests for all families, regardless of levels of inbreeding, prior to outplanting (Woods et al. 2002). Thus, families resulting from different cross types had seedlings of comparable size at the start of the field tests. No thinning was conducted prior to data collection at age 10 years.

Data analysis of the field tests

Initial tree heights were adjusted to remove effects of test site and blocks within each site. Adjusted heights were computed as the sum of the mean and the residual tree height, which were estimated from the following linear model:

$$\text{Height} = \text{mean} + \text{site} + \text{block(site)} + \text{residual}.$$

TASS growth projections utilize a frequency distribution of initial relative height growth vigors. To produce this frequency distribution, the adjusted heights were converted into relative heights from 0 to 1.1, and divided into classes. The more height classes used, the better the precision of the simulation; however, the smaller the interval of the classes, the fewer trees in each class and thus the poorer the representation of that class. This problem was solved by initially using larger intervals, i.e., 22 classes, to generate a smooth frequency distribution and to model the distribution for each level of inbreeding. The estimated distribution was then used to generate the frequency-distribution tables with a finer interval, i.e., 55 classes, for simulation.

The following four-parameter Weibull function was used with SigmaPlot (v5.0) to model the height distribution for each level of inbreeding:

$$y = a\left(\frac{c-1}{c}\right)^{\frac{1-c}{c}}\left[\frac{x-x0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c-1} e^{-\left[\frac{x-x0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c}} + \frac{c-1}{c}$$

where *y* is the probability density of *x* (height); *a*, *b*, *c*, and *x*0 are the four parameters to be estimated for each level of inbreeding. The estimated parameters are listed in Table 2 and height distributions and their estimated distribution curves are shown in Fig. 1 for various levels of inbreeding.

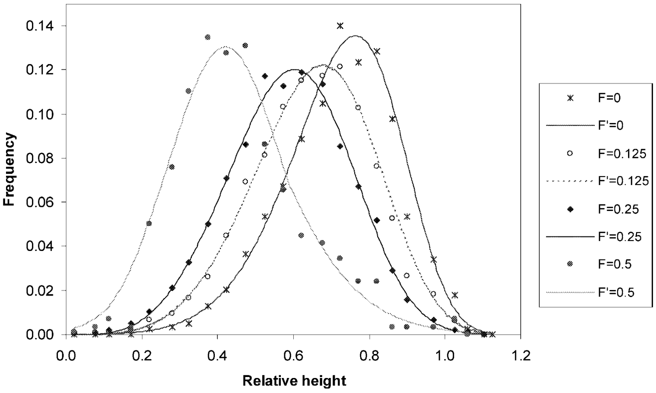

Fig. 1 Observed (*F*) and estimated (*F'*) frequency distributions for relative height at age 10 for various levels of inbreeding

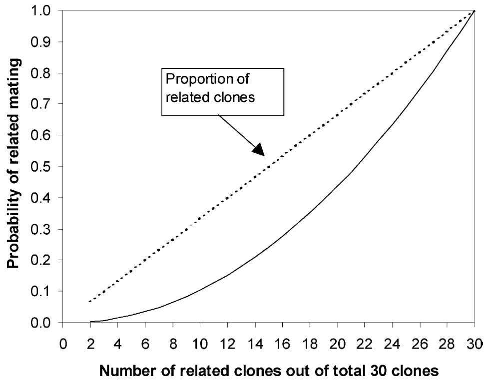

Fig. 2 Probabilities of matings between relatives in a seed orchard with the number of related clones varying between 2 and 30 out of the total 30 clones, assuming the same number of individuals in each clone and random mating among all individuals across clones

Probability of mating among related clones

The probability of mating between any two related individuals (P_i) in a clonal seed orchard was estimated using the following formula, assuming completely random mating among individuals in the seed orchard:

$$P_i = C_r^2 / C_n^2$$

where *C* is mating combinations; *r* is the number of related clones (or related individuals) and *n* is the total number of clones (or individuals) in the seed orchard. This formula calculates the proportion of the combinations of any two related individuals over all possible pairwise combinations in the seed orchard. The proportion of related matings in a 30-clone seed orchard as a function of the numbers of related clones is illustrated in Fig. 2. It shows that the probability of mating among relatives is much smaller than the proportion of related clones included in a seed orchard, especially when the proportion of related clones is small. For example, if 30% of individuals in a seed orchard are related, the probability of mating between relatives is only 9%, assuming random mating.

