VARIATION IN EARLY FLOWERING OF INBRED JACK PINE FAMILIES

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Abstract.--Inbreeding of jack pine delays initiation of first flowering and depresses fecundity. However, by 6 years of age the percentage of second selfed generation trees flowering does not differ from that of outcrossed trees although the number of strobili per tree continues to be lower on the inbred trees. Dominance for initial female flowering is indicated. Crosses between selfed lines restore early flowering behavior and fecundity to that of open-pollinated progenies of the ancestral parent trees. Thus, inbreeding and outcrossing of inbreds can be used as complementary breeding strategies in jack pine.

Additional keywords: initial flowering, fecundity, selfing, outcrossing, inbreeding depression, heterosis, *Pinus banksiana*.

Forest tree breeders have tried to avoid or at least minimize inbreeding in production populations for reforestation purposes because inbreeding can result in depression of many important characteristics. However, early results show that inbreeding has potential as a complementary breeding strategy for developing breeding populations for improvement of jack pine (*Pinus banksiana* Lamb.) (Rudolph 1976, 1981). Whereas inbreeding as a strategy in many other pines has been limited by the apparent difficulty, long generation turnover time, and high cost of producing inbred materials (Snyder 1968; Franklin 1969), in jack pine these limitations are not serious for several reasons. The trees flower at a young age making possible the rapid turnover of generations (Rudolph 1966b, 1979a; Jeffers and Nienstaedt 1972). Seed yield and quality, although reduced by inbreeding, are adequate for breeding purposes (Rudolph 1966b, 1967, 1976). Phenotypic selection for growth rate in open-pollinated jack pine populations appears to be ineffective (Canavera 1975) which makes the use of outcrossed progenies of inbreds one possible alternative.

Inbreeding, particularly in those pines in which the sexual generation interval is not long, offers the tree breeder many advantages. It will, in general, result in increased variability in the breeding populations which implies increased heritability and, therefore, improved selection efficiency. Preliminary tests of young jack pine indicate that even without selection, crosses between inbreds show heterosis, that is, relief of inbreeding depression, and restoration of vigor to at least the average original ancestral condition (Rudolph 1976, 1981). Inbreeding can be useful for eliminating

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deleterious genes from breeding populations and, with the short time between generations in jack pine, selfing in breeding populations can be continued into later generations, eliminating most deleterious genes and increasing homozygosity and the potential for greater hybrid vigor in crosses between advanced generation inbreds. Such purging of deleterious genes from a breeding population can potentially reduce population size but the size can be supplemented early in the breeding program without significant loss in performance. Thus, inbreeding should be done early in a breeding program rather than later.

In assessing the potential application of inbreeding, it is important to evaluate effects in terms of inbreeding depression, heterosis upon outcrossing of the inbreds, and changes in means and in the within and between family variance. In jack pine, such evaluations through the S2 generation have been made for cone set, seed yield and quality and seedling growth in the greenhouse (Rudolph 1976); for autumn coloration of foliage in the nursery (Rudolph 1980); and for height growth in the first 4 years (Rudolph 1981). In the normally early flowering jack pine, it is especially important to determine the effects of inbreeding on early cone and seed production if inbreeding is to be applied as a complementary breeding strategy. Here flowering and cone production in the first 6 years in S2 progenies and Sl outcross progenies are compared to that in progenies of the original S0 parent trees.

MATERIALS AND METHODS

The S_0 trees were grown from seed collected in 1950 on the Chippewa National Forest in Minnesota. Descriptions of the S0 progenies have been reported previously (Rudolph 1966a, 1966b, 1967). Controlled self-pollinations to produce the S_1 generation were made in 1962 and 1963. Details pertaining to the Sl generation have been reported earlier (Rudolph 1966a, 1967).

Controlled pollinations on 29 S1 trees were made in 1974. The only selection criterion applied in choosing the $_{Si}$ trees for the pollinations was the presence of both male and female strobili. However, trees of exceptionally poor growth and form (most of which also flowered poorly) were avoided. Pollinations on each of the 29 S1 trees included selfing to produce S2 seed and crosses with a mixture of pollen from the 29 S1 trees to produce what will be referred to as S1 x Sl-mix seed. Thus, the Sl x S_1 pollinations on the average could include over 3 percent self s. However, earlier studies have shown that only a small portion of these would result in viable seedlings (Rudolph 1979b) and so would not significantly influence the results reported here.

