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Relationship between inbreeding depression and inbreeding coefficient in maritime pine (*Pinus pinaster*)

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Abstract The relationship between inbreeding depression and inbreeding coefficient (F) for several important traits was investigated in an 11-year trial of maritime pine (*Pinus pinaster*). Five levels of inbreeding ($F=0$; 0.125; 0.25; 0.5; 0.75) were obtained in a mating design involving ten plus-trees, or their progenies, as parents (total of 51 families). For $F=0.75$, the mean inbreeding depressions were 27% for height, 37% for circumference at breast height (63% for bole volume), 23% for basal straightness (better straightness of the inbred trees), and 89% for female fertility (number of cones). Large differences were observed among inbred families for the same level of inbreeding. The evolution of depression with F was more or less linear, depending on the traits. Significant differences among F -levels appeared very early for height (from 5-years of age). Inbreeding depression was much more expressed during unfavorable years than during favorable years for yearly height growth. When compared with other *Pinus* species, maritime pine appears to be less affected by inbreeding, especially for the percentage of filled seeds and general vigor. A reduced genetic load in maritime pine may result from the evolutionary history of the species and its scattered distribution.

Key words *Pinus pinaster* · Inbreeding depression · Filled seeds · Adult growth · Genetic load

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Introduction

Inbreeding depression and heterosis are two facets of the same phenomenon. Inbreeding depression has often been studied in major forest-tree species (Fowler 1965, in *Pinus resinosa*; Andersson et al. 1974, in *Picea abies*; Libby et al. 1981, in *Sequoia sempervirens*; Sorensen and Miles 1982, in *Pseudotsuga menziesii*, *Pinus ponderosa* and *Abies procera*; Wilcox 1983 and Sniezko and Zobel 1988, in *Pinus radiata*; Lundkvist et al. 1987, in *Pinus sylvestris*; Matheson et al. 1995 in *Pinus elliottii*; Griffin and Cotterill 1988, in *Eucalyptus regnans*), whereas heterosis after inbreeding has been much less explored. Inbreeding has been generally investigated because of its impact on breeding and selection strategies and seed orchard production. In conventional clonal or seedling seed orchards, selfing or sib-mating may occur (Baradat 1987a, b; Baradat and Letrilliart 1987) and result in depressed seedlings for vigor or other traits (Wilcox 1983; Lundkvist et al. 1987; Matheson et al. 1995). Many forest-tree improvement programs are based on recurrent schemes (Lowe and van Buijtenen 1986; Cotterill et al. 1989; Shelbourne et al. 1989; McKeand and Bridgewater 1992; White et al. 1993) and have reached the second generation. Thus, relatedness among individuals of the breeding populations will rapidly increase in future generations. A better knowledge of inbreeding effects is necessary to formulate new breeding strategies for the future (Sniezko and Zobel 1988; Williams and Savolainen 1995). In particular, sub-lining strategies have been proposed to manage relatedness and inbreeding (van Buijtenen 1976; Burdon and Namkoong 1983; McKeand and Bridgewater 1992; White et al. 1993). On the other hand, inbreeding could be used as an efficient tool in forest-tree breeding [Barker and Libby 1974; Lindgren 1975 and Eriksson et al. 1984 (cited by Lundkvist et al. 1987); Durel 1992a; Williams and Savolainen 1995] since it increases between-family variance and heritabilities (Wilcox 1983; Sniezko and Zobel 1988), allowing potentially better efficiency in

selection schemes. In certain cases, the phenotypic values of self- and outcross-families allow one to compute selection indices on additive genetic values of selfed-individuals (Durel 1992a) which lead to higher genetic gains than among outbred-individuals.

Most studies on inbreeding depression in forest trees compare a given level of inbreeding with non-inbred material (Fowler 1965; Snyder 1972; Libby et al. 1981; Rudolph 1981; Sorensen and Miles 1982; Wilcox 1983; Lundkvist et al. 1987; Griffin and Cotterill 1988). Individual comparisons of self- and open- (or polycross-) pollinations of genotypes are often reported. In the present paper, our objective was to determine the relationship between inbreeding depression and a range of inbreeding levels, assessed by the coefficient of inbreeding (F). In addition, different types of families with the same F -level, such as sib- and parent-offspring matings, were considered. The mating design allowed us to estimate accurate values of inbreeding depression since the small number of genotypes were involved among all the crosses. Comparisons were also made with a previous study in maritime pine (Jouve, unpublished data) where S_1 and full-sib progenies of the same genotypes were compared in two contrasted sites (humid podsolic sand and dry dune). To our knowledge, we report here the first comprehensive study concerning the evolution of inbreeding depression with various values of the inbreeding coefficient (F) for economically important traits at the adult stage in forest trees.

Materials

Mating design

Five levels of F inbreeding coefficient were obtained from a mating design. A set of related trees were selected as parent trees for the controlled crosses. A given include set comprised : the parental tree (P_0), individuals from the selfed offspring of P_0 (S_1), full-sibs having P_0 as parents (FS and FS'), and half-sib of FS and FS' having P_0 as parent (HS). These trees were controlled crossed in order to obtain the different F -values (Table 1a). Standards ($F=0$) were obtained by crossing offspring of P_0 (i.e. FS) with unrelated P_1 trees. It can be seen from Table 1a that a given level of F can be obtained for different combinations. For example, $F=0.25$ is obtained by crossing two full-sibs (brothers-sisters) or by crossing a parent with its offspring. This mating scheme was repeated three times, for three different P_0 parents (Table 1b). All mating, involving over 51 families, were successful.

