INBREEDING IN *PINUS RADIATA*.
II: TIME COURSE OF INBREEDING DEPRESSION AND EFFECT ON GROWTH CURVE

HARRY X. WU

CSIRO Division of Forestry and Forest Products,
P. O. Box 946, Mount Gambier, South Australia 5290, Australia

A. C. MATHESON and D. SPENCER

CSIRO Division of Forestry and Forest Products,
P. O. Box 4008, Canberra, A.C.T. 2600, Australia

ABSTRACT

Changes in the effects of inbreeding on sectional area at breast height (1.3 m) each year from ages 1 to 13 were studied in *Pinus radiata* D.Don of five different inbreeding levels: $F = 0$, outcrosses; $F = 0.125$, resulting from half-sib matings; $F = 0.25$, resulting from full-sib matings; $F = 0.5$, resulting from selfing; and $F = 0.75$, resulting from two generations of selfing. The experimental material was derived from eight founder clones. Inbreeding had a significant impact on both cumulative and annual increments in sectional area from age 3, i.e., 2 years after outplanting when most trees had reached 1.3 m in height. At age 4, trees with the highest inbreeding levels ($F = 0.5$ and 0.75) had significantly less sectional area increment than those with lower inbreeding levels ($F = 0$, 0.125, 0.25). From age 11 on, trees had differentiated into three groups: outcrosses ($F = 0$) were best, sib matings ($F = 0.125$ and 0.25) were intermediate, and selfs ($F = 0.5$ and 0.75) were worst for cumulative and yearly increment of sectional area. An apparent bimodal time trend in inbreeding depression (ID) of annual increment was observed, being highest at the initial stage of stand development, disappearing at about the time of crown closure in the outcrossed population, and reappearing later. This bimodal trend was believed to be associated with the different times at which crown closure began for the different inbreeding levels, and indicates that inbreeding depression is a dynamic process associated with stage of stand development, spacing, and design of field layout.

In fitted Gompertz growth curves, all parameters were affected by inbreeding, including maximum growth rate, time to reach maximum growth rate, and the maximum sectional area achievable. There were significant differences in response to inbreeding among pedigrees.

Keywords: inbreeding depression; competition; growth curve; evolution of depression; *Pinus radiata*.

INTRODUCTION

Inbreeding depression can differ depending on stages in the life cycle at which measurements are made. In a compilation study of 54 species from 23 families of vascular plants, Husband

& Schemske (1996) observed that, for outcrossed species, ID was severe during seed development (affecting viability), was much less severe during growth, and became severe again at the onset of reproduction. Age-specific inbreeding depression may be associated with the age-related changes in genetic control within a growth period which have been observed in conifers such as *Pseudotsuga menziesii* (Mirb.) Franco (Namkoong *et al.* 1972), *Pinus ponderosa* P.Lawson et Lawson (Namkoong & Conkle 1976), *Pinus taeda* L. (Foster 1986), *Picea abies* (L.) Karsten (Bentzer *et al.* 1989), and *Pinus radiata* (Cotterill & Dean 1988; Matheson *et al.* 1994). In *P. radiata*, Matheson *et al.* (1994) observed that non-additive genetic variance began at a low level, increased to a maximum of more than twice the additive variance at age 4, then declined slowly to negligible levels by ages 9 to 12. These changes have sometimes been linked to the stand developmental phases originally proposed by Franklin (1979).

In conifers, inbreeding depression has been observed at all life stages: embryo development, seedling to adult growth, and reproduction (Franklin 1970; Griffin & Lindgren 1985; Williams & Savolainen 1996; Durel *et al.* 1996). Selfing usually results in marked reduction of sound seeds, and selfed seedlings usually exhibit severe early inbreeding depression in growth rate. Severe inbreeding depression also has been observed to affect reproduction age. Within the vegetative growth period, there seems no apparent trend in inbreeding depression with tree age in conifers. In *Ps. menziesii*, *Pinus ponderosa*, and *Abies procera* Rehder, the effect of inbreeding depression on height increased slightly between ages 2 or 3 and 10 (Sorensen & Miles 1982). A mild increase of depression with tree age also has been reported in *Picea mariana* (Mill.) B.S.P. (Park & Fowler 1984) and *Pinus elliottii* Engelm. (Matheson *et al.* 1995). In contrast, inbreeding depression in *Picea abies* declined between ages 19 and 61 (Eriksson *et al.* 1973), and similarly, Wilcox (1983) reported that inbreeding depression effect on height for *P. radiata* in the nursery was 18.2% for selfs and dropped to 8.4% at age 4 in the plantation. However, mortality was believed to have biased the results of these last two studies (Williams & Savolainen 1996). In *P. sylvestris*, Lundkvist *et al.* (1987) observed that inbreeding depression was constant from ages 6 to 14 years at one site, but increased at another site during the same period—possibly owing to increased competition at that site. In *Pinus pinaster* Aiton, Durel *et al.* (1996) observed inbreeding depression was more severe during adverse years than favourable ones, but there was constant inbreeding depression between ages 5 and 11. However, most experimental estimates of the time course of inbreeding depression suffer from the same problem as those of many age-age correlations (Matheson *et al.* 1994)—namely, too few age points. Thus, a method of estimating the annual depression would be an advantage in plotting the age trend of inbreeding depression.

Study of the development of inbreeding depression would be useful for understanding its dynamics and the genetic and environmental mechanisms underlying its progress. Also, changes in inbreeding depression with tree age are important to long-term population management. Early severe inbreeding depression combined with a lesser effect later on would be easier to accommodate within a breeding programme as early purging of deleterious alleles would then be effective.