Control-pollinated and open-pollinated (S1 O.P.) cones were collected in early October 1975. Cone set and seed yield and quality have been reported (Rudolph 1976). Open-pollinated seed from the S_O trees (parents of the S1 trees) growing on the Argonne Experimental Forest in northeast Wisconsin was included when available. Seed from some S0 trees was not available for comparison because the plots had been thinned previously. For the same reason, seed and seedlings of the selfed SO trees were not available for comparison in this study. The seed was germinated in late April, 1976 on moist filter paper in petri-dishes and before the seed coats were shed the seedlings were planted into "Jiffy 7"[2] peat pellets in the greenhouse. Ten replications of four trees were planted for all seed lots that had sufficient seedlings but some lots contained less than the required 40 seedlings so not all lots contained 10 replications. The same design was used in the nursery planting and in the field test.

Greenhouse thermostats were set at 22°C during the day and 17°C at night, but on warm, sunny days temperatures occasionally reached 30°C. A 50 percent shade screen covered the greenhouse. The seedlings were fertilized with a water-soluble 20-10-10 fertilizer as needed to maintain vigorous growth.

When 10 weeks old the seedlings were transplanted into nursery beds at the Forestry Sciences Laboratory, Rhinelander, Wisconsin in late June and early July 1976. Spacing between plants was 25 x 25 cm (10 x 10 in). Initial flowering was scored in May 1)77 at the beginning of the second growing season in the nursery.

The seedlings were planted in a field near Rhinelander, Wisconsin in May 1978 at a spacing of 2.44 x 2.44 m (8 x 8 ft). Seventeen S2, 15 S1 x S1-mix, 19 S1 open-pollinated, and 18 SO open-pollinated progenies were available for the field test.

Female strobili in six replications were counted in 1978, 1979, and 1980. In 1981, because of the large number of strobili, only trees in the first replication were scored. Cones were counted in the autumn in 1979 and 1980, and in 1981 the developing cones were counted in June. Male strobili production in 1978, 1979, 1980 and 1981 was scored on the same trees as were scored for female strobili. The number of male clusters per tree was assigned to one of six classes: 0, 1-5, 6-25, 26-50, 51-100, >100.

Flowering comparisons were made between progeny groups in which trees were available in the groups to be compared. Because the number of families within groups differs, the degrees of freedom for the various comparisons differ as well. Flowering variation in each progeny group was evaluated by the standard deviation. Comparisons between progeny groups were made by "t" tests, recognizing that the data sets may not be normally distributed but assuming that they are not heavily skewed.

Although the data are not completely balanced genetically or in experimental design, estimates are thought to be based on enough trees to make relative early flowering comparisons between progeny groups reliable.

RESULTS AND DISCUSSION

Female flowering was initially observed in May 1977 when the seedlings were approximately 12 months old. Overall, one percent of the trees bore female strobili but the percentage of trees flowering was twice as great in the S1 and S0 open-pollinated progenies as in the S2 progenies (table 1).

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[2] Mention of trade names does not constitute endorsement of the products by the USDA, Forest Service.

Table 1.--Percentage of trees with female strobili in the first 6 years in S0, S1 outcrossed, and S_2 jack pine progenies sown in 1976

Year	S_1 Selfed (S_2)	S_1 x S_1-Mix	S_1 O.P.	S_0 O.P.
1977	0.6	0.8	1.2	1.2
1978	7.2	13.2	14.4	18.2
1979	58.3 1/	87.6	84.4	90.8
1980	77.0 2/	97.6	95.2	97.7
1981	94.3	100.0	97.1	98.6

1/ Differs significantly from S_1 x S_1-mix, S_1 O.P., and S_0 O.P. progenies at 1% level.

2/ Differs significantly from S_1 x S_1-mix progenies at 5% level and from S_1 O.P. and S_0 O.P. at 1% level.

All flowering trees had only one strobilus except one had two, and all were borne on the main stem (Rudolph 1979a). In 1978, at the beginning of the third growing season, the percent of trees with female strobili increased up to 18 in the outcrossed progenies but was less than half as frequent in the S2 progenies (table 1). Although the differences in percentage of trees bearing female strobili in 1977 and 1978 in the S2 and outcrossed progenies are of biological interest, they are not statistically significant possibly because of the wide variation in earliness of flowering among families.