The parental trees (P_0, P_1, \dots, P_i) were plus-trees randomly chosen from the 350 trees that constitute the first generation of the maritime pine breeding program (Baradat and Pastuszka 1992). Conversely, their progenies (FS, FS', HS) were previously selected by multi-trait index selection (height and straightness) in progeny tests. S_1 -trees were chosen for the presence of both female and male strobili with no attempt to select for superior height, growth or straightness. Controlled crosses were made in the Spring of 1978 and cones collected in the Autumn of 1979. Seeds were extracted in the Winter of 1979 and the number of seeds per cone (NCS), the total number of seeds (SN), the number of empty seeds (NES) and seed weight (SW) for each cross were recorded. The proportions of filled seeds were computed as : $FS = [SN - NES]/SN$.

Table 1 Mating design of the trial. Parents were related trees : P_0 = plus-tree, S_1 = selfed offspring of P_0 , FS = offspring of P_0 in a full-sib family, FS' = full-sibs of FS, HS = offspring of P_0 and half-sibs with FS and FS' . Panel **a**: value of F inbreeding coefficient for each type of mate. Panel **b**: number of crosses for each types of mate. The three successive numbers correspond to three different P_0 pivot-trees

a			
Parents	P_0	S_1	FS
P_0	0.5	0.5	0.25
S_1	0.5	0.75	0.25
FS	0.25	0.25	0.5
FS'	0.25	0.25	0.25
HS	0.25	0.25	0.125
P_1	0	0	0

b			
Parents	P_0	S_1	FS
P_0	/	2 + 3 + 2	2 + 2 + 2
S_1	/	2 + 2 + 3	/
FS			1 + 1 + 1
FS'	/	/	2 + 2 + 1
HS			4 + 4 + 3
P_1	/	/	4 + 4 + 4

Experimental design and measurements

Seeds from the 51 progenies were sown in the nursery during the Spring of 1981. Abnormal seedlings appeared after germination (chlorotic seedlings) but died during their first season. During the Winter of 1981–1982, seedlings were transplanted in a field test near the I.N.R.A. station (20 km south-west from Bordeaux, elevation 55 m) at a spacing of 4×1.10 m. The soil was a semi-humid podsolic sand of low fertility. The trial consisted of a 70 incomplete block design in which 49 other progenies were involved (a total of 100 progenies). Each block comprised nine random row plots of five trees.

The following traits were measured at 11 years of age : total height (HT), circumference at breast height (CIR), stem straightness assessed by the butt angle of lean (BAL) on the first meter, number of cones per tree (NC). Bole volume (VOL) was assessed by a conic equation. Retrospective measurements of height were made on two trees per plot using whorls as morphologic markers : successive total heights were measured for the last 7 years, giving estimates of six annual height increments (from the 6th to the 11th growing season).

Data analysis and statistical models

For seed-related traits (i.e. NSC, SW and FS), one-way analyses of variance (ANOVAs) were computed with the F -level as a factor. The F -level was always attributed to the seeds themselves (i.e. the progenies).

For most adult traits, raw data were adjusted to block effects. Data assessed on a subsample of trees (successive heights and height increments) were not adjusted to block effects since only two trees per plot were measured. A hierarchical ANOVA was performed with a nested design (F -levels, and families within F -level). The family factor was always considered as a random effect. Conversely, the F factor was successively considered as a fixed and random effect. In the last case, comparison between the F -effect mean square and the familial mean square allowed us to determine the relative contribution of the variation due to inbreeding and the family variation to the total variation. But no variance was computed for the F effect. Mean comparisons were made with a Newman and Keuls test. All the statistical analyses have been performed with the software package

OPEP (Baradat 1989). To avoid confusion between F (inbreeding coefficient) and F (value of the Fisher test), the former will be referred as F and the latter as Fisher in the text.

Inbreeding depression was computed as follows:

$(m_0 - m_i) \times 100/m_0$, where m_i is the family mean for which $F = i$, and m_0 is the family mean for which $F = 0$.

Linear and curvilinear regressions were made with family means as dependent variables and F -values (F and F^2) as independent variables. Consequently, variation among families for the same F -level was taken into account in the regression test (t -test).

Results

Evolution of inbreeding depression according to F -levels

Seed traits

For SW, no significant differences appeared among the different inbred families (Table 2). For NSC, a significant difference was observed (5% level), based on the Newman and Keuls test. The first four levels of inbreeding for progenies ($0 \leq F \leq 0.5$) were not significantly different (Table 2), whereas the mean for $F = 0.75$ was significantly lower with an inbreeding depression of about 40%. Thus, significant differences appeared only when maternal parents of the seeds were S1-trees instead of outcrossed trees (see Table 1). For FS, a very highly significant Fisher-test was obtained when the F -level of progenies was considered. The mean values ranged from 94% to 57% (Table 2).