Unlike consanguineous matings, the probability of selfing is not a function of the number of related clones included in a seed orchard. The percentage of seed resulting from self-pollination varies among clones, but in seed orchard seedlots of Douglas fir averages between 5% and 10% (Neale and Adams 1985; Prat and Caquelard 1995; Show and Allard 1982; Sorensen 1999; Stoehr et al. 1998).

Table 2 Parameters estimated for the four parameter Weibull function

Level of inbreeding	Parameters				R^2 (*P* value)
	a	b	c	×0	
F=0	0.1355	0.9595	7.0047	0.7642	0.986 (0.0001)
F=0.125	0.1221	0.7021	4.5520	0.6673	0.997 (0.0001)
F=0.25	0.1202	0.6101	3.9180	0.6034	0.995 (0.0001)
F=0.5	0.1261	0.3522	2.2260	0.4039	0.966 (0.0001)

Table 3 Scenarios used for simulations comprising different proportions of seedlings with inbreeding coefficients of F=0 ($P_{F=0}$), 0.125 ($P_{F=0.125}$), 0.25 ($P_{F=0.25}$) and 0.5 ($P_{F=0.5}$) used for TASS

Scenario	$P_{F=0}$	$P_{F=0.125}$	$P_{F=0.25}$	$P_{F=0.5}$
1	0.65	0.3	0	0.05
2	0.7	0.25	0	0.05
3	0.75	0.2	0	0.05
4	0.8	0.15	0	0.05
5	0.85	0.1	0	0.05
6	0.9	0.05	0	0.05
7	0.7	0	0.25	0.05
8	0.75	0	0.2	0.05
9	0.8	0	0.15	0.05
10	0.85	0	0.1	0.05
11	0.9	0	0.05	0.05
12	0.9	0	0	0.1
13	0.95	0	0	0.05
14	1	0	0	0
15	0.5	0.25	0.2	0.05
16	0.6	0.2	0.15	0.05
17	0.65	0.2	0.1	0.05
18	0.65	0.15	0.15	0.05
19	0.7	0.15	0.1	0.05
20	0.75	0.15	0.05	0.05
21	0.75	0.1	0.1	0.05
22	0.8	0.1	0.05	0.05
23	0.85	0.05	0.05	0.05

Development of scenarios

For simulations using the TASS model, a total of 23 scenarios were developed that varied in the proportions of inbred seedlings at the levels of F=0.125 ($P_{F=0.125}$), F=0.25 ($P_{F=0.25}$) and F=0.5 ($P_{F=0.5}$) (Table 3). Scenarios 1 through 6 vary only for $P_{F=0.125}$, scenarios 7 through 11 vary for $P_{F=0.25}$ only, and scenarios 15 to 23 vary for both $P_{F=0.125}$ and $P_{F=0.25}$. There were more scenarios involving relatively small proportions of inbred trees (i.e., from 0 to 0.2 for $P_{F=0.125}$ and from 0 to 0.15 for $P_{F=0.25}$) than those with higher proportions as relatively low levels of inbred trees are likely more realistic. The range of proportions of inbreds covered in these scenarios varies well beyond what will realistically occur.

On the basis of the modeled frequency distribution for each inbreeding level shown in Fig. 1, the scenarios listed in Table 3 were translated into lists of trees, with each tree having a certain relative height value reflecting its relative growth vigor. These lists were used as the initial TASS input data sets.

Simulation design

Growth and yield of stands representing the different scenarios were simulated in TASS using a factorial design including three site qualities: site indices of 20, 28 and 35 m height at 50 years, and three planting densities: 625, 1,111 and 1,890 trees per hectare. Each scenario was simulated 30 times for each of the nine combinations of site index and planting density. This was because the neighborhood of individual trees in the simulated stand was randomly assigned for each simulation separately, resulting in different competitive environments for each individual tree. In total, 6,210 simulations were conducted.

The output of TASS includes number of trees per hectare, merchantable stem volume, and total stem volume at 5-year intervals up to age 100 years. The present study, however, focused only on changes in number of trees per hectare (*N*) with age, and the merchantable stem volume per hectare (MV) at the end of rotations of 60, 75 and 90 years.