The percentage of trees in the outcrossed progenies with female strobili increased sharply in the fourth growing season in 1979 when 84 to 91 percent of the trees flowered. The frequency in the S2 progenies was significantly lower at 58 percent. The frequency of female flowering trees further increased in 1980 and the difference between S2 and outcrossed progenies was again significant. At the beginning of the sixth growing season in 1981, at least 94 percent of the trees in all progeny groups bore female strobili and differences between the S2 and outcrossed progenies were no longer significant. Thus, whereas inbreeding depression in earliness of initial female flowering, expressed as percentage of trees with female strobili, was evident through the fifth year, it was essentially eliminated by the sixth year. This suggests that the trees need to reach some "threshold" size before they begin to flower and that essentially all the trees reached this size by age 6. Noteworthy as well is the fact that the percentage of trees with female strobili in the S1 x S1-mix progenies is as high as it is in the S0 open-pollinated progenies. Thus, crosses between selfed lines on the average show restoration of the predisposition for early female flowering to the average level of progenies of the ancestral parents. Similar relief from inbreeding depression and heterosis for early height growth has already been noted in this population (Rudolph 1981).

The average number of female strobili produced remained at about one per tree in the second and the third growing season, i.e., in 1977 and 1978. Thereafter, the average number increased rapidly up to as many as 63 per tree in the S1 open-pollinated progenies at the beginning of the sixth season (table 2). The number of female strobili per tree in the fourth, fifth, and sixth years was significantly lower in the S2 progenies than in the outcrossed progenies. In the sixth year the S2 progenies still had only about one-third as many female strobili per tree as the S1 and So open-pollinated progenies. Thus, significant inbreeding depression in fecundity is still evident at 6 years. The lower strobilus production in the S2 progenies may be partially due to the S2 trees being significantly shorter (Rudolph 1981) and thus having smaller crowns and fewer potential positions for female strobili. Variation in fecundity within progeny groups as expressed by the standard deviation did not differ among the progeny groups.

Table 2.--Average number of female strobili per flowering tree in the first 6 years in So, S1 outcrossed, and S2 jack pine progenies sown in 1976

Year	S_1 Selfed (S_2)	S_1 x S_1-Mix	S_1 O.P.	S_0 O.P.
1978	1.1 (0.67)[1]	1.2 (0.88)	1.2 (0.59)	1.3 (0.55)
1979	2.1[2] (1.35)	4.8 (1.46)	4.8 (1.89)	5.2 (1.71)
1980	8.1[2] (5.56)	21.4 (6.80)	21.3 (6.70)	22.9 (6.84)
1981	21.4[3] (19.36)	47.6[4] (18.32)	63.1(19.87)	61.0(23.13)

[1] Numbers in parentheses are the standard deviations.

[2] Differs significantly from S_1 x S_1-mix, S_1 O.P., and S_0 O.P. progenies at 1 percent level.

[3] Differs significantly from S_1 O.P., and S_0 O.P. progenies at 1% level.

[4] Differs significantly from S_1 O.P. progenies at 1% level.

None of the cones from the 1977 strobili reached maturity in 1978, possibly due to lack of pollen in the nursery in 1977 and the transplanting shock in 1978. In 1979, the fourth year, an average of slightly more than one cone matured per cone-bearing tree in all progeny groups (table 3). The number increased in 1980 up to about four in the S1 and S_0 open-pollinated progenies. Production in the S2 progenies was significantly less at two per tree. The projected number of mature cones produced per flowering tree (based on counts of developing cones in June 1981) decreased in 1981 to a maximum average of less than two in S and SO open-pollinated progenies and only 0.3 in the S_2 progenies (table 3J. The projected number of mature cones in 1981 is based on flowering trees because, due to possible additional cone losses, the number of mature cone-bearing trees could not be ascertained in early June 1981. The standard deviation for average number of mature cones per tree was consistent among progeny groups (table 3).

Table 3.--Average number of mature cones per cone-bearing tree in the first 6 years in S0, S1 outcrossed, and S2 jack pine progenies sown in 1976

Year	S_1 Selfed (S_2)	S_1 x S_1-Mix	S_1 O.P.	S_0 O.P.
1979	1.1 (0.61)[1]	1.3 (0.74)	1.4 (0.57)	1.5 (0.74)
1980	2.0[2] (1.01)	3.2[3] (1.02)	3.7 (1.04)	3.9 (1.16)
1981[4]	0.3[2] (0.65)	1.4 (0.71)	1.7 (0.79)	1.5 (0.98)

[1] Numbers in parentheses are the standard deviations.