Adult traits

The results of the ANOVA performed for the five levels of inbreeding coefficient (F) are shown in Table 3. For all traits except BAL, the F effect was highly significant with family variation (F as random effect). Vigor traits (HT and CIR), showed the most important Fisher-test values. This value was also high for survival rate, and much lower for the number of cones (NC). Notation of NC was probably performed when trees were too young in relation to the maturation stage. For BAL, the Fisher-

Table 2 Mean values of the seed traits for the different F -levels. Means with the same letter do not differ significantly from each other (Newman and Keuls test, $P = 0.05$). Abbreviations are : SW (100-seeds weight), NSC (number of seeds per cone), FS (proportion of filled seeds)

F-value	Traits					
	SW		NSC		FS	
0	5.54	A	142.5	A	94.1	A
0.125	5.82	A	135.1	A	89.7	B
0.25	5.72	A	128.0	A	88.4	BC
0.5	5.51	A	127.2	A	74.7	BC
0.75	5.09	A	85.1	B	56.9	C

Table 3 Results of ANOVA with nested design (F -level and family within F -level) : Fisher-test values for the adult traits and residual Mean Square between brackets. Degrees of freedom : 4 for F -level, 46 for family and 1494 for the residual. Abbreviations are : HT (total height at 11-years old), CIR (circumference at breast height), BAL (butt angle of lean), NC (number of cones per tree)

Source of variation	Traits				
	Survival rate	HT (cm)	CIR (cm)	BAL (°)	NC
F -level	11.1***	33.5***	27.7***	1.9 ^{ns}	3.7**
Family	1.9***	5***	3.9***	5.4***	3.2***
Residual	(0.09)	(9320)	(62.5)	(17.5)	(0.84)

test was not significant when F was considered as a random effect, but was significant when F was considered as a fixed effect. The family effect was significant for all the studied traits. It was not very different among the traits (Table 3), but was the largest for BAL. Between family variances were not computed since no clear genetic components could be estimated because of the complex mating design and the small number of families used per F -level.

For each F -level, the mean values, the standard deviations, the Newman and Keuls groups, and the inbreeding depression values are indicated in Table 4. The survival rate appeared to be moderately influenced by inbreeding: the inbreeding depression was about 20% for the highest F -level (i.e. $F = 0.75$). Thus, approximately one quarter of the S2 trees had disappeared 10 years after planting, whereas only 7% of the non-inbred trees had died. The rise of the inbreeding depression was mainly observed between the two last F -levels (0.5–0.75). For vigor traits, inbreeding depression was more consistent (27% and 37% for HT and CIR respectively with $F = 0.75$). Inbreeding seemed to affect radial growth more strongly than vertical growth, but the precision of the two estimates was not sufficient to define a significant difference. The combination of both traits in a conic volume equation showed a much higher inbreeding depression for volume growth (VOL) : about 63% with $F = 0.75$ (Table 4).

For the butt angle of lean (BAL), an inbreeding depression of 23% was observed for $F = 0.75$. This decrease of the angular deviation to butt vertically is therefore a favorable effect for straightness of the stem: "the more inbred, the more straight". But a large variation was observed among families and this inbreeding depression was not significant.

The number of cones per tree (NC) was the most depressed trait (Table 4). Many S2 trees showed no cones, whereas non-inbred trees bore an average of 0.6 cone. Again, the maturation phase was probably not fully achieved for many trees (11-years old) and these results are only indicative. Male flowering was more often observed, even on S2 trees, and male strobili frequently appeared on the top branches and the axis-I shoot, indicating that the adult distribution of sexual

Table 4 Mean values of the adult traits for the different F -levels with standard deviations (between brackets), Newman and Keuls groups and inbreeding depressions computed as described in Materials and Methods. Abbreviations are: HT (total height at 11-years of age), CIR (circumference at breast height), VOL (bole volume), BAL (butt angle of lean), NC (number of cones per tree)

Traits	F -value	Mean value	N & K groups ^a	Inbreeding depression
Survival rate	0	92.7 (1.9)	A	
	0.125	92.8 (1.6)	AB	0%
	0.25	94.7 (1.2)	AB	-2%
	0.5	86.0 (2.4)	B	7%
	0.75	74.3 (4.6)	C	20%
HT (cm)	0	811 (12)	A	
	0.125	788 (10)	AB	3%
	0.25	767 (11)	B	5%
	0.5	687 (13)	C	15%
	0.75	596 (18)	D	27%
CIR (cm)	0	37.7 (0.9)	A	
	0.125	35.9 (0.8)	AB	5%
	0.25	33.9 (0.8)	B	10%
	0.5	28.8 (0.9)	C	25%
	0.75	23.9 (1.3)	D	37%
VOL (l)	0	47.1 (2.0)	A	
	0.125	42.0 (1.7)	AB	11%
	0.25	37.5 (1.8)	B	20%
	0.5	25.9 (2.1)	C	45%
	0.75	17.2 (3.0)	D	63%
BAL (°)	0	9.0 (0.5)	A	
	0.125	7.8 (0.5)	A	13%
	0.25	7.4 (0.5)	A	18%
	0.5	7.2 (0.6)	A	20%
	0.75	6.9 (0.8)	A	23%
NC	0	0.62 (0.09)	A	
	0.125	0.52 (0.08)	A	16%
	0.25	0.40 (0.08)	AB	37%
	0.5	0.29 (0.10)	AB	53%
	0.75	0.07 (0.14)	B	89%

^a Means with the same letter do not differ significantly from each other (Newman and Keuls test, $P = 0.05$)

organs (male strobili on basal branches and female flowers on top shoots) had not yet been achieved for the highly inbred trees. The inbreeding depression for $F = 0.75$ was the highest (about 89%), with large variation among S2-families.