In this study, we investigated the effect of development of inbreeding depression on cross-sectional area at breast height up to age 13 in *P. radiata* for five inbreeding levels, with emphasis on the following questions: (1) how early can the effect of inbreeding depression on vigour be identified (measured as dbh or sectional area); (2) does the magnitude of

inbreeding depression differ among tree ages or developmental stages; (3) if inbreeding depression is development stage-specific, what are the genetic and environmental mechanisms behind it; (4) what is impact of inbreeding on the growth curve; and (5) what are the interactions between pedigree (descendants of a particular parental clone) and inbreeding levels for three growth-curve parameters?

MATERIAL AND METHODS

The *P. radiata* inbreeding experiment was set up in the 1970s at the Gippsland Forest Experimental Station in Victoria, Australia. The detailed mating design and field experiment have been described by Wu *et al.* (in press). In summary, five inbreeding levels were created from eight founder clones: a mating of Design II (4×4 factorial cross) using eight grandparents to create a non-inbred population (F = 0, population 1), matings among half-sib progenies of eight founder clones to create population 2 with F = 0.125, matings among full-sib progenies of founder clones to create population 3 with F = 0.25, selfing of eight founder clones to create the first-generation selfed population 4 with F = 0.5 (referred to as S_1), and selfing of progenies derived from first-generation selfs to create the second-generation selfed population 5 with F = 0.75 (referred to as S_2). In 1981, all five populations were planted at Symmonds near Mt Gambier in South Australia with four replicates. Two populations (outcrosses and S_1 population) were also planted nearby at Kilsbys in 1982 with six replicates. A split-plot design was used for the field experiment with populations forming main plots and families within population forming subplots. Subplots were formed as a single row of six trees with spacing of 3 m beween rows and 2 m between trees within rows.

To study the age trend of inbreeding depression and its effect on growth curves, the second and third trees in each plot of six were felled for sampling. If the second or third tree was not available (because it was dead or a filler), then the next available tree was selected. After felling, disks were cut at breast height (1.3 m) for analysis. While still fresh, the disks were photocopied, a computer digitiser was used to measure the width of each annual ring, and the total cross-sectional area within each annual ring was computed.

The age (year) of each annual ring was determined from the outermost ring. We observed that 86% of F = 0, 79% of 0.125, 75% of 1.25, 67% of 0.5, and 52% of 0.75 had an annual ring at breast height by age 2 (1 year after planting), while 98%, 98%, 97%, 94%, and 92% of trees had rings at age 3 (2 years after planting) respectively. In populations 1 to 5 respectively, 200, 192, 228, 143, and 206 trees were felled and their disks were analysed. The annual increment of cross-sectional area was computed and cumulative sectional area was used for fitting growth curves. Cumulative and annual depression were computed for four inbreeding levels (F = 0.125, 0.25, 0.5, 0.75) according to $1-(w_s/w_c)$ where w_s and w_c are mean sectional areas for inbred and outcrossed progeny (F = 0), respectively.

Two families in population 1 and four families in population 4 (half of population 4) were planted only at the Kilsbys site. It was desirable to incorporate these families into the analysis of the main Symmonds trial. Six families in population 1 and four families in population 4 were planted at both sites and served as connections between two plantings. For common families in population 1 (F = 0) and population 4 (F = 0.5), a linear model was fitted to investigate family × site interactions in the two populations separately. It was found the interaction of family with site was not significant in either population (population 1 and 4)

at any age and so combining data across sites was possible. Data from the Kilsbys site were adjusted and combined with data from the Symmonds site for sectional area of both populations as described by Wu *et al.* (in press).

The Gompertz growth curve was fitted for each individual tree to study effects of inbreeding on growth curves. The Gompertz curve has the form:

$$W = \alpha \, e^{-\beta \, e^{-\kappa t}}$$

where W is the sectional area (cm^2) at age t (year) and α, β, and κ are positive parameters. Using the Gompertz growth curve, when age t = (1/κ)ln β the rate of growth has a maximum value of $\kappa\alpha$/e. Also, when age t continues to increase after the maximum growth rate has been reached, the rate of growth declines, and when age t increases indefinitely to t = ∞, the rate of growth approaches 0 and W has a maximum asymptote value W = α. Thus, the growth pattern depicted by the Gompertz curve describes very well the diameter or sectional growth pattern of a single tree. The Gompertz curves were fitted by the Gauss-Newton Numerical Search Method (Neter *et al.* 1989), and the SAS NLIN Procedure (SAS Institute Inc. 1989) was used for the iterative calculation. We calculated the rate of maximum increment (RMG, cm^2/year), the time to reach maximum increment rate (TMG, year), and the sectional-area asymptote (AMG, cm^2) for each tree from fitted growth curves.

Analysis of variance was conducted to investigate the effects of inbreeding level and pedigree (founder clone), and their interaction, for both cumulative and annual increments of sectional area, on the three fitted Gompertz growth curve parameters (α, β, and κ), and RMG, TMG, and AMG according to following linear model:

$$Y_{ijkl} = \mu + R_i + C_j + P_k + CP_{jk} + RP_{ik} + RC_{ij} + E_{ijkl}$$

where μ is grand mean, R_i is effect of replication, C_j is founder clone (pedigree) effect, P_k is population (inbreeding level) effect, CP_{jk} is the interaction effect between founder clone and population, RP_{ik} is the interaction between replication and inbreeding level, RC_{ij} is the interaction of replicate with founder clone, and E_{ijkl} is the residual. All effects were assumed random except for grand mean and inbreeding levels (population effects). As an indication of true difference between various inbreeding levels, Tukey-Cramer multiple-comparisons were used to compare pair-wise differences among inbreeding levels.

