ACCELERATED GROWTH AND INITIAL FLOWERING OF S_2 *PINUS BANKSIANA* SELECTED FOR PRECOCIOUS FLOWERING

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Abstract: --An accelerated growth protocol was applied in a greenhouse to hasten flowering in 13 S_2 lines of jack pine *(Pinus banksiana* Lamb.) selected for precocious flowering. Seeds were sown on October 1. After the artificial "summer, fall, winter, and spring," seedlings were placed outdoors between June 20 and November 1. Ovulate strobili were counted between November 25 and December 8; about 21 percent of all seedlings flowered at 14 months of age. The percentage of trees flowering within a family line ranged from 5% to 70%. The average number of strobili per tree was 0.4, increasing to 1.7 when only the flowering trees were considered. Seedling heights were measured at the end of the simulated dormancy period in the greenhouse and on November 1 when they were brought inside. Correlation coefficients for the relationship of precocious flowering and height were significant for all trees pooled. Most within-family correlation coefficients for precocious flowering and height were positive and significant.

Keywords: flower production, inbreeding depression, jack pine, ovulate strobili, selfing.

INTRODUCTION

A tree must achieve a certain but, as yet, undefined state of vegetative development in the crown and root system before it is able to enter a generative phase and initiate its first flower primordia. Attainment of a minimum height or size has been used to explain the phase-change phenomenon (Zimmerman 1972, Hackett 1985, Chalupka & Cecich 1997). The ratio of seedling size to age can be increased by using "Accelerated Growth" protocols (Rudolph 1966, Hanover et al. 1976, Wheeler et al. 1982) that allow seedlings to go through several growing cycles in a relatively short time, or permit them to grow continuously. Hackett (1985) concluded that "the best strategy for obtaining rapid sexual maturation in many tree species is to grow the seedlings as rapidly as possible to a certain species- or genotype-dependent minimum size and then apply the flower-inducing treatment that is appropriate for the species."

Bolstad et al. (1992) and Cecich et al. (1994) used a growth chamber to investigate the control of precocious flowering in jack pine *(Pinus banksiana* Lamb.) and noted that the first flowering was a function of relative size within a half-sib family. There was a strong positive correlation between seedling height and flowering, but no evidence of a threshold or minimum height necessary for flowering to occur. When thousands or tens of thousands of seedlings are to be exposed to accelerated growth conditions, a growth chamber may not be practical because of its limiting size. A greenhouse then becomes more useful for conducting the protocols, recognizing that there can be greater environmental variation within a greenhouse. With this in mind, we conducted a verification of our growth chamber model and accelerated growth protocol (Cecich et al. 1994) in a greenhouse using 13 lines of S_2 jack pine seedlings that had been selected only for precocious flowering. Flowering is postponed or depressed by inbreeding (Rudolph 1981), especially within the S_1 generation when most lethal and deleterious alleles are expressed (Fisher 1965). Therefore, we considered it to be important to determine if depressed flowering could be partially offset by choosing taller seedlings. In this paper we

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use an accelerated growth protocol to determine if there is a positive relationship between precocious flowering and seedling height for the 13 S_2 lines of jack pine.

MATERIALS AND METHODS

On October 7, 1993, 6,873 jack pine *(Pinus banksiana* Lamb.) seeds from the 13 selfed S_1 family lines (Rudolph et al. 1989) were sown on moist perlite in covered petri dishes. Temperature was held at a constant 25° C. After successful germination, the embryo radicles extended 1 to 2 cm from the seedcoats and the seeds were transplanted to a 1:1:1 mix of soil, sand, and perlite in Spencer-Lamier root trainers (#1100-4) on a bench in the greenhouse.