Table 5 Linear and curvilinear regression coefficients and correlation coefficients between family mean values and F (or F and F^2) inbreeding coefficient. Abbreviations are: FS (proportion of filled seeds), HT (total height at 11-years of age), CIR (circumference at

Trait	Linear regression		Curvilinear regression		
	Regression coeff.	Correlation coeff.	Regression coeff.		Multiple correlation coeff.
			F	F^2	
FS	-0.48 ***	-0.63	n.s.	n.s.	/
HT	-287.5 ***	-0.85	n.s.	n.s.	/
CIR	-19.2 ***	-0.86	n.s.	n.s.	/
VOL	-41.4 ***	-0.85	n.s.	n.s.	/
BAL	n.s.	n.s.	-8.2*	9.9*	0.32
NC	-0.83 ***	-0.45	n.s.	n.s.	/

Linear and curvilinear regressions

For seed traits, regression of elementary values on F was performed only for FS (Table 5). The regression coefficient was highly significant (P -value < 0.0001) but the R^2 -value was only 0.4. A large variability among families was observed for the highest F -levels (Fig. 1). For adult traits, linear regressions of the family mean values on the F -values were always significant except for straightness (Table 5). Correlation coefficients ranged from -0.45 to -0.86 (P -value < 0.001), so R^2 -values ranged from 0.20 to 0.74. The largest values were observed for vigor traits (HT, CIR and VOL) with P -values < 0.0001 . The family mean values and the linear regression are presented in Fig. 2a for HT. For all the traits, a curvilinear regression model on F and F^2 was tested. The supplementary variable F^2 was never significant for the vigor traits and NC. The two independent variables showed significant regression coefficients for BAL (Table 5), but the multiple correlation coefficient was only equal to 0.32. A large variability among families was observed for BAL at the different F -levels and particularly for $F = 0.75$ (Fig. 2b). Therefore, we could predict that the curvilinear regression would be very inaccurate.

When the linear regression was significant, a predicted value of inbreeding depression for $F = 1$ was computed. This value was equal to 35%, 50% and 88% for height, circumference, and volume respectively. It was over 100% for the number of cones.

Growth-curve analysis

The subdivision of total height in annual increments allowed us to study the growth curves between 5- and 11-year-old trees (Fig. 3). The curves corresponding to the five levels of inbreeding had quite similar patterns but presented different growth rates. No significant difference was observed between the three first F -levels whatever the age. The two other F -levels ($F = 0.5$ and 0.75) showed significantly lower growth curves, starting

breast height), VOL (bole volume), BAL (butt angle of lean), NC (number of cones per tree). A t -test was performed on each regression coefficient: n.s.: not significant at 5% level, *: significant at 5% level; ***: significant at 0.1% level

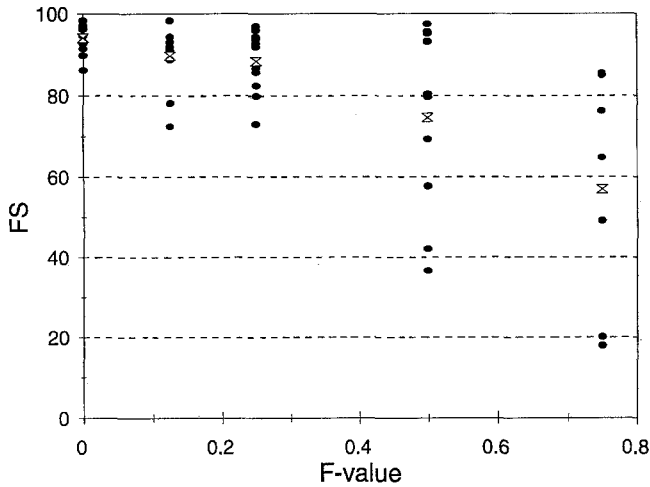


Fig. 1 Proportions of filled seeds (FS) for the 51 progenies against their F-values. Diabolo signs represent mean values