Data analysis

ANOVA was applied to examine the effects of site index, planting density, inbreeding scenario and their interactions on final MV at the three harvesting ages. The analysis was conducted using the GLM procedure in SAS (SAS Institute Inc. 1989). The linear model was as follows:

$$y_{ijkl} = \mu + s_i + d_i + sd_{ij} + c_k + sc_{ik} + dc_{jk} + sdc_{ijk} + e_{ijkl}$$

where y_{ijkl} is the MV of the *l*th replication of the *k*th scenario at the *i*th site index and the *j*th planting density; μ is the general mean; s_i is the fixed effect of the *i*th site index; d_i is the fixed effect of the *j*th planting density; sd_{ij} is the interaction between the *i*th site index and the *j*th planting density; c_k is the fixed effect of scenario; sc_{ik} is the interaction between the *i*th site index and the *k*th scenario; dc_{jk} is the interaction between the *j*th planting density and the *k*th scenario; sdc_{ijk} is the three-way interaction among the *i*th site index; the *j*th planting density and the *k*th scenario; and e_{ijkl} is the random error term.

Multiple regressions were used to estimate the effects of site index and planting density on the changes in proportions of outcrossed and inbred trees in a stand from initial plantation to harvest. To make effects of site index and planting density comparable, both of these independent variables were standardized.

Multiple regressions were also applied to estimate the average effects of different proportions of inbred trees at inbreeding levels F=0.125 ($P_{F=0.125}$) and F=0.25 ($P_{F=0.25}$) with MV as the dependent variable. Prior to regression, the effects of site, density and site*density were removed from MV. The adjusted MV values were the sum of the mean of the predicted MV value and the residual from the following linear model:

$$\mathrm{MV} = \mathrm{mean} + \mathrm{site} + \mathrm{density} + (\mathrm{site} * \mathrm{density}) + \mathrm{residual}.$$

REG procedures in SAS were used to perform the regression. The linear model of the multiple regressions was:

$$y = b1 * P_{F=0.125} + b2 * P_{F=0.25} + a.$$

where *y* is the adjusted MV, *b*1 and *b*2 are regression coefficients, and *a* is the intercept. The parameters *a*, *b*1 and *b*2 were used to develop an inbreeding depression index (*D*) that estimated the effect of inbreeding on final MV at harvest.

Results and discussion

General ANOVA

The effects of scenario and its interactions with site index or planting density on MV at three harvesting ages (60, 75 and 90 years) were all significant (Table 4). The effects of site, density and their interaction were also highly significant.

The coefficient of variation of MV among scenarios, reflecting the effect of inbreeding, decreased with increasing site index and planting density at each harvesting age, and with greater harvesting ages (Fig. 3). This is because competition among trees starts earlier and is more severe on more productive sites with higher planting densities, and competition lasts longer at older harvesting ages, consequently more inbred trees are purged on better sites with higher planting density or with longer rotations than on poorer sites with lower planting densities or shorter rotations.

Table 4 Results of ANOVA of merchantable volume per hectare (MV) at three harvesting ages: 60, 75 and 90 years

Source	*df* [a]	Type III F		
		Age 60	Age 75	Age 90
Site	2	3423726.0*	3992844.0*	4517549.0*
Density	2	291521.8*	250573.7*	215564.7*
Site∗density	4	13809.7*	9837.9*	5836.7*
Scenario	22	640.8*	658.9*	682.1*
Site∗scenario	44	52.7*	54.3*	52.6*
Density∗scenario	44	4.4*	6.9*	10.3*
Site∗density∗scenario	88	1.5*	2.2*	3.2*

* Significant at $P<0.005$

[a] Degrees of freedom for error term are 6003

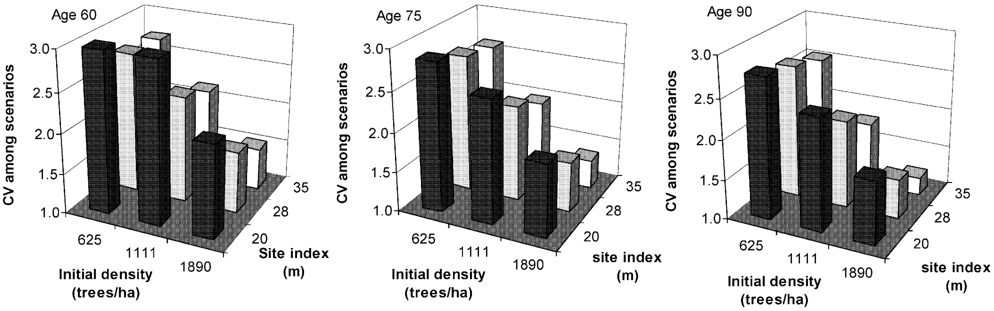

Fig. 3 Changes in coefficient of variation among scenarios for merchantable volume with site index and planting density at three harvesting ages (60, 75 and 90 years)