The protocol for accelerated-growth (Figure 1) was conducted in a 13 m x 7 m pipe-frame structure (greenhouse) covered with a double layer of 6-mil-thick polyethylene. This structure had an attached headhouse that contained the heating and cooling system and the controller for the drip irrigation. Starting in February 1994, the seedlings were fertilized with a commercial formulation of NPK (14:14:14) plus minors every 2-3 months until the end of the experiment. During the extended photoperiod in the greenhouse, the tops of the plants received about 50 E m^{-2} s^{-1} of supplemental light from high-pressure sodium vapor lamps. The plants were moved outdoors on June 20, 1994, when they had finished the second shoot elongation phase (SE2), so that they would be exposed to the decreasing natural photoperiod of summer during the bud development phase (B2). They were returned to the greenhouse on November 1, 1994, after receiving a brief period of chilling outdoors.

Seedling height (mm) was measured in early May 1994, at the end of the first dormancy period (D1) and at the end of the second dormancy period (D2) in early November 1994. The height increment for SE2 was determined by subtracting D1 from D2. The first flowers (ovulate strobili) that the S_2 seedlings produced were observed and counted between the dates of November 25 and December 8, 1994. A threshold height for each family was determined by transforming the height of the shortest tree bearing strobili in each family to a percent of the mean height for the family.

The mean estimate of height was calculated for each height class within a family and for the pooled observations. Height classes were necessary to calculate the percentage of trees flowering relative to height and to determine the number of strobili per flowering tree. Percent data were arcsine transformed prior to analysis. Flowering data were transformed to $\sqrt{y+0.5}$ because of the large number of zeros. Pearson's product-moment correlations (SAS Institute 1994) were calculated for height and the flowering variables.

RESULTS

Germination

Eight days after sowing, 1944 seedlings (28 %) germinated and 1593 were vigorous enough to be transplanted to the containers. Fourteen months after sowing ("1 yr"), 833 seedlings (12.1 %) survived (Table 1). The precocious flowering response was observed on these surviving seedlings. Seedling survival of the 13 families ranged from 0.6 % in Family 89 to 20.4 % in Family 16. Inbreeding depression for germination was evident (Table 1), when the percent of all S_2 seeds germinated (28.3 percent) was compared with open-pollinated data from S_1 (66.4 percent) and So parents (62.2 percent) of the current lines (Rudolph 1976). Rudolph's germination data were based on "large" seeds; that is, those that were retained by a sieve with 1-mm holes. These data were adjusted so that an estimate for germination of 100 percent of the seeds was obtained. The "small" seeds almost never germinated and were routinely removed (Rudolph 1976). Unfortunately, there were no out-crossed or open-pollinated progeny in the present seed lots for a direct comparison of performance between So and the S2 plants in the current environment. The original parent materials for these lines were lost to plantation thinning, wind damage and disease.

Location	Greenhouse								Outdoor		Greenhouse
Growth Phase	GP-1	D – 1						SE-2	B-2	D-2*	***
Week #	1- 20	21	22, 23	24, 25	26	27 - 29	30	31 - 37	38 - 50	51 -60	61 —>
Light (h)	18	15	ambient	ambient	ambient	ambient	ambient	18	ambient	ambient	18
(oC) Day Night	25 15	25 15	25 15	15 7	7 7	7 7	15 7	25 15	ambient	ambient	25

GP = Growth period — GP-1: 20 weeks

D= Dormancy — D-1: Feb 21 to May 1; D-2: Sept 19 to Nov 1

SE = Shoot Elongation period — SE-2: May 2 to June 19

B= Bud Development period — B-2: June 20 to Sept 18

* = covered with pine mulch

*** = Flower observation: Nov 25 to Dec 8

Figure 1. Protocol for growing jack pine in accelerated growth conditions to promote the expression of precocious flowering.

Flowering

173 (21%) of the 833 surviving S2 seedlings produced a total of 301 ovulate strobili at the age of 14 months from seed (average of 0.4 strobili per tree, Table 2). Seedlings in Family 77 produced the most strobili (138), with 70% of the seedlings flowering. The poorest flowering family was Family 110; only 5 percent of its trees flowered. When only the flowering trees were considered, the mean number of strobili per tree was estimated to be 1.7. For the individual families, the mean estimate for the number of strobili per flowering tree ranged from 1.0 in Family 1081 to 2.5 in Family 89. These two families also had the least number of individual seedlings. Family 77 had the next highest mean estimate for the number of strobili per flowering tree (2.2). One seedling in Family 77 had 14 strobili and several had between 4 and 7. The most strobili that were observed on seedlings from other families were 2 or 3. We never observed strobili on seedlings with juvenile foliage (primary needles).