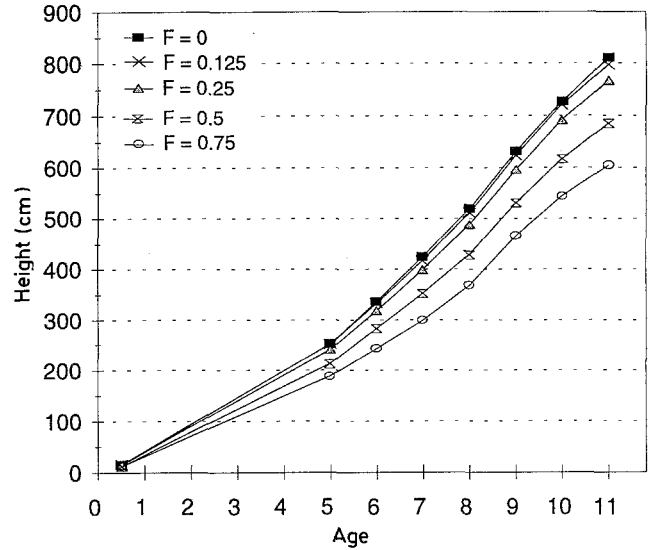


Fig. 3 Average height growth curves for the five F-levels

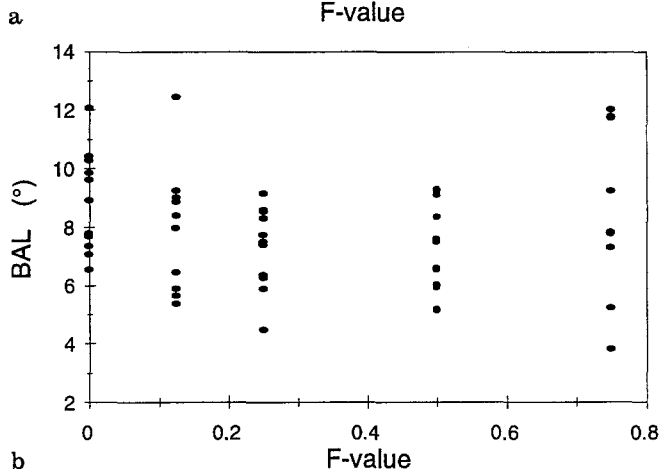
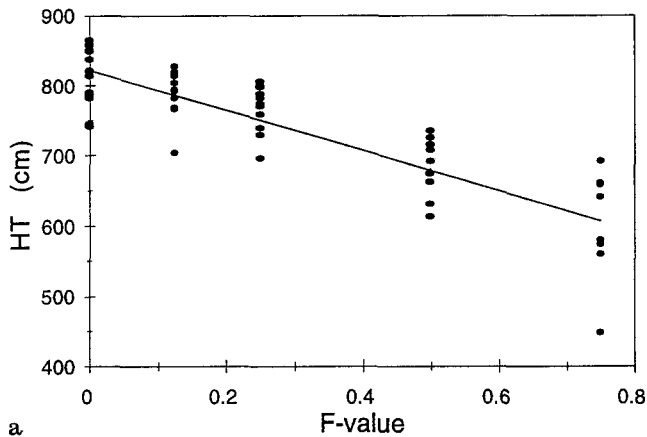


Fig. 2a,b Family mean values for height (a-HT) and straightness (b-BAL) against their F-values. A linear regression is drawn for height

at 5 years (Newman and Keuls test, 5% level). The different curves clearly reflected accumulation of inbreeding depression effects on each annual height increment.

Annual increments were then analyzed independently. Annual increments of inbred trees were always

shorter than those of non-inbred trees. Mean values are given in Fig. 4a for each F-level and year. An interesting pattern was observed: inbreeding depression was more pronounced during unfavorable years than during favorable years. The “biological index” of each year was determined by the average length of the shoot increments of all trees during that year. Indeed, in the 6th, 7th and 11th years (i.e. growing seasons), height growth was low (< 78 cm) and differences between inbred families were significant, even between the low inbreeding levels ($F = 0.125$ and 0.25) and the non-inbred trees in the 11th year. On the other hand, in the 9th growing season, the “biological index” was high (average growth of 108 cm) and the discrimination among the F-levels was lower since only two groups were significantly different: ($F = 0, 0.125, 0.25$) and ($F = 0.5, 0.75$) (Fig. 4a). For intermediate years (e.g. 10th growing season), differences were significant between $F = 0.5$ and $F = 0.75$, but not between the lower values of the F coefficient.

The same pattern was observed when considering the inbreeding depression values (Fig. 4b). In the 6th, 7th and 11th years (unfavorable years), the depression was about 20% for $F = 0.5$ and ranged from 28% to 36% for $F = 0.75$. In the 9th year (favorable year), the depression was only 10% and 15% for the same F-levels.

Discussion

Inbreeding depression as a function of the inbreeding coefficient

Inbreeding clearly affected the phenotypic mean of most traits. But inbreeding effects were much more important for fertility and bole volume than for straightness. Inbreeding is known to affect traits related to fitness (Falconer 1960; Charlesworth and Charlesworth 1987).