RESULTS
Time Course of Inbreeding Depression

Analysis of variance of cumulative growth and annual growth increment showed that inbreeding started to have a significant effect (p<0.05) on sectional area at breast height from age 3 (2 years after planting) (Tables 1 and 2). At age 3, the cumulative growth of outcrosses (F = 0) and half-sib mated (F = 0.125) offspring was significantly larger than that of full-sib mated (F = 0.25), selfed (F = 0.5), and double-selfed (F = 0.75) offspring. Two groups had formed, one consisting of offspring with lower inbreeding levels (F = 0 and 0.125), and the other consisting of offspring with higher inbreeding levels (F = 0.25, 0.5, and 0.75). At age 4, the two selfed populations (F = 0.5 and 0.75) began to differentiate from the full-sib mated population as well as the outcrosses and half-sib populations. Significant differentiation between the two selfed populations (F = 0.5 and 0.75) and the other three less-inbred populations persisted from age 4 onwards (Table 1). However, there were never any significant differences between the two selfed populations (F = 0.5 *v.* F = 0.75). Among the

TABLE 1–Results from analysis of variance (variance ratio and significance level) for cumulative cross-sectional area at breast height from age 2 (1 year after outplanting) to age 13

Sources of variation	d.f.	Age											
		2	3	4	5	6	7	8	9	10	11	12	13
Replication	3	2.34	3.49*	1.30	1.09	1.83	2.63	3.55*	3.93*	3.39*	2.96	2.33	2.20
Population	4	4.26	8.02**	34.8**	34.5**	20.1**	12.5**	9.25**	8.69**	9.81**	11.8**	13.8**	15.4**
Pedigree	7	1.08	0.46	1.08	2.29*	2.91*	2.92*	2.93*	3.12**	3.36**	3.64**	3.88**	4.08**
Population×pedigree	23	0.59	0.76	0.89	1.92**	2.84**	3.38**	3.54**	3.33**	3.09**	2.94**	2.68**	2.54**
Replication×population	12	0.94	1.24	1.74*	1.87*	1.95*	1.53	1.33	1.23	1.11	0.92	0.86	0.81
Replication×pedigree	16	0.52	1.11	1.36	1.10	0.92	0.89	0.84	0.89	0.89	0.88	0.89	0.87

* $p < 0.05$
** $p < 0.01$

Tukey-Cramer multiple comparison

$F = 0$		a	a	a	a	a	a	a	a	a	a	a	a
$F = 0.125$		a	a	a	ab	ab	a	a	a	ab	b	b	b
$F = 0.25$		a	b	b	b	b	a	a	a	b	b	b	b
$F = 0.50$		a	b	c	c	c	b	b	b	c	c	c	c
$F = 0.75$		a	b	c	c	c	b	b	b	c	c	c	c

Inbreeding levels with same letter were not significantly different at $p < 0.05$.

TABLE 2–Results from analysis of variance (variance ratio and significance level) for annual increment of cross-sectional area at breast height from age 2 (1 year after outplanting) to age 13

Sources of variation	d.f.	Age											
		2	3	4	5	6	7	8	9	10	11	12	13
Replication	3	2.15	3.37*	0.64	1.28	1.98	2.01	2.84*	2.98*	1.56	5.27**	2.63	2.85*
Population	4	2.88	8.20**	35.7**	27.2**	2.92*	0.70	1.08	2.76	16.9**	25.0**	35.8**	24.0**
Pedigree	7	1.25	0.53	1.33	3.11**	2.95*	2.06	1.92	3.18**	3.88**	4.48**	4.57**	4.16**
Population×pedigree	23	0.55	0.80	0.98	2.54**	3.43**	3.83**	2.47**	1.76*	1.69*	2.03**	1.47	1.47
Replication×population	12	1.80	1.30	2.12**	1.86*	2.14**	1.65	1.79*	1.45	0.90	0.53	0.94	1.07
Replication×pedigree	16	0.55	1.15	1.29	0.79	0.84	0.85	0.80	1.12	0.76	0.76	0.59	0.80

* $p < 0.05$
** $p < 0.01$

Tukey-Cramer multiple comparison

F = 0		a	a	a	a	a	a	a	a	a	a	a	a
F = 0.125		a	a	b	b	a	a	a	a	b	b	b	b
F = 0.25		a	b	b	b	a	a	a	a	b	b	b	b
F = 0.50		a	b	c	c	b	a	a	a	c	c	c	c
F = 0.75		a	b	c	c	c	a	a	a	c	c	c	c

Inbreeding levels with same letter were not significantly different at p<0.05.

three less-inbred populations (F = 0, 0.125, 0.25), the differences of increment were inconsistent among ages; there were significant differences between F = 0.25 and the other two least-inbred populations (F = 0 and 0.125) at age 4, and between F = 0 and F = 0.25 at ages 5, 6, and 10. However, from age 11 onwards, the outcrossed population (F = 0) consistently had significantly larger increment than the half-sib (F = 0.125) and full-sib mated (F = 0.25) populations. Thus, after age 11, there were three differentiated groups among five inbred levels: outcrosses (F = 0, best growth), the two sib-mated populations (F = 0.125 and 0.25, intermediate), and the two selfed populations (F = 0.5 and 0.75, worst). Within each group, no significant differences were observed for sectional area from age 11 onwards.

The results for annual increment of sectional area were similar to those for cumulative increment except that there were no significant differences among five inbreeding levels at three consecutive ages (7, 8, and 9). At age 3, the outcrossed and half-sib mated populations (F = 0 and 0.125) had significantly larger increment than the others (F = 0.25, 0.5, and 0.75). At ages 4 and 5, the increment in the outcrosses (F = 0) was larger than in the two sib-mated populations (F = 0.125 and 0.25), while in both outcrosses (F = 0) and the sib-mated population (F = 0.125 and 0.25) the increment was larger than for the two selfed populations (F = 0.5 and 0.75). However, at age 6, the increment was similar between outcrosses and the two sib-mated populations (F = 0, 0.125, and 0.25) although increment in the two selfed populations (F = 0.5 and 0.75) was still less than in the three less-inbred populations. At ages 7, 8, and 9, there was no difference in growth increment among the five inbreeding levels. Nevertheless, from age 10 onwards, after 3 years of no significant differences, growth increment was differentiated again among three groups: outcrossed (F = 0) had the highest, sib-mated populations (F = 0.125 and 0.25) were intermediate, and selfed populations (F = 0.5 and 0.75) had the least.