Table 5 Results of multiple regressions of changes in percent trees (from initial plantation to mature stands) with various levels of inbreeding on site index and planting density for harvesting ages at 60, 75 and 90 years. All partial R^2 and model R^2 were significant at the level of $P<0.0001$

Age	F	Intercept	Regression coefficient (partial R^2)		R^2
			Site	Density	
60	0	2.68	1.69 (0.29)	1.99 (0.40)	0.69
	0.125	−0.35	−0.25 (0.29)	−0.28 (0.43)	0.24
	0.25	−0.75	−0.49 (0.35)	−0.58 (0.35)	0.25
	0.5	−1.54	−0.97 (0.11)	−1.13 (0.14)	0.71
75	0	3.75	2.12 (0.12)	2.57 (0.16)	0.72
	0.125	−0.53	−0.37 (0.15)	−0.44 (0.15)	0.28
	0.25	−1.07	−0.65 (0.10)	−0.82 (0.15)	0.26
	0.5	−2.10	−1.09 (0.10)	−1.30 (0.16)	0.72
90	0	4.79	2.79 (0.12)	2.77 (0.13)	0.71
	0.125	−0.74	−0.56 (0.30)	−0.57 (0.41)	0.30
	0.25	−1.43	−0.92 (0.30)	−0.93 (0.43)	0.25
	0.5	−2.56	−1.29 (0.36)	−1.25 (0.34)	0.70

Changes in percentage of inbred trees versus outcrossed trees with age

In general, the percentage of trees that were inbred declined with age, and this decline continued monotonically until harvest age. Percent changes in inbred trees in a stand, from initial plantation to harvest, were negatively correlated with site index and planting density at all the three harvesting ages (Table 5). This is explained by earlier canopy closure on better sites and with a higher planting density, resulting in earlier simulated competition among trees and more competition-induced mortality of slower growing trees.

The regression coefficients declined with both the level of inbreeding and harvest age for both site index and planting density. This suggests that the higher the inbreeding level of the tree, the more likely the tree is to be purged from the stand; and a higher proportion of inbred trees will be purged with later harvest age. The relative contributions of site index and planting density were of similar magnitude based on their regression coefficients and partial r^2 values, although planting density had slightly greater contributions than site index in most cases (Table 5).

These relationships were illustrated for one intermediate scenario (scenario 19) in Fig. 4. This scenario simulates initial conditions with 70% outcrossed trees (shown on the right axis), 15% trees with F=0.125, 10% trees with F=0.25 and 5% trees with F=0.5 (shown on the left axis). The decline in the total percentage of inbred trees (i.e., the increase in percentage of outcrossed trees) from age 5 to age 100 was less than 3% at the lowest site index (20 m) and the lowest planting density (625 trees/ha) simulated in this experiment, but was nearly 15% at the highest site index (35 m) with the highest planting density (1,890 trees/ha). Consequently, the inbreeding depression for final stand yield was much smaller in the latter case.

Effects of inbred trees on final yield of merchantable volume

The effects of inbreeding on stand productivity across different site indices and planting densities were estimated by multiple regression of adjusted MV on proportions of inbreds at the levels of inbreeding F=0.125 ($P_{F=0.125}$) and F=0.25 ($P_{F=0.25}$). The multiple regressions were