The height variation of the S2 jack pine lines during the first year is summarized in Table 3. We attempted to determine a threshold value for seedling height at which precocious flowering is likely to occur. The height of the shortest seedling with a strobilus in each family, as a percent of the mean height for that family, ranged from 70 to 117 percent. The shortest seedlings flowering in 11 of the 13 families were shorter than the family means. On average, the shortest tree bearing a strobilus was about 90 percent as tall as the mean height of all seedlings pooled (Table 3).

Table 1. Germination and survival of S2 jack pine seeds grown in conditions of accelerated growth.

Line	Number of seeds sown	Number germinated	Germination rate (%)	Number potted	Survival at 1 year	Survival rate at 1 year (%)
15	650	208	32.0	147	115	17.7
16	245	80	32.7	80	50	20.4
44	976	380	38.9	169	90	9.2
60	420	129	30.7	129	70	16.7
66	465	143	30.8	125	82	17.6
72	122	25	20.5	25	13	10.7
77	600	104	17.3	104	92	15.3
89	477	25	5.2	25	3	0.6
105	470	126	26.8	109	76	16.2
1081	724	64	8.8	64	19	2.6
1082	960	369	38.4	344	103	10.7
110	293	42	14.3	42	39	13.3
114	471	249	52.9	230	81	17.2
Totals & Means	6873	1944	28.3'	1593	833	12.1

x. Rudolph's (1976) germination data were adjusted to represent 100 percent of the seeds, not just the large seeds. After that adjustment, 28.1 percent of his S2 jack pine seeds germinated.

Correlation of height and precocious flowering

The correlations of November height (D2) and the SE2 height increment with strobili per tree for all 833 trees in the study were highly significant (r = 0.210 and 0.270, respectively, P> 0.01). There was no significant correlation between strobili per tree and May height (D1). To further examine flowering behavior in relation to height, we put the tree height measurements into classes based on mean heights per class. The percentage of trees flowering in a height class ranged from zero to 33 percent (r = 0.875, P>0.01). The correlation of height with the number of strobili per flowering tree (r = 0.848) was also highly significant.

When correlations were calculated within families, only two families (15 and 114) showed a significant positive correlation of first flowering with D1 height (Table 4); Family 16 had a significant negative correlation. However, when seedling height was measured at the end of the second dormancy period in early November (D2), all of the correlation coefficients became more positive and 7 of the 13 families had significant positive correlations. The correlation coefficients for the SE2 data with that of flowering were similar to those generated with the D2 data. There were no negative correlation coefficients when the SE2 increment was evaluated with flowering (Table 4).

The height distribution for each family was sorted into classes and correlation coefficients were calculated in relation to the number of strobili per flowering tree and the percent of trees flowering (Table 5). Families 72, 89 and 1081 were not included because they only had a few seedlings and two height classes. Correlation coefficients for flowering with D2 heights of Families 15 and 16 became more negative when compared to the D1 height measurements. Family 114 retained strong positive correlations for both measurement dates. Family 77 was the "best" family in terms of producing the most strobili; however, Family 16, also a good strobilus producer, demonstrated a significant negative correlation of precocious flowering with height. The correlations of the SE2 height increments with flowering provided fewer significant *r*-values than the height measurements taken at D2 (Table 5).

Table 2. A summary of the flowering responses of 13 S_2 jack pine lines.