There were also significant differences among pedigrees (derived from particular founder clones) from age 5 onwards for cumulative growth and annual increment except at ages 7 and 8 for annual increment (Tables 1 and 2). Significant interactions between pedigrees and inbreeding levels were also observed and this significant interaction was observed from age 5 (Tables 1 and 2).

Effect of Inbreeding on Growth Curve

The five populations of different inbreeding levels could be divided into three groups from both annual sectional increment curves (Fig. 1) and cumulative growth curves (Fig. 2): these groups were outcrosses (F = 0), the two sib-mated populations (F = 0.125 and 0.25), and the two selfed populations (F = 0.5 and 0.75). From cumulative growth curves, outcrosses had the highest cumulative sectional area for all tree ages, the two sib-mated populations were intermediate, while the two selfed populations had the lowest (Fig. 2). The differences between the three groups of cumulative curves were also reflected in the annual sectional increment curves (Fig. 1). Annual increment of sectional area at breast height increased from age 3 for all inbreeding levels to a maximum level at about age 5–6, depending on inbreeding level (Fig. 1). Among the inbreeding levels, maximum sectional area increment and the age at which it was achieved varied. The outcrosses reached maximum increment of sectional area first, at age 5; the two sib-mated populations took either 5 (F = 0.125) or 6 (F = 0.25) years, and the two selfed populations (F = 0.5 and 0.75)
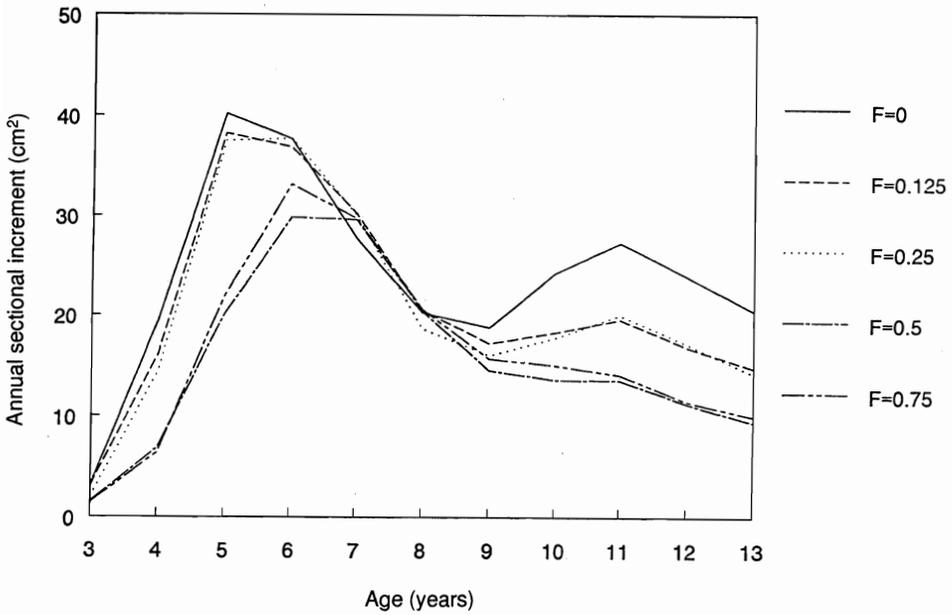
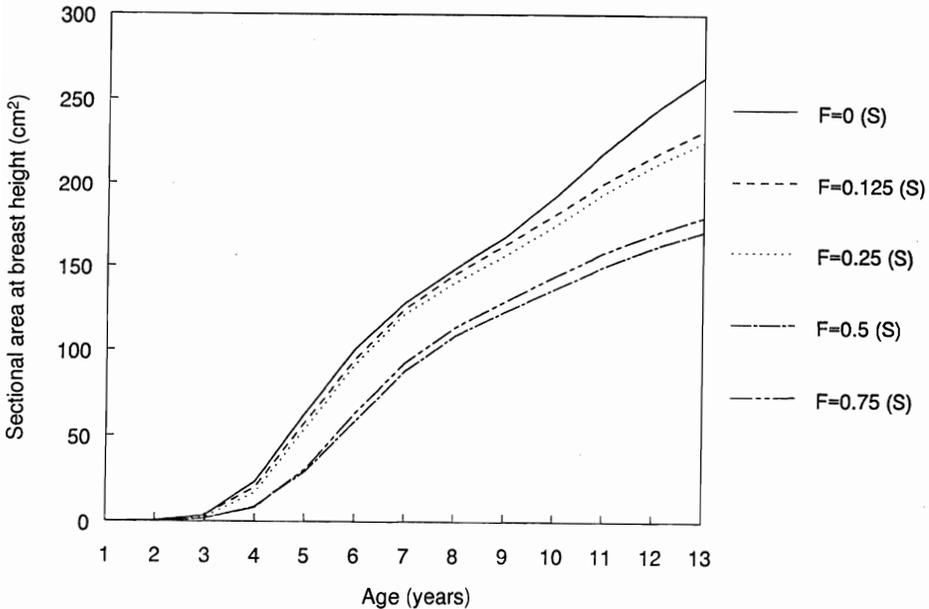FIG. 1—Annual sectional area increment among five inbreeding levels from age 3 to age 13.