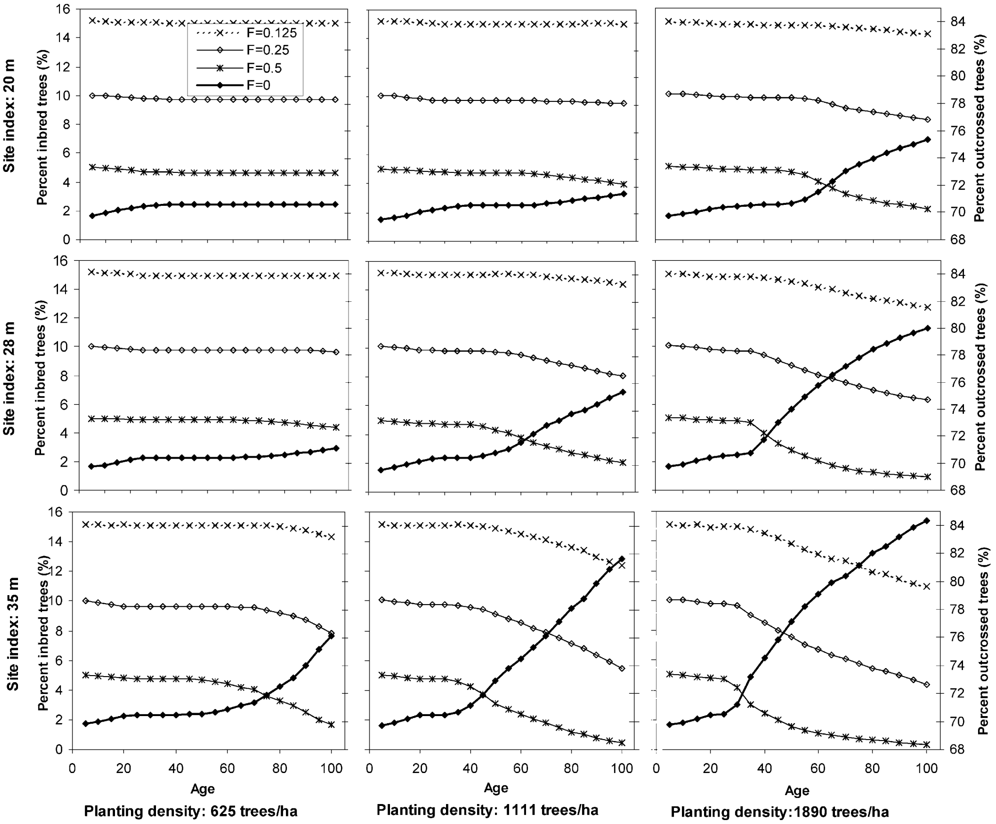

Fig. 4 Changes in percentages of inbred trees (*left axis*) and outcrossed trees (*right axis*) with age at various levels of inbreeding at different site indices with different planting densities for scenario 19 (Table 3) as an example

Table 6 Results of multiple regressions of MV on simulated proportions of initially-planted trees with F=0.125 ($P_{F=0.125}$) and F=0.25 ($P_{F=0.25}$) for three harvesting ages

Harvesting age	Independent variable	Parameter estimate (m^3/ha)	Partial r^2 (P value)	Model r^2 (P value)
60	Intercept	374.425		
	$P_{F=0.125}$	−49.634	0.409 (0.0001)	
	$P_{F=0.25}$	−84.546	0.587 (0.0001)	0.996 (0.0001)
75	Intercept	500.703		
	$P_{F=0.125}$	−62.933	0.418 (0.0001)	0.995 (0.0001)
	$P_{F=0.25}$	−105.441	0.577 (0.0001)	
90	Intercept	606.635		
	$P_{F=0.125}$	−72.858	0.421 (0.0001)	0.995 (0.0001)
	$P_{F=0.25}$	−121.322	0.574 (0.0001)	

nearly perfect, with r^2 greater than 0.99 for all three harvesting ages (Table 6). Contributions of both $P_{F=0.125}$ and $P_{F=0.25}$ to the model were statistically significant.

The regression coefficients of the two independent variables ($P_{F=0.125}$ and $P_{F=0.25}$) were negative with a stronger effect (more negative coefficient) for $P_{F=0.25}$ than for $P_{F=0.125}$, as expected. If both $P_{F=0.125}$ and $P_{F=0.25}$ equal zero, the predicted MV equals the intercept. Thus, the effects of inbreeding on MV can be expressed by the percentages of the regression coefficients over the intercept. The effects of inbreeding on MV were −13.3, −12.5 and −12.0% for $P_{F=0.125}$, and −22.5, −21.0 and −20.0% for $P_{F=0.25}$ at the harvesting ages 60, 75 and 90 years, respectively. The negative effects of $P_{F=0.125}$ on MV were close to the corresponding inbreeding coefficient (F=0.125), whereas the negative effects of $P_{F=0.25}$ were considerably smaller than the corresponding inbreeding coefficient (F=0.25), and thus deviated from a linear relationship with inbreeding coefficient at higher levels of inbreeding (Fig. 5).

This result is inconsistent with the linear relationship observed between growth traits and inbreeding coefficients in early stages of inbreeding experiments prior to crown closure (Durel et al. 1996). The most likely explanation for these different results are simulated effects of competition against inbred trees after crown closure, particularly for those trees at higher levels of inbreeding. For example, trees with F=0.25 had a greater probability of being purged through competition than those with F=0.125 (as illustrated in Fig. 4).