Line	Number of trees	Number of flowering trees	Percentage of trees flowering	Number of strobili	Strobili per tree	Strobili per flowering tree
15	115	33	28.6	47	0.4	1.4
16	50	14	28	24	0.5	1.7
44	90	5	5.5	8	0.1	1.6
60	70	9	12.8	11	0.2	1.2
66	82	8	9.8	11	0.1	1.4
72	13	2	15.4	4	0.3	2.0
77	92	64	70	138	1.5	2.2
89	3	2	66.7	5	1.7	2.5
105	76	8	10.5	13	0.2	1.6
1081	19	2	10.5	2	0.1	1.0
1082	103	15	14.6	25	0.2	1.7
110	39	2	5.1	3	0.1	1.5
114	81	9	11.1	10	0.1	1.1
Totals & Means	833	173	20.8	301	0.4	1.7

Table 3. Height variation among S_2 jack pine lines selected for precocious flowering.

		May height (mm)		Nov. height (mm)		Ht of shortest	Shortest tree as
Line	n	Mean	s.d.	Mean	s.d.	tree flowering	a % of mean ht.
15	115	66	20	325	136	275	85
16	50	95	23	399	155	330	83
44	90	93	29	342	66	304	89
60	70	106	29	328	119	256	78
66	82	94	33	354	138	289	82
72	13	78	26	301	132	366	117
77	92	75	18	342	138	240	70
89	3	74	8	280	146	310	92
105	76	104	30	379	146	382	101
1081	19	78	23	291	119	263	90
1082	103	90	21	294	108	286	97
110	39	108	27	376	140	360	96
114	81	65	24	240	99	236	98
Mean		87	24	327	126	300	90.2

Table 4. Correlation of D1 and D2 heights and the SE2 height increment with the number of flowers per tree.

		Correlation Coefficients		
Line	n	D1	D2	SE2
15	115	0.225 *	0.366 **	0.338 **
16	50	- 0.280 *	0.003	0.192
44	90	0.147	0.093	0.035
60	70	0.055	0.288 *	0.327 **
66	82	- 0.013	0.374 **	0.405 **
77	92	0.107	0.272 **	0.241 *
105	76	0.223	0.328 **	0.287 *
1082	103	0.155	0.322 **	0.323 **
110	39	0.120	0.145	0.092
114	81	0.362 **	0.371 **	0.322 **

Sources 72, 89 and 1081 had insufficient observations.
*, ** Indicate significance at P> 0.05 and 0.01, respectively.
No height classes were used in the analysis.

Table 5. Correlations by classes of D1 and D2 height and the SE2 height increment with flowering at 14 months from seed.

	May height (D1)		November height (D2)		SE2 height increment	
Line	# Flr/Fltr	% Trs-Flrg	# Flr/Fltr	% Trs-Flrg	# Flr/Fltr	% Trs-Flrg
15	0.801 *	0.869 *	0.312	0.920 **	0.293	0.919 **
16	- 0.575	- 0.817	0.004	- 0.850 *	0.626	0.270
44	0.677	0.769 *	0.754 *	0.856 *	- 0.204	- 0.204
60	0.264	- 0.041	0.775 *	0.820 *	0.926 **	0.914 **
66	- 0.189	0.528	0.696	0.820 *	0.383	0.531
77	0.474	0.650	0.741	0.978 **	0.725	0.688
105	0.185	0.379	0.828 *	0.857 *	0.640	0.830 *
1082	0.206	0.235	0.603	0.697	0.295	0.461
110	0.927 *	0.927 *	0.391	0.387	0.131	0.131
114	0.881 **	0.850 *	0.823 *	0.801 *	0.867 *	0.835 *

*, ** Indicate significance at P> 0.05 and 0.01, respectively.
Sources 72, 89 and 1081 had only two classes and were not included in analysis.
Flr/Fltr = Number of flowers (strobili) per flowering tree in a height class.
Trs-Flrg = Percent of trees flowering in a height class.