FIG. 2—Growth curves of average sectional area at breast height from age 3 to age 13 for five inbreeding levels.

took 6 years. Just as the age of maximum sectional area increment was influenced by inbreeding level, so too was the maximum increment rate—i.e., the highest increment rate (40.2 cm²) was for the outcrosses, intermediate for the two sib-mated populations (38.3, 37.7

cm^2 for F = 0.125 and F = 0.25, respectively), and lowest for the two selfed populations (30.0, 33.1 cm^2 for F = 0.5 and F = 0.75, respectively). In contrast to their different increment rates at younger ages, annual increments for sectional area among the three groups were similar at ages 7, 8, and 9. However, the increment curves diverged again from age 10 onward, i.e., outcrosses once again had the highest increment, sib-mated populations had intermediate increment, and selfed populations had the lowest. This was mainly because annual increment at ages 10, 11, and 12 increased among outcrosses compared with sib-mated populations, which more or less maintained their increment at the same level, and selfed populations whose annual increment continued to decline (Fig. 1). Another noticeable difference among the three groups was that the increment curve for outcrosses had two peaks, and the curves for sib-mated populations were close to unimodal with a small rise at age 10 while the curves for selfed populations were unimodal with only one peak.

Inbreeding significantly affected all three parameters of the fitted Gompertz growth curves (Table 3). The α and κ parameters were significantly different among the three inbred groups (i.e., outcrosses (F = 0), two sib-mated populations (F = 0.125 and 0.25), and two selfed populations (F = 0.5 and F = 0.75), while β was significantly different only between the two selfed populations (F = 0.5 and 0.75) and the other three less-inbred populations (F = 0, 0.125, and 0.25). According to the fitted growth curves, predicted maximum sectional area increment (RMG) was 33.85, 34.79, 33.91, 29.24, and 30.72 cm^2 per year for inbreeding levels F = 0, 0.125, 0.25, 0.5, and 0.75, respectively. Inbreeding significantly affected predicted rate of maximum increment; in outcrosses, half-sib, and full-sib mated populations it was significantly larger than in the two selfed populations (Table 3). However, there were no significant differences within each group, i.e., within the three lower inbreeding levels (F = 0, 0.125, and 0.25) or within the two selfed populations. The predicted ages to reach

TABLE 3–F-statistics and significance levels for analyses of Gompertz growth curve parameters, maximum increment (RMG), time to reach maximum increment (TMG), and sectional area asymptote (AMG, same as α) for cross-sectional area at breast height

Sources of variation	d.f.	α	β	κ	RMG	TMG
Replication	3	2.28	0.58	2.43	2.92*	4.76*
Population	4	28.8**	873**	28.1**	2.77*	12.04**
Pedigree	7	4.97**	1.68	2.82**	2.46*	2.16*
Population×pedigree	23	1.67*	2.24**	1.84**	3.05**	1.68*
Replication×population	12	0.55	1.29	0.81	1.93*	0.88
Replication×pedigree	16	0.86	0.99	0.79	1.06	0.84

* p<0.05
** p<0.01

Tukey-Cramer multiple comparison

F = 0	321.9a	4.76a	0.32a	33.8a	7.86a
F = 0.125	252.4 b	6.29a	0.42 b	34.8a	7.15 b
F = 0.25	244.3 b	6.34a	0.42 b	33.9a	7.18 b
F = 0.50	180.8 c	9.56 b	0.49 c	29.2 b	7.30 b
F = 0.75	186.9 c	11.20 b	0.51 c	30.9 b	7.33 b

Inbreeding levels with same letter were not significantly different at p<0.05.

maximum increment (TMG) and predicted asymptote for sectional area (AMG) were also significantly different among populations (Table 3). TMG increased with inbreeding level from half-sib mated (7.15 years) and full-sib mated (7.18 years) to the selfed populations (7.30 years for F = 0.5 and 7.35 years for F = 0.75), but was actually greatest for outcrosses (7.86 years). This conflicts with the empirical increment growth curve fitted. This is because the two peaks of annual increment for outcrosses violated the assumed single growth peak for fitting the Gompertz growth curve, and hence the fitted growth curve predicted TMG later for outcrosses. Thus, Gompertz growth curves better fitted the data for inbred populations than those for outcrosses. Predicted AMG was largest for outcrosses (321.9 cm^2), intermediate for the half-sib (252.4 cm^2) and full-sib mated populations (244.3 cm^2), and smallest for the S_1 (180.8 cm^2) and S_2 populations (186.9 cm^2).

Time Course of Inbreeding Depression

There were marked changes of inbreeding depression (ID) in both cumulative and annual sectional area increment among ages (Fig. 3 and 4). ID for cumulative increment increased from age 3 to a maximum at age 4 (14.2%, 27.6%, 62.7%, and 65.0% depression for F = 0.125, 0.25. 0.5, and 0.75, respectively) and then declined from ages 5 to 9 (2.9%, 6.6%, 26.6%, and 22.9% depression at age 9). ID then increased again so that at age 13 it was 11.9%, 14.4%, 35.2%, and 31.8% for F = 0.125, 0.25, 0.5, and 0.75, respectively (Fig. 3). The age-trend of ID can be more clearly observed in annual increment depression (Fig. 4). As for cumulative increment, ID for annual sectional area increment also increased from age 3 and had the highest level at age 4 (17.6%, 26.4%, 65.0%, and 67.3% depression for F = 0.125, 0.25. 0.5, and 0.75, respectively). However, it declined from age 4 to age 6 (2.2%, –0.1%, 20.8%. 12.0% depression at age 6). At age 7, negative ID (–9.0%, –8.4%, –6.7%, –7.1%
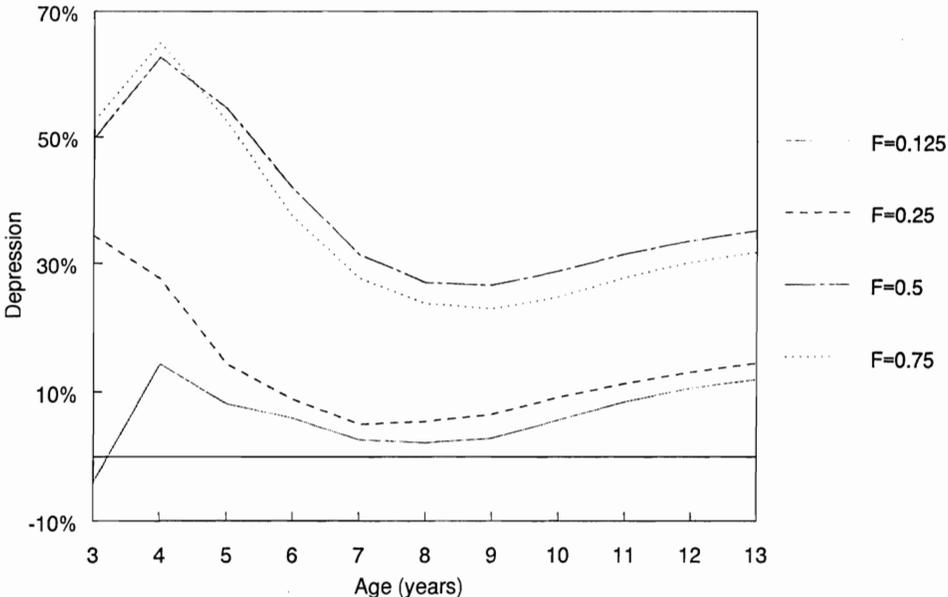