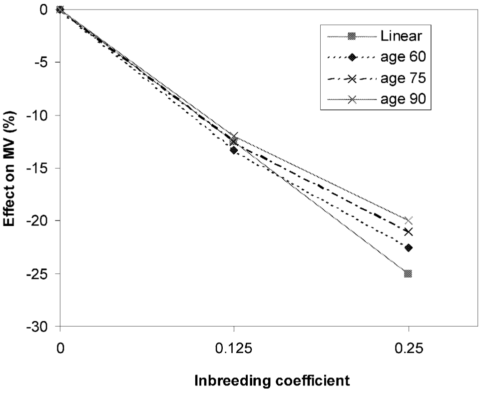

Fig. 5 Relationships between inbreeding coefficient and effects of inbreeding on merchantable volume (*MV*) at three harvesting ages (60, 75 and 90 years)

Based on the ratios of the regression coefficients of $P_{F=0.125}$ and $P_{F=0.25}$ to the intercepts, a stand-level inbreeding depression index for a given seedling mixture can be developed in terms of the effect on final yield of MV for the three harvesting ages as

$$D_{60} = 0.133P_{F=0.125} + 0.225P_{F=0.25} \tag{1}$$

$$D_{75} = 0.125P_{F=0.125} + 0.210P_{F=0.25} \tag{2}$$

$$D_{90} = 0.120P_{F=0.125} + 0.200P_{F=0.25} \tag{3}$$

With these indices, reductions in MV at a given harvesting age due to inbreeding can be estimated. For example, the inbreeding depression index of scenario 19 is 0.038 for harvest at age 90, indicating that the reduction in MV due to inbreeding will be 3.8%.

In practice, it is useful to predict the effects of inbreeding on MV at a given site index and planting density. The results of a multiple regression of MV on site index, planting density, inbreeding depression index and their interactions as well as their transformations showed that, the values of r^2 were all close to 1 and highly significant for the three harvesting ages. Thus, the effects of inbreeding on TASS-simulated MV can be predicted well based on those independent variables.

Predicting inbreeding depression indices of a seed orchard with related clones

Assuming random mating among clones or individual trees in a seed orchard, the $P_{F=0.125}$ and $P_{F=0.25}$ of a seedling mixture (after nursery culling) grown from an orchard seed lot can be estimated as:

$$P_{F=0.125} = S_{F=0.125}\left(C^2_{NC_{F=0.125}} \middle/ C^2_{NC_t}\right) \tag{4}$$

$$P_{F=0.25} = S_{F=0.25}\left(C^2_{NC_{F=0.25}} \middle/ C^2_{NC_t}\right) \tag{5}$$

where C is number of possible combinations; $NC_{F=0.125}$ and $NC_{F=0.25}$ are the numbers of clones that have a coancestry of 0.125 and 0.25 respectively; NC_t is the total number of clones in the seed orchard; and $S_{F=0.125}$ and $S_{F=0.25}$ are the probabilities of producing acceptable seedlings from consanguineous mating relative to unrelated mating. These latter two values have been estimated as 0.55 and 0.41, respectively, from our previous studies (Woods et al. 2002).

The inbreeding depression index (D) of a seed orchard containing related clones can be estimated by combining equations 4 and 5 with equations 1, 2 and 3 for the harvest ages 60, 75 and 90 as follows:

$$\begin{aligned} D_{60} = {} & 0.133S_{F=0.125}\left(C^2_{NC_{F=0.125}} \middle/ C^2_{NC_t}\right) \\ & + 0.225S_{F=0.25}\left(C^2_{NC_{F=0.25}} \middle/ C^2_{NC_t}\right) \end{aligned} \tag{6}$$

$$\begin{aligned} D_{75} = {} & 0.125S_{F=0.125}\left(C^2_{NC_{F=0.125}} \middle/ C^2_{NC_t}\right) \\ & + 0.210S_{F=0.25}\left(C^2_{NC_{F=0.25}} \middle/ C^2_{NC_t}\right) \end{aligned} \tag{7}$$

$$\begin{aligned} D_{90} = {} & 0.120C_{F=0.125}\left(C^2_{NC_{F=0.125}} \middle/ C^2_{NC_t}\right) \\ & + 0.200S_{F=0.25}\left(C^2_{NC_{F=0.25}} \middle/ C^2_{NC_t}\right) \end{aligned} \tag{8}$$

For example, if there are 30 clones in total in a seed orchard, 5 clones are half-sibs and 3 clones are full-sibs, the inbreeding depression index of the seed orchard for harvest age 75 is:

$$\begin{aligned} D_{75} = {} & 0.125 \times 0.55\left(C^2_5/C^2_{30}\right) \\ & + 0.210 \times 0.41\left(C^2_3/C^2_{30}\right) = 0.0022 \end{aligned}$$

This means that the effect of inbreeding on final yield of merchantable stem volume is less than 0.3%. However, if related individuals have a higher probability of mating with each other due to similar floral phenology as mentioned earlier, the above equations could underestimate the inbreeding depression index (discussed later).