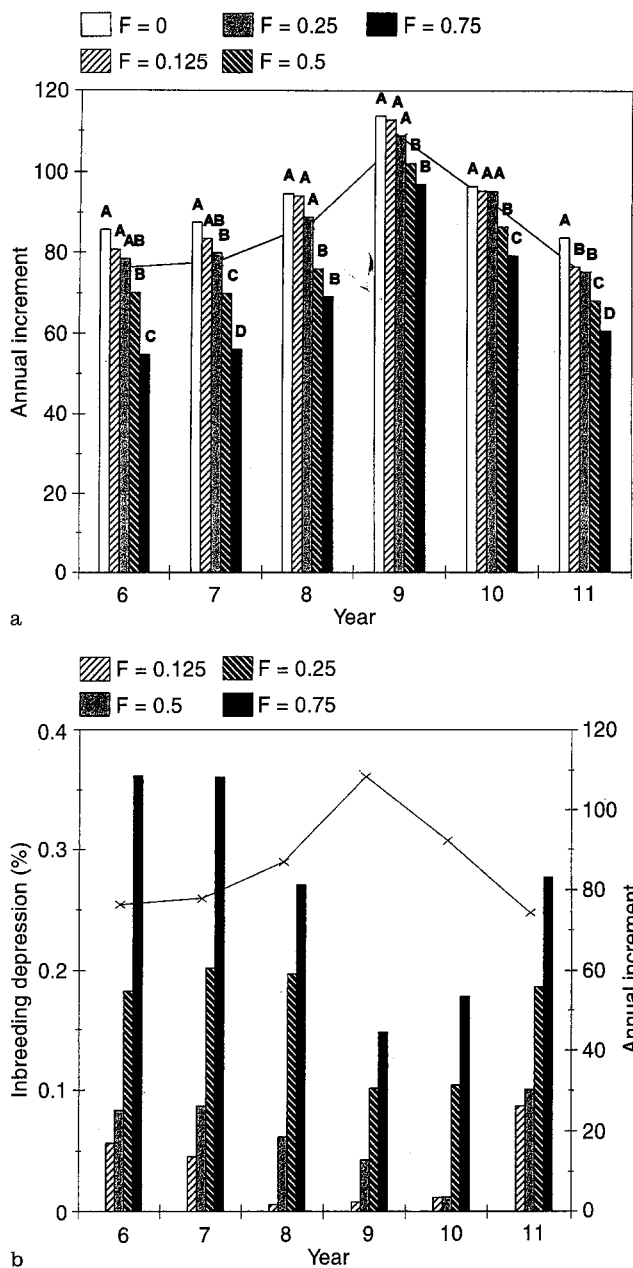


Fig. 4a Mean values of annual height increments for the five F -levels from the 6th to the 11th year. Vertical bars with different letters are significantly different (Newman and Keuls test at 5%). **b** Mean values of inbreeding depression for the four F -levels from the 6th to the 11th year. In both panels, the line indicates the mean values of annual height increments over all individuals

The larger inbreeding depression on radial growth than on height growth also reflected this general rule: radial growth is more related to competition ability than height growth in maritime pine (von Euler et al. 1992), and fitness strongly depends on this competition ability.

Linear regression of family means on the F -values allowed us to explain a great part of the variation for vigor traits: for each trait, linear prediction accounted for more than 70% of the total familial variation. But the slope was steeper for bole volume than for its two

components (i.e. height and circumference). Curvilinear regressions were not significant for these traits. A curvilinear response would be interpreted as interlocus interaction or epistasis (Hallauer and Miranda 1981). But the absence of any significant deviation from linear regression does not mean that epistasis does not exist, since different interaction effects may compensate for each other. In maize, linear regression is very generally observed for many traits, and Hallauer and Miranda (1981) suggested that the effect of epistasis on inbreeding depression was probably small.

For fertility (NC) and filled-seed percent (FS), linear regressions were also significant, but they accounted for only 40% (or less) of the total familial variation. Families were widely distributed at the different F -levels but especially at high values of F (i.e. $F = 0.5$ and 0.75).

For straightness, the curvilinear regression was significant, but it seems hazardous to suggest the presence of epistasis, because of the very large variation observed among families at the different F -levels (Fig. 2b).

Inbreeding depression must be compared with genetic gain to evaluate its impact in a multi-generation breeding program. In maritime pine, genetic gains were computed in a third generation seedling seed orchard (Durel 1992b): a gain of about +12% was expected for height growth at 9-years of age as compared to the unimproved genetic material. Similarly, combined index selection was performed to cull individuals for the third generation breeding population. A genetic gain of about +4% was expected on height growth as compared to the second generation (Durel 1992b). On the other hand, an increase of 10% of the inbreeding coefficient would lead to a loss of -3.5% on height growth due to inbreeding depression. Fortunately, such an inbreeding coefficient has not yet been reached in the maritime pine breeding population. But these values clearly reflect the negative impact of inbreeding depression on the breeding program of a forest tree, where only a limited number of individuals are managed due to practical constraints. Subdivision of the population in breeding groups (or sublines - van Buijtenen 1976; Burdon and Namkoong 1983; White et al. 1992) appears to be a very efficient strategy to control inbreeding effects.