FIG. 3—Cumulative depression of sectional area at breast height for five inbreeding levels from age 3 to age 13.

FIG. 4—Effect of inbreeding depression on annual sectional area increment (cm²) at breast
height for five inbreeding levels from age 3 to age 13. MI = mean overall yearly
increment.

depression for F = 0.125, 0.25. 0.5, and 0.75, respectively) was observed, although the
negative values (perhaps more properly called inbreeding "vigour") were not statistically
significant (Table 2). At age 8, the effect of inbreeding was also non-significant (Table 2).
ID for annual sectional area increment began to increase from age 9 although it was still
non-significant at that age, but it became significant again from age 10 onwards and at age
13 was 28%, 30.8%, 54.1%, and 51.3% for F=0.125, 0.25, 0.5, and 0.75, respectively. In
summary, we observed an apparent bimodal pattern for ID of annual sectional area increment
in *P. radiata* whether the two sites (Symmonds and Kilsbys) were analysed separately or
combined. To demonstrate the relationship between the trend of growth and inbreeding
depression, the mean current sectional area increment (MI) (Durel *et al.* 1996) was also
calculated (Fig. 4). There was no clear association between MI and ID.

Clonal Effects and Interaction Between Inbreeding Level and Clone on Growth Curve

Significant differences were observed among pedigrees (each derived from a particular
founder clone) for the rate of maximum increment (RMG), TMG, and AMG predicted from
the fitted growth curves (Table 3). Interactions between inbreeding level and pedigree for
these three parameters of the growth curve were also statistically significant. For RMG, four
pedigrees (derived from clones 2, 3, 4, and 8) declined from inbreeding level F = 0 to F = 0.75
(Fig. 5) while for three pedigrees (derived from clones 5, 6, and 7) RMG increased from
F = 0.5 to 0.75 despite declining between F = 0 to F = 0.5. This was especially so for pedigrees
5 and 7. We also observed that RMG differed more among pedigrees at higher inbreeding
levels, especially F = 0.75. For example, RMG ranged from 25 to 40 cm² for F = 0.75 while
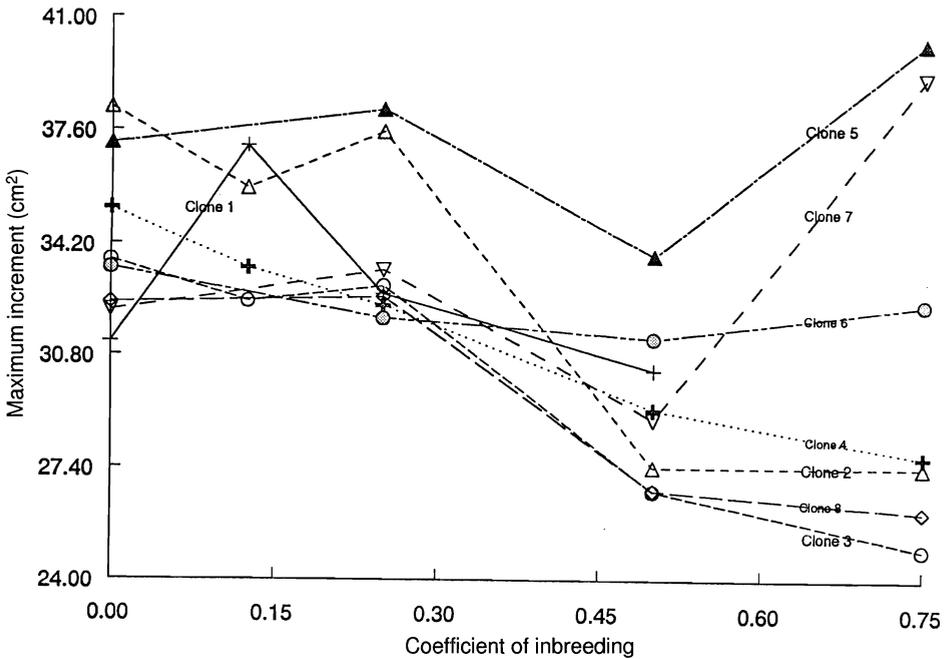it varied only from 31 to 38 cm² for F = 0 (Fig. 5).

FIG. 5–Effect of inbreeding on maximum increment of sectional area among eight *Pinus radiata* pedigrees.

For AMG, there were also two patterns among the eight pedigrees. For pedigrees 2, 3, 4, and 8, AMG decreased from F = 0 to 0.75, while it declined initially from F = 0 to F = 0.5, then increased from F = 0.5 to F = 0.75 for pedigrees 5, 6, and 7. This increase was particularly apparent for pedigree 7 (Fig. 6).