The impact on genetic gain of a seed orchard by including related clones

The reason for including related materials in an orchard is the superiority in breeding values of elite families and clones within these families. The impact on genetic gain due to including relatives (GR%) is a tradeoff between the superior breeding values and the inbreeding index. It can be estimated for the inclusion of related clones from one pedigree using the following equation:

$$\mathrm{GR}(\%) = P_{m(r*ur)} \times B_r/2 - D$$

where $P_{m(r*ur)}$ is the probability of related and unrelated clones mating assuming equal numbers of individuals in all clones and random mating among all individuals; B_r is the mean superiority of related clones in breeding value over potential unrelated selections and D is the inbreeding

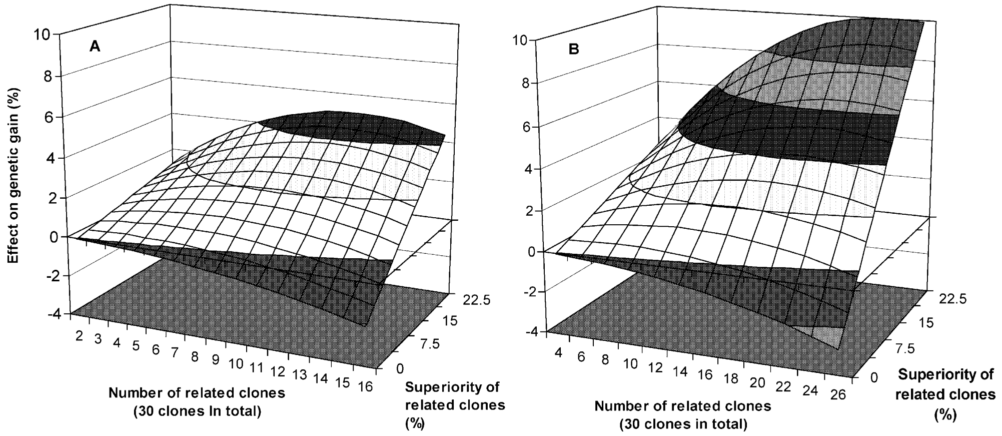

Fig. 6 Effects of including related clones from one (**A**) or two (**B**) pedigree(s) on genetic gain in a 30-clones seed orchard with different numbers of related clones and various levels of superiority in breeding values of related clones over the next best clones that are candidates for the seed orchard

depression index of the seed orchard. Both B_r and D are expressed as percentages.

If related clones from two pedigrees are included in a seed orchard, the genetic gain arising from mating between clones across the two pedigrees needs to be accounted for. Thus, the genetic gain of a seed orchard involving related clones from two pedigrees is

$$\mathrm{GR}(\%) = \big(P_{\mathrm{m(r1*ur)}} \times B_{\mathrm{r1}}/2 + P_{\mathrm{m(r2*ur)}} \times B_{\mathrm{r2}}/2 + P_{\mathrm{m(r1*r2)}} \times (B_{\mathrm{r1}} + B_{\mathrm{r2}})/2\big) - D$$

where $P_{\mathrm{m(r1*ur)}}$, $P_{\mathrm{m(r2*ur)}}$ and $P_{\mathrm{m(r1*r2)}}$ are the probabilities of mating between clones from pedigree 1 and unrelated clones, between clones from pedigree 2 and unrelated clones, and between clones from pedigree 1 and clones from pedigree 2, respectively; and B_{r1} and B_{r2} are the superiority values of mean breeding values of related clones from pedigree 1 and pedigree 2. This equation could be expanded to estimate the genetic gain of a seed orchard involving related clones from more than two pedigrees as follows:

$$\mathrm{GR}(\%) = B_{\mathrm{r}}\big(P_{\mathrm{m(r*ur)}}/2 + P_{\mathrm{m(r)}}\big) - D$$

where $P_{\mathrm{m(r*ur)}}$ and $P_{\mathrm{m(r)}}$ are the probabilities of matings between related clones and unrelated clones, and between related clones from different pedigrees, respectively; and B_{r} is the mean superiority values of breeding values of related clones over pedigrees.