Evolution of inbreeding depression with age and climatic variations

Inbreeding depression was rather stable over years for total height and varied from 15.1% to 17.4% for $F = 0.5$ without any clear trend. Lundkvist et al. (1987) observed the same stable evolution in *P. sylvestris* between 6- and 11-years of age in one site, but inbreeding depression for height increased during the same period in another site. A slight increase with age has also been reported by different authors (Table 6: Sorensen and Miles 1982; Park and Fowler 1983; Matheson et al. 1995). The increase of inbreeding depression could be attributed to

Table 6 Inbreeding depression values for vigor traits of forest-tree species reported by different authors (for $F = 0.5$ except in particular cases)

Species	Traits		Reference
	Height (age)	Volume (age)	
<i>Pinus radiata</i>	9% (4)	29% (4)	Wilcox 1983
<i>Pinus sylvestris</i>	19% (14)	46% (14)	Lundkvist et al. 1987 (a)
	32% (11)	66% (11)	
<i>Pinus elliottii</i>	21% (1)		Snyder 1972
	34% (5)		
<i>Pinus elliottii</i>	11%–31% (13)	43%–87% (13)	Matheson et al. 1995 (b)
<i>Pinus ponderosa</i>	30% (3)		Sorensen and Miles 1982
	36% (10)		
<i>Pinus banksiana</i>	18% (4)		Rudolph 1981 (c)
<i>Picea abies</i>	33% (2)		Andersson et al. 1974 (d)
	19% (1 ^{1/2})		
	18% (1 ^{1/2})		
<i>Picea mariana</i>	15% (2)		Park and Fowler 1983
	20% (6)		
<i>Abies procera</i>	27% (3)		Sorensen and Miles 1982
	30% (10)		
<i>Pseudotsuga menziesii</i>	24% (2)		Sorensen and Miles 1982
<i>Sequoia sempervirens</i>	29% (10)		
	20–35% (1)		Libby et al. 1981
<i>Eucalyptus regnans</i>	11% (3 ^{3/4})	37% (3 ^{3/4})	Griffin and Cotterill 1988

(a) Data from two different sites
(b) Computed as $1/2 \cdot b\% \cdot 100$ (adjusted), where $b\%$ is the regression coefficient of familial mean on F -values (see Matheson et al. 1995, Table 2)

(c) S2 progenies ($F = 0.75$)

(d) Computed as $1/2 b$, where b is the regression coefficient of familial mean on F -values.

Only the three data samples with more than ten crosses were considered (Andersson et al. 1974, Table 2)

increased competition with other trees. Competition effects for water and nutrients are greater in the field when the stand is older (Lundkvist et al. 1987). In our test, the quite stable inbreeding depression over years for total height could indicate that competition was still not very pronounced. For maritime pine, Lemoine (1980) observed that competition effects are quite limited before 10 years in a normal spacing stand (density < 2500 trees/ha).

Sorensen and Miles (1982) also analyzed inbreeding effects on growth rate. They concluded that relative inbreeding depression at any age could combine a true genetic inbreeding depression of growth rate and an additional effect resulting from inbred and non-inbred trees being at different points on a quite common growth curve. This second effect agrees with the hypothesis of a lag between inbred and non-inbred trees for their physiological state.

Subdivision of height growth in annual height increments showed that inbreeding depression was much more sensitive to annual variation than could be observed for total height. Environmental factors could accentuate inbreeding depression on growth. Unfavorable years were much more discriminant and led to a larger inbreeding depression than more favorable years. This result is in good agreement with the general observation of the better stability of hybrids over fluctuating environments in comparison with inbred lines (Falconer 1960; Wallace 1963; Hallauer and Miranda 1981). Accordingly, heterosis is more generally observed in unfavorable environmental conditions in inter-racial hybrids of maritime pine (Harfouche 1995; Harfouche et al. 1995).

Inbreeding depression in maritime pine in comparison with other conifer species

The proportion of filled seeds was highly influenced by the inbreeding level of the crosses, whereas seed weight appeared to be more independent. In *P. radiata*, Griffin and Lindgren (1986) observed 43% and 42% of filled seed in S1 and S2 progenies (expressed in proportions of filled seeds of outcrosses). These values are much lower than those we obtained in maritime pine: 80% and 61% for S1 and S2 progenies when expressed as the proportions of filled seeds of outcrosses. The authors also cited other studies on *Pinus* species: 22% in S1 progenies of *P. elliottii* (Squillace and Kraus 1963); 60% in S1 and 19% in S2 progenies of *Pinus monticola* (Bingham 1973); 77% in S1, 36% in S1 × S1, 50% in S1 × parent and 30% in S2 progenies of *Pinus thunbergii* (Katsuta 1966). In Douglas-fir, Woods and Heaman (1988) obtained very strong depression for the mean yield of filled seeds per cone in half-sib matings, full-sib matings, S1 and S2: respectively 67%, 53%, 4% and 0%. Similar results were obtained by Snyder (1968) on self-pollination of *P. elliottii* with only 10% of seedlings produced by wind-pollination.

In comparison with other conifer species, maritime pine appears to be less affected by inbreeding depression. This result has been confirmed on a large number of other self-fertilizations (data not shown), and may be the consequence of a reduced genetic load: in maritime pine, less embryonic lethal genes than in other *Pinus* species (Bramlet and Popham 1971; Park and Fowler 1984; Savolainen et al. 1992) are probably involved in the genetic determinism of filled seeds.

Survival rate in the field was not strongly affected by inbreeding (7% in S1 and 20% in S2). Most lethal or sublethal alleles have probably been eliminated in the seeds and in the nursery by natural selection. Actually, survival rate was mostly an indication of the loss of small and weak seedlings. Sorensen and Miles (1974) also reported larger inbreeding depression for survival rate in the first year than in the following years, in Douglas-fir and *P. ponderosa* (see also Sorensen and Miles 1982). In *P. elliotii*, Matheson et al. (1995) found little evidence of inbreeding depression for survival in a field trial. Conversely, in *Pinus leucodermis*, Morgante et al. (1993) reported that selection against inbred progenies did not act during seed maturation and germination, although mortality in this last phase was considerable. However, heterozygotes were favored and inbreds were eliminated before trees were 5-years old.