DISCUSSION

It was a great advantage in this study of age trends in inbreeding depression that the trial was not thinned, and so the same trees could be used throughout for calculating inbreeding depression. Studies of the time course of inbreeding depression are frequently unsatisfactory because they involve either estimates of mortality or growth measurements at too few ages. Mortality is important in long-term experiments where the growth of inbred (especially selfed) and outcrossed progeny is compared. In some studies, early and late measurements have not been based on the same set of trees because the weakest plants died before later measurements could be taken, thus creating a bias in estimates of inbreeding depression. Examples involve *Picea abies* (Eriksson *et al.* 1973) and *Pinus radiata* (Wilcox 1983). In other studies, inbreeding depression was reported for only a few ages (Sorenson & Miles 1982; Lundkvist *et al.* 1987) which hinders the observation of genuine trends of depression with stand development. Thinning would also affect the progress of inbreeding depression.

Inbreeding significantly depressed sectional area increment at breast height from age 3 onwards. Significant inbreeding depression was not observed at ages 1 and 2, possibly because most trees were still less than 1.3 m in height at age 1 and many trees (48%, 33%,
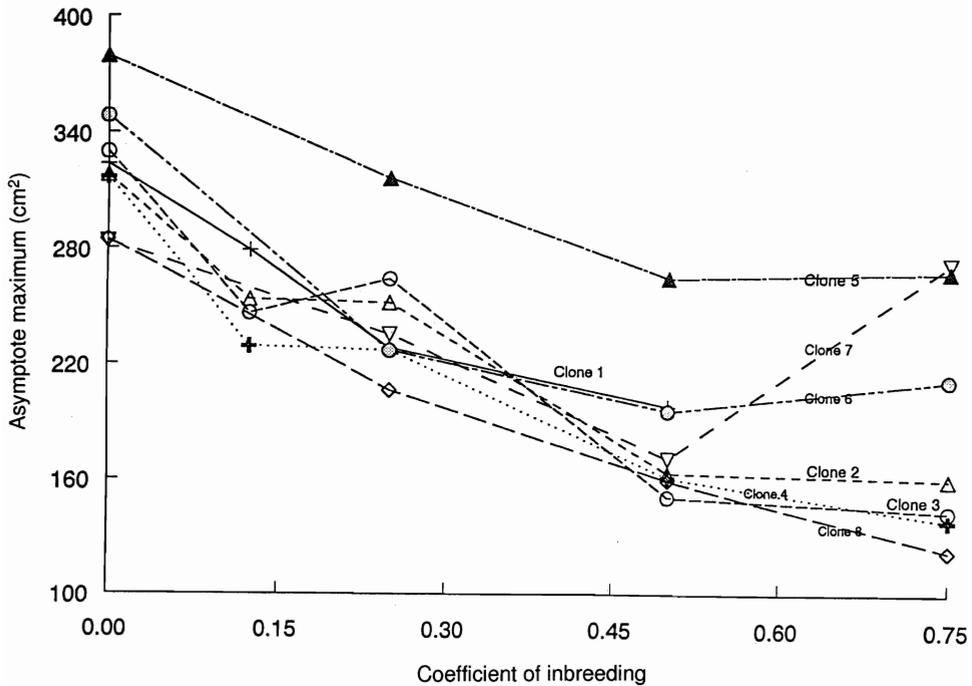
FIG. 6–Effect of inbreeding on sectional area asymptote among eight *Pinus radiata* pedigrees.

25%, 21%, and 14% of trees for S_2, S_1, full-sib, half-sib, and outcrossed populations, respectively) were still under 1.3 m at age 2. This also indicates that inbreeding depressed tree height and consequently extended the time for trees to reach the breast height. Results from height measurements in the same trials have shown that inbreeding depression was apparent in seedlings of 3, 6, and 24 months. Strong inbreeding depression observed in these young *P. radiata* seedlings was consistent with severe selfing depression observed in seedlings of many other conifers (Franklin 1970; Sorensen & Miles 1974; Sorensen *et al.* 1976; Williams & Savolainen 1996) and reported in an earlier selfing study in *P. radiata* (Wilcox 1983). Severe early depression in *P. radiata* allows some nursery culling of selfed or other less-inbred seedlings and could be used to keep some of the slowest-growing inbreds out of plantations.

Inbreeding strongly affected growth curves, with all three parameters of the Gompertz growth curve showing significant effects. We observed that, according to the statistical significance of the three Gompertz parameters, both empirical curves and fitted Gompertz growth curves could be divided into three groups: outcrosses, the two sib-mated populations, and the two selfed populations. Inbreeding not only delayed the time to reach maximum increment, but also reduced the magnitude of maximum increment. However, there were no differences in growth curves between the half-sib and full-sib populations, and between the S_1 and S_2 populations. Sorensen & Miles (1982) observed that inbreeding depression at a specified height was lower than at the same ages for *Pseudotsuga menziesii, Pinus ponderosa,* and *A. procera*. They concluded that relative inbreeding depression at any age consists of a general depression of growth rate plus an effect resulting from self and outcross

plants being at different points on a more or less common growth curve. The delayed time to reach maximum sectional area increment reported here in *P. radiata* empirical curves agrees with the hypothesis of a lag between inbred and non-inbred trees for their growth pattern (or physiological state, Durel *et al.* 1996). However, we observed that maximum increment of sectional area was also different between different inbreeding levels.