The impact on realized genetic gains of a seed orchard with a total of 30 clones involving a subset of related clones from one pedigree (with coancestry coefficient of 0.125) or from two pedigrees (with coancestry coefficients of 0.125 and 0.25 respectively) are shown as examples in Fig 6. There is as expected a trade-off between the positive effect of adding higher breeding value clones to an orchard and the negative effect of mating between these related clones. However, the genetic gain of the seed orchard at harvest age 75 can be improved if the related clones have an average breeding value that is just 2.5% higher than the next best unrelated clones among available candidates and there are fewer than 8 related clones from one pedigree or 16 related clones from two pedigrees. There are optimal combinations between the numbers of related clones and their superiority in breeding values. With the same breeding value and the same number of related clones included in a seed orchard, the genetic gain is higher if these related clones are from two or more pedigrees.

Our simulation is based on the assumption of random mating in a seed orchard. Previous studies, however, have shown that variation in reproductive phenology has significant effects on the mating system of Douglas fir (El-Kassaby and Davidson 1991; El-Kassaby et al. 1986; Erickson and Adams 1989; Sorensen 1997). Related individuals tend to have similar reproductive phenology, and thus may have a higher probability of pollinating each other than unrelated individuals, particularly if their reproductive phenology is extremely early or late. On the other hand, pollen grains from unrelated individuals may have a higher probability of fertilizing the eggs than pollen from related trees when both are available. This is because pollen tube formation is found to be triggered by a secretion from the ovule, and cross-pollination results in more rapid pollen tube development than self pollination in this species (Takaso and Owens 1994). Additionally, in advanced-generation seed orchards, individuals with extreme floral phenology are unlikely to be included.

Even if related individuals have a higher likelihood of mating with each other than predicted assuming random mating due to the greater synchronization of reproductive phenology among related clones, their impact on genetic gain is limited as the values of D_{75} are very small. For example, D_{75} is 0.0036 with 5 clones from pedigree 1 and 5 clones from pedigree 2. Genetic gain increases by 3.96% if related clones have a breeding value 15% greater than the next best unrelated clones that are the candidates for the seed orchard. If the value of D_{75} is doubled for the consideration of a greater synchronization of reproductive phenology among related clones, it reduces genetic gain by only an additional 0.36%. Meanwhile, the probability of related matings may be reduced through effective seed orchard design for wind-pollinated orchards (Vanclay 1991) or through controlled crossing for seed production.

The results from this study also have implications for use of the multiple population breeding strategy (MPBS) proposed for the conservation of genetic diversity (Namkoong 1989; Namkoong et al. 1980). In the MPBS, the breeding population is divided into subpopulations, which are kept separate and selected in different directions for different adaptive traits or market values. This strategy may be modified to produce outcrossed hybrids between subpopulations for plantation establishment (Eriksson et al. 1993) by incorporating clones from different subpopulations into a seed orchard. However, inbreeding is likely to occur over multiple generations as subpopulations will usually have a small effective population size, and clones within a subpopulation may be related each other. Based on results from the current study, the negative inbreeding effects on seed produced in such a seed orchard should be minor in term of final productivity, particularly since the level of relatedness of the clones from the same subpopulation is expected to be much smaller than the levels simulated in this study.

Conclusions

The probability of related individuals mating is small in a seed orchard involving a relatively small proportion of related clones. The probability of producing an acceptable seedling from related matings is even lower due to the effects of inbreeding on seed production, seedling survival and nursery culling. After plantation establishment, a large proportion of inbred trees will be purged through competition-induced mortality after crown closure. Better sites, higher planting densities, and later harvesting ages increase the chance of eliminating inbreds from the final stand. Consequently, the effects of inbreeding on productivity of the final stand are much smaller than the inbreeding depression of individual trees in early life stages. The effects of inbreeding depression for a seedling mix involving inbreds or a seed orchard involving related clones can be predicted in terms of final yield. Achieving increased genetic gain by including some related clones with slightly higher breeding values in seed orchards would more than offset the effects of inbreeding resulting from related matings. Thus, the negative impact of including a small number of related clones in a seed orchard on commercial stand productivity is not a concern as long as the effective population size of the seed orchard can still be accepted.

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