[2] Differs significantly from S_1 x S_1-mix progenies at 5% level and from S_1 O.P and S_0 O.P. progenies at 1% level.

[3] Differs significantly from S_1 O.P. progenies at 1% level and from S_0 O.P. progenies at 5% level.

[4] Number of mature cones in 1981 is projected per flowering tree rather than per the then unknown number of mature cone-bearing trees.

In evaluating female strobili and mature cone production in this population, a most striking finding is that only less than 8 percent of the 1980 female strobili were projected to develop into mature cones in 1981. Whereas some of this catastrophic loss is undoubtedly due to abortion (Cecich 1979), much of it is likely due to a sharp increase in conelet and cone insects in the plantation area since 1979 (Rauf et al. 1981). Thus, although precocious female flowering and fecundity can reach operational seed orchard levels by the fifth or sixth year, conelet and cone insect control methods will need to be developed before the above results can apply to seed orchard planning and management.

Production of male strobili began in 1978, the beginning of the third growing season, when up to 63 percent of the S1 x S1-mix trees flowered (table 4). This percentage of male flowering trees in the third year is much higher than previously reported (Rudolph and Yeatman 1982). Usually notable male flowering does not begin until the fourth year. Whereas no complete explanation for this extraordinarily high percentage of male flowering trees in the third year is apparent in this study, it is possible that moisture stress may be involved. The 1976 and 1977 growing seasons were very dry so it is possible that the trees were subjected to moisture stress during initiation and differentiation of reproductive structures.

By the sixth year, more than 93 percent of the trees in the outcrossed progenies bore male strobili (table 4). The percentage of trees flowering in the S2 progenies was slightly over half that in the outcrossed progenies in the third and fourth years and increased to about three-fourths in the sixth year. Thus, inbreeding depression was still evident.

Table 4.--Percentage of trees with male strobili in the first 6 years in S_o, S1 outcrossed, and S2 jack pine progenies sown in 1976

Year	S_1 Selfed (S_2)	S_1 x S_1-Mix	S_1 O.P.	S_0 O.P.
1978	29.7[1]	62.8	60.4	53.7
1979	39.0[2]	73.2	69.0	65.1
1980	11.0[1]	18.3	22.7	15.5
1981	73.6[3]	93.3	97.1	94.3

[1] Differs significantly from S_1 x S_1-mix, and S_1 O.P. progenies at 1% level.

[2] Differs significantly from S_1 x S_1-mix, and S_1 O.P. progenies at 5% level.

[3] Differs significantly from S_1 O.P. progenies at 5% level.

The number of male strobili clusters per flowering tree was substantial beginning with the initial male flowering in the third year (table 5). Male production did not continue to increase steadily in the first 6 years as did female flowering; in 1980 fewer males per tree were produced than in 1979. Such year-to-year fluctuations again may be related to growing conditions during initiation and differentiation of flower primordia.

Table 5.--Average number of male strobili clusters 1/ per flowering tree in the first 6 years in So, S_I outcrossed, and S2 jack pine progenies sown in 1976

Year	S_1 Selfed (S_2)	S_1 x S_1-Mix	S_1 O.P.	S_0 O.P.
1978	1.27[3] (0.48)[2]	1.60 (0.18)	1.53 (0.24)	1.55 (0.20)
1979	1.40[4] (0.86)	1.93[6] (0.26)	1.86 (0.24)	1.74 (0.24)
1980	0.89[3] (1.13)	1.33 (0.61)	1.45 (0.42)	1.25 (0.53)
1981	1.89[5] (1.15)	2.96 (0.55)	2.72 (0.90)	2.97 (0.71)

[1] Number of male clusters, classes: 0 = 0; 1 = 1-5; 2 = 6-25; 3 = 26-50; 4 = 51-100; 5 = >100.

[2] Numbers in parentheses are the standard deviations.

[3] Differs significantly from S_1 x S_1-mix progenies at 5% level.

[4] Differs significantly from S_1 x S_1-mix progenies at 1% level.

[5] Differs significantly from S_1 x S_1-mix progenies at 5% level and from S_1 O.P. progenies at 1% level.

[6] Differs significantly from S_0 O.P. progenies at 5% level.