For vigor traits, inbreeding depression in maritime pine appeared to be smaller than in other conifers (Table 6). In maritime pine, Jouve (unpublished data) obtained inbreeding depression values (for $F = 0.5$) of 14% and 21% on primary and secondary shoots (mean values on seven annual shoots), and 21% on circumference at breast height. In another site, which was poorer and drier, the same progenies gave similar inbreeding depression values : 15% on total height and 19% on circumference. Hence, in comparison with other conifers, maritime pine seems to be less sensitive to inbreeding for vigor. This result agrees with the lower inbreeding effect noted for the percentage of filled seed.

For fertility (numbers of cones), a strong inbreeding depression (53%) appeared for $F = 0.5$, and reached a very high value (89%) for $F = 0.75$. Since there is probably a time lag between the physiological state of the inbred and non-inbred progenies, these values were probably over-estimated. But inbreeding depression is known to affect mostly traits related to fitness (Falconer 1960). By comparison between self- and outcross-progenies, Jouve (unpublished data) reported a depression of 35% for the number of cones in a dry site where early fertility was observed on most of the progenies.

Straightness was not so affected by inbreeding, as was also found by Jouve (unpublished data) in his study. An inbreeding depression of 12% on straightness was recorded by Wilcox (1983) in *P. radiata*. In *P. epliotii*, Matheson et al. (1995) reported many instances of non-significant inbreeding depression (and only few significant ones) for straightness in half-sib and parent-offspring crosses measured at different ages. In maritime pine, unfavorable genetic and phenotypic correlations between vigor and straightness are very often observed in progeny tests (Danjon 1994). Such correlations could have an important effect in the reduction of the BAL value in inbred families (on average): inbreeding depression on vigor could result in a correlated improvement of straightness. Actually very large variations among S2 progenies were observed : the straightest and the most crooked families were S2 (see Fig. 2b).

Genetic load in maritime pine

A lower genetic load in maritime pine than in other *Pinus* species could originate from the strong geographic subdivision of this species over its natural area. Different gene diversity studies have been performed in maritime pine (Baradat and Marpeau-Bezard 1988; Bahrman et al. 1992, 1994). Recently, Petit et al. (1995) have confirmed the high level of differentiation of this species ($G_{ST} = 0.17$) as found with different markers (isozymes, denaturated proteins and terpenes). This high level of differentiation is combined with a rather low level of mean diversity by comparison with other conifers (Hamrick et al. 1992). In maritime pine, the total diversity (h_T , Nei 1987), average on eight isozyme loci, was equal to 0.211 (Petit et al. 1995), and this value was overestimated since the loci were selected for their polymorphic pattern whereas monomorphic isoenzyme loci were discarded (Petit, personal communication).

The plus-trees involved in the present study and in that of Jouve belonged to the Landes forest (South-West of France) which corresponds to the northern part of the natural distribution. Before domestication in the 19th century, maritime pine was distributed in small distinct regions and along rivers (Guinaudeau 1964); it covered about 100 000 hectares. During the 19th century, artificial afforestation has been widely pursued in the Aquitaine region, and at the present time 1.5 million hectares are planted. The seeds used for the large-scale afforestation all came from the local population. In a study performed in two coastal dune stands of the Médoc region (West of Bordeaux), Baradat and Marpeau-Bezard (1988) estimated an inbreeding coefficient of about 0.3 with a terpenic marker (3-carene). These stands belonged to the original populations of maritime pine, and originated from natural regeneration. Thus, the rather geographic structure of maritime pine in South-West of France before domestication, combined with stand disturbances like fires, could have produced an increase of inbreeding in natural stands along the coast. A significant part of the genetic load could have been removed by natural selection against unfavorable and lethal alleles expressed at a recessive state.

Two other particular *Pinus* species can be compared with maritime pine. In *P. radiata*, Wilcox (1983) reported that the low inbreeding depression observed could be related to its very small natural range. This should maintain a high level of relationship among trees and low frequencies of unfavorable alleles by natural selection. For this species, Moran and Bell (1987) observed that the total genetic diversity assessed by allozyme variation was only 0.12, a low estimate compared to most conifers. But 16.2% of the total genetic diversity could be apportioned between populations, a high proportion for a conifer (Moran et al. 1988). An extreme example is given by red pine (*P. resinosa*) where Fowler (1965) reported quite similar values of percent full seed and hypocotyl length (2 months old) for self and open or cross progenies. He stressed the type of regener-

ation of this species : red pine regenerates after stand disturbances, such as fires, which often reduce the population size to a few trees. Fowler and Morris (1977) advocated the lack of genetic diversity in this species (see also Mosseler et al. 1992) to the occurrence of a genetic bottleneck during Holocene glaciation. Thus, inbreeding in successive generations would accelerate elimination of deleterious mutant alleles.

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