DISCUSSION

Precocious flowering is usually associated with seedling height or size (Chalupka & Cecich 1997). A significant positive correlation was noted for the relationship of precocious flowering and relative height of jack pine seedlings within a family, rather than absolute size (Bolstad et al. 1992, Cecich et al. 1994). That is, the taller seedlings within a family tended to express precocious flowering and to produce more strobili per seedling. However, they found no evidence of a threshold height. If a threshold height does exist within a family, its expression is probably dependent on the environment in which the seedlings are growing. This view is supported by comparing the flowering response of one year-old nursery-run seedlings grown in a nursery (1 in 1000; Rudolph 1979) or grown under conditions of accelerated growth (Cecich & Bauer 1987). In the latter study, seedlings from a nursery-run seed mix were grown under conditions of accelerated growth for one winter, strobili were hand pollinated with an unrelated pollen mix, and the seedlings placed in the nursery for one year. The seeds were collected from those plants and exposed to an accelerated growth protocol. When those seedlings were 14 months-old, about 30 percent of them produced ovulate strobili; but seedling height was not measured (Cecich & Bauer 1987).

What are some factors that may be considered as potential "thresholds" to precocious flowering behavior in jack pine? Morphologically, an ovulate strobilus has its origin as a lateral long-branch primordium (Doak 1935). This was indirectly substantiated when no ovulate strobili were observed on jack pine seedlings having a "foxtail" morphology; that is, during a period in which they had no lateral branches (Cecich 1981). In the present and previous studies of jack pine, we never observed strobili on seedlings with only juvenile foliage (primary needles that are not of fascicular origin). Of four species of *Cupressus* that were induced to flower with photoperiod and gibberellin treatments (Pharis & Morf 1967), the one species that maintained its juvenile needles did not flower. Our measurements of seedling height and observations of precocious flowering within a family showed that, on average, the shortest individual bearing a strobilus was about 90 percent as tall as the mean height for that family. These morphological characters may not provide absolute threshold values, but they are associated with the expression of precocious flowering.

The relationship of precocious flowering with height suggests that they are phenotypically correlated in jack pine. When all families were pooled in the present study, seedling height was highly correlated with precocious flowering at 14 months of age (r = 0.210**), the percentage of trees flowering within a height class (r = 0.875**) and also with the number of strobili per flowering tree (r = 0.848**). Although the pooled observations indicate that precocious flowering seedlings are likely to be found in the upper 50 percentile of the height distribution (Table 3), more precision in predicting precocious flowering can be gained by selecting within families. There were families in which no significant correlation existed between the flowering variables and seedling height or the correlations were negative. Thus, family relationships are important and should be considered when evaluating and selecting seedlings for potential precocious flowering behavior. Selecting the larger seedlings in a family before their first flowering should increase the likelihood of selecting precocious flowering trees and trees with more initial strobili. Schmidtling (1981) found that precocious flowering and growth traits in *Pinus taeda* L. were negatively correlated, indicating that the relationship of height to precocious flowering may not be universal.

In the present study, about 21 percent of all seedlings produced strobili at 14 months (Table 2). One must not lose sight of the fact that these current seedlings were the product of 2 cycles of inbreeding. Compared to the flowering response of wild type seedlings grown under similar conditions (30 percent; Cecich & Bauer 1987), inbreeding depression for the expression of precocious flowering in these 14-

month-old S_2 jack pine seedlings appears to be present. Unfortunately, direct comparison to the performance of the So parents of these lines cannot be made, because they no longer exist.

Perhaps the genes that control precocious flowering (Mandel & Yanofsky 1995, Weigel & Nilsson 1995) and their relationship to seedling height were more strongly fixed in these S_2 jack pine lines than in the previously-used lines that were based on S_1 and open-pollinated progenies (Cecich et al. 1994). The *LFY* gene that regulates precocious flowering (Weigel & Nilsson 1995) apparently encodes a developmental switch that can convert all lateral shoot primordia into solitary flowers, leading to precocious flowering. Since the ovulate strobili of gymnosperms have their morphological origins as lateral long-branch primordia (Doak 1935), it is not unreasonable to speculate that the *LFY* gene is being expressed and fixed in these inbred jack pine lines, especially one such as Family 77 that flowers so abundantly. These data also suggest that the isolation of flowering genes, such as *LFY* or *AP1* (Mandel & Yanofsky 1995), from the taller inbred seedlings would be more economically feasible than from open-pollinated half-sib families without any selection for precocious flowering.

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