The S_2 progenies had fewer male clusters per flowering tree than the outcrossed progenies (table 5), probably for the same reasons that S_2 trees also had fewer female strobili. The standard deviation in the S2 progeny group was consistently higher than in the outcrossed progeny groups indicating that variation in male strobili fecundity is increased by inbreeding.

Correlations between progeny groups within years in the number of female strobili per flowering tree show mixed relations (table 6). Noteworthy are numerous negative correlations in 1978 and 1979, particularly those involving S2 progenies, that change to positive and, in some cases, significant correlations by 1981. This suggests that the most prolific early flowering outcrossed families have the most depression upon inbreeding in terms of early female strobili production but these later become the most prolific among the inbreds. Dominance for early flowering may be indicated.

Table 6.--Correlations between progeny groups within years in number of female strobili per flowering tree

Progeny group	S_1 selfed (S_2)	S_1 x S_1-mix	S_1 O.P.	S_0 O.P.
		1978		
S_1 selfed (S_2)	1.000	-0.446 (9)1/	-0.384 (13)	-0.202 (11)
S_1 x S_1-mix		1.000	0.172 (11)	-0.241 (9)
S_1 O.P.			1.000	-0.200 (14)
S_0 O.P.				1.000
		1979		
S_1 selfed (S_2)	1.000	-0.718*(9)	-0.540 (13)	-0.088 (11)
S_1 x S_1-mix		1.000	0.752** (11)	0.198 (9)
S_1 O.P.			1.000	0.298 (14)
S_0 O.P.				1.000
		1980		
S_1 selfed (S_2)	1.000	0.096 (9)	0.009 (13)	0.281 (11)
S_1 x S_1-mix		1.000	0.662*(11)	0.559 (9)
S_1 O.P.			1.000	0.333 (14)
S_0 O.P.				1.000
		1981		
S_1 selfed (S_2)	1.000	-0.160 (8)	0.344 (12)	0.587*(11)
S_1 x S_1-mix		1.000	0.734**(10)	0.094 (8)
S_1 O.P.			1.000	0.143 (14)
S_0 O.P.				1.000

1/ Numbers in parentheses are the degrees of freedom.
* Significant at the 5% level.
** Significant at the 1% level.

Correlations between years within progeny groups in number of female strobili per flowering tree were not found between 1978 and subsequent years (table 7). However, correlations between 1979 and 1980 and 1980 and 1981 were positive and significant in all progeny groups. This suggests that once most of the trees are producing female strobili, ranking of the families for number of female strobili per flowering tree did not significantly change between 1979 and 1980 or between 1980 and 1981. However, correlations between 1979 and 1981, although positive, fall short of significance. Longer term observations would be needed to clarify possible genotype x year interactions.

Table 7.--Correlation between years within progeny groups in number of female strobili per flowering tree

Year	1978	1979	1980	1981
		S_1 selfed (S_2) (15)[1]		
1978	1.000	0.192	-0.060	-0.185
1979		1.000	0.554*	0.319
1980			1.000	0.831**
1981				1.000
		S_1 x S_1-mix (13)		
1978	1.000	0.239	0.246	0.213
1979		1.000	0.714**	0.392
1980			1.000	0.417
1981				1.000
		S_1 O.P. (17)		
1978	1.000	0.438	0.431	0.176
1979		1.000	0.851**	0.445
1980			1.000	0.588**
1981				1.000
		S_0 O.P. (16)		
1978	1.000	0.250	0.089	-0.148
1979		1.000	0.661**	0.350
1980			1.000	0.806**
1981				1.000

[1] Numbers in parentheses are the degrees of freedom.

* Significant at the 5% level.

** Significant at the 1% level.

CONCLUSIONS

Inbreeding of jack pine to the second selfed generation (S2) results in significant depression or delay in initiation of early flowering and fecundity. However, by 6 years of age the percentage of S_2 trees flowering does not differ significantly from that of outcrossed trees--essentially all produce female strobili. The number of strobili per tree continues to be lower at 6 years. Although depressed, flowering in the S2 generation is adequate for breeding purposes. For potential production populations, as in seed orchard operations, the results show that crosses between selfed lines restore the early flowering behavior and fecundity to at least that of open-pollinated progenies of the ancestral parent trees. Thus, inbreeding and hybridization of inbreds in jack pine can be used as a complementary breeding strategy and may have potential for production of progenies from crosses among inbred lines in seed orchards. Studies to determine the effectiveness of selection and breeding for early flowering and its relation to tree growth are underway.

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