An important finding in this study was the bimodal age trend of inbreeding depression for sectional area increment. We observed that inbreeding depression for annual increment was highest early in stand development, disappeared at about the time of crown closure among outcrosses, and reappeared as the stand developed further under inter-tree competition. This bimodal trend of inbreeding depression in sectional increment was attributed to differences in the timing of onset of stand competition at the different inbreeding levels. Although no steps were taken to measure stand development in terms of tree competition, crown closure for *P. radiata* at a spacing of 2 × 3 m on such a site near Mt Gambier is typically at about age 5 or 6 years. By ages 7 and 8, crowns of outcrosses at Symmonds were mostly completely closed. This competition must be delayed in the slower-growing half- and full-sib mated populations and even more delayed in S_1 and S_2 populations. Thus, the significant difference observed between outcrosses and sib-mated populations in the free growth period at ages 4 and 5 must be reduced at age 6 owing to greater competition among outcrosses than among sib-matings (Table 2). This difference in competition level might have contributed to a non-significant difference in growth increment between outcrosses and sib-matings at age 6. In the meantime, the S_1 and S_2 populations would not yet have achieved crown closure at age 6 and would thus still have relatively free growth due to smaller tree size (Fig. 1). Consequently, the difference in annual increment between selfed populations (F = 0.5 and 0.75) and the three less-inbred populations was much less at age 6 than at age 5 (Fig. 1), although increment in the three less-inbred populations was still larger than two selfed populations. For example, the MIs in outcrossed, half-sib, full-sib, S1, and S2 populations were 40.2, 38.3, 37.5, 20.2, and 22.0 cm² at age 5 and they were 37.7, 36.9, 37.7, 29.8, and 33.2 cm² at age 6; thus the average difference between the three less-inbred populations and two selfed populations was reduced to 5.9 cm² at age 6 from 17.6 cm² at age 5. By age 7, tree competition in S_1 and S_2 should have started to affect growth and we observed that growth rate in S_1 and S_2 declined slightly. However, at this time competition within outcrosses, and within half-sib and full-sib populations, was becoming more intense. Thus, different competition levels might have obscured the differences in increment between the various inbreeding levels and consequently obscured inbreeding depression. There was no more inbreeding depression at age 7. Indeed, we observed that the increment in the outcrosses at age 7 was smaller than in the inbred populations, which produced an inbreeding "vigour" (Fig. 4). We might expect that the inbreeding depression would reappear when crowns were closed for all populations although competition levels would still differ, and inbreeding depression was again evident in sectional area increment from age 10 onwards (Fig. 1 and 4).

A substantial decrease in increment at ages 8 and 9 was observed for all five populations. This complicated the observed pattern of inbreeding depression. Climatic data within the growth period indicated that age 8 was the driest year among the 13 growing seasons, both overall and during the 5 months of most active growth for *P. radiata* (July–November). That year annual rainfall was only 586 mm with 240 mm in July–November compared with averages of 701 and 371 mm, respectively, for all 13 years. However, rainfall was normal

for the age 9 year. Since availability of water resources is the most limiting factor for *P. radiata* growth in the Mt Gambier region, it seems likely that the drought year at age 8 could have affected growth in year 9. Interestingly, there was no inbreeding depression at age 8 or 9 when these were adverse years, judging from mean annual growth (biological index). This contrasts with observations in *P. pinaster* (Durel *et al.* 1996) where adverse years had the most inbreeding depression. We believe that both adverse growing conditions and different competition levels between the five inbreeding levels at ages 8 and 9 might have complicated the pattern of inbreeding depression in this study. From age 10 onwards, rainfall became more or less normal and the annual increment of outcrosses increased more than that of half-sib and full-sib mated populations, while that of the S_1 and S_2 populations was the lowest. Three inbred groups were again differentiated in growth rate (Fig. 1).

Thus, inbreeding depression is a dynamic process which is affected by stand development, particularly competition. Since competition is related to trial layout, plot size, and spacing, the time course of inbreeding depression in this study must have been affected by the split-plot layout of the field design. If outcrosses had been planted together with inbred trees, competition may have increased inbreeding depression due to the suppression of inbred trees by neighbouring outcrossed trees. This might have been the situation for Eriksson & Lindgren (1975, cited by Lundkvist *et al.* 1987) and for Lundkvist *et al.* (1987). The increase of inbreeding depression in one trial of *P. sylvestris* from age 6 to 11 was attributed by Lundkvist *et al.* (1987) to the increased competition with other plants for water and nutrients under field conditions. However, on another site where single-tree plots were used, inbreeding depression did not increase between ages 6 and 14. This may indicate that competition between the trees did not dominate tree growth in that particular trial. Sorensen & Miles (1982) reported a slight increase of inbreeding depression with age, but crowns had not closed at the last measurement (age 10). Constant inbreeding depression in *P. pinaster* was attributed to very limited stand competition (Durel *et al.* 1996). In a similar split-plot design for a *P. elliottii* inbreeding trial, Matheson *et al.* (1995) observed similar stable depression from ages 2 to 13 years for height and diameter and a slight increase of depression for volume, perhaps due to slight inter-tree competition in that study.

The dynamic relationship between stand development and inbreeding depression observed in *P. radiata* has practical applications for the understanding of inbreeding depression in conifers. Data from one or a few ages may not be sufficient to depict trends. The variation of inbreeding depression at different ages (developmental stages) and at different spacing and layout makes reliable comparisons among inbreeding experiments difficult. Furthermore, thinning would have an important impact on trends.

It was notable that the S_2 stock were bigger than the S_1 on average, and had less inbreeding depression (Fig. 3), although this difference was not statistically significant. This is similar to the comparison for height at age 13 (Wu *et al.* in press) and might indicate purging was effective in eliminating some deleterious alleles in the S_1. We also observed that different pedigrees behaved differently with respect to inbreeding depression. For example, predicted maximum increment (MGR) for pedigrees 5 and 7 was substantially greater in the S_2 than the S_1 and was even larger for the S_2 than for outcrosses. The maximum growth asymptote (AMG) for pedigree 7 was also larger in the S_2 than the S_1 and was similar to that of outcrosses. This may indicate that selfing combined with between- and within-family selection would be effective for purging deleterious alleles in *P. radiata